FULL ABSTRACTS

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Developmental plasticity, the ability to produce multiple phenotypes from a single genotype, is a ubiquitous feature of multicellular life. At its extreme, developmental plasticity takes the form of polyphenism, or the production of discrete alternative morphs, often with disparate ecological roles. The nematode *C. elegans* has a polyphenism in its adult mouthparts, allowing individuals to develop into microbivores or into omnivores capable of predation, depending in part on the environment of them may be hiding in plain sight, disguised as developmental genes.

Selfish genetic elements promote their own transmission while being neutral or detrimental to the fitness of the organism. In extreme cases, selfish elements promote their transmission by killing individuals that do not inherit them, leading to a genetic incompatibility between carriers and non-carriers. Genetic incompatibilities are found across the tree of life, but in only a few cases have the underlying genetic mechanisms been resolved. We discovered a novel genetic incompatibility between strains of *C. elegans*. We present here the life cycle and infection strategies of these new pathogens that allow them to colonise and eventually kill the nematode host. We have performed RNAseq experiments to address pathogen-induced changes of host gene expression. We show that the transcriptional response to oomycete infection consists of upregulation of already known antimicrobial peptides and other putative immunity genes, as well as members of a previously uncharacterised family of chitinase-like (chil) genes. Using reporter constructs and smFISH, we demonstrate the chil genes are induced at the epidermis. We also demonstrate that chil induction is an oomycete-specific response and cannot be mounted against other pathogens or upon environmental stresses. Intriguingly, we present evidence that infection is not required for chil gene induction since a non-infectious pathogen extract is also capable of triggering the same transcriptional response. Using functional genetics and atomic force microscopy, we demonstrate that the chil genes play a role to antagonise the oomycete infection by modifying the biochemical properties of the cuticle. Our work introduces some new natural pathogens of *C. elegans* and paves the way for the discovery of the molecular components of oomycete recognition and transcellular signalling involved in the innate immunity response.

**A wolf in sheep's clothing: a selfish element disguised as a linked pair of developmental genes underlies a genetic incompatibility in C. elegans.**

Selfish genetic elements promote their own transmission while being neutral or detrimental to the fitness of the organism. In extreme cases, selfish elements promote their transmission by killing individuals that do not inherit them, leading to a genetic incompatibility between carriers and non-carriers. Genetic incompatibilities are found across the tree of life, but in only a few cases have the underlying genetic mechanisms been resolved. We discovered a novel genetic incompatibility between strains of *C. elegans*. The incompatibility is caused by a selfish element composed of two genes: a maternal-effect toxin and a zygotically expressed antidote. In crosses between strains that carry the element and ones that do not, the element acts via maternal effect to kill the progeny that do not inherit it. We identified the genes underlying the incompatibility, and were surprised to find that the toxin and the antidote were encoded by *sup-35* and *pha-1*, respectively. *pha-1* was originally proposed to be an essential developmental gene due to specific pharyngeal defects observed in mutants, and its defects were known to be fully suppressed by mutations in *sup-35*. Our results indicate that the phenotypes previously associated with mutations in *pha-1* are in fact a consequence of *sup-35* toxicity, which is rescued by *pha-1*. The lethality associated with *pha-1* mutations is known to require additional genes, suggesting that the *sup-35/pha-1* selfish element exerts its toxicity by hijacking a conserved developmental pathway. We de novo assembled the haplotypes of strains not carrying an active *sup-35/pha-1* element using a combination of Illumina short reads and Oxford Nanopore long reads, and found structural variation and remarkable nucleotide divergence, illustrating the ability of selfish elements to reshape the genome. We also identified strains carrying loss of function variants in *sup-35* that abolish the incompatibility, further supporting its role as a toxin rather than a developmental gene. Our results illustrate how the study of natural genetic variation can illuminate our understanding of gene function. Furthermore, our results suggest that selfish elements conferring genetic incompatibilities may be more common than previously thought, and that some of them may be hiding in plain sight, disguised as developmental genes.
ecological strategies are molecularly controlled and change in evolutionary time. The *P. pacificus* mouth polyphenism was previously found to be regulated by a novel sulfatase (EUD-1), which indirectly inactivates a nuclear hormone receptor (NHR-40). Using a forward screen for suppressors of *eud-1*, we found that the polyphenism switch is also controlled by a novel sulfoxotransferase, which we named SEUD-1 (*suppressor of eud-1*). Like *eud-1*, *seud-1* is the result of lineage-specific gene duplications: it is a novel homolog of *ssu-1*, a gene implicated in another environmentally-conditioned developmental decision (dauer formation) in *C. elegans*. To gain increased resolution of the intercellular signaling mechanism of the polyphenism switch, we performed expression studies of *seud-1*. To test whether SEUD-1 and EUD-1 compete for the outcome of the switch, we manipulated the relative numbers of their wild-type alleles using crossing and transgenic experiments. Phenotypes resulting from all possible genotype combinations showed that the relative numbers of functional alleles of the two genes determined the ratios of adult morphs. Given the sensitivity of phenotypes to competing gene dosage, we hypothesized that gene-copy number could provide a sensitive target for natural selection to drive the evolution of plasticity phenotypes. Hybrid crosses between *P. pacificus* and a sibling species, particularly one that has incurred a recent duplication of *seud-1*, indeed indicated that gene duplication can amplify a plasticity regulator’s molecular and phenotypic effects. Our results suggest that plasticity phenotypes sensitive to gene dosage can evolve directly by gene amplification, in addition to the expected selection for cis-regulatory changes. Given the repeated duplications of *eud-1* and *seud-1* homologs, we speculate that gene duplication and amplification, followed by fixation and divergence, offers a mechanism for the birth of novel developmental regulators, including polyphenism switch genes. In summary, our study provides the first genetic insight into how the molecular regulation of polyphenism evolves to produce new plasticity phenotypes.

5 A family of novel sulfolipids drives *C. elegans* defensive responses to a predator. A. Pribadi1,2, Z. Liu1, M. Kariya3, F. Schroeder2, J. Srinivasan4, S. Chalasani1,2 1) Molecular Neurobiology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA; 2) Division of Biological Sciences, UCSD, La Jolla, CA; 3) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY; 4) Department of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA.

Animals discover predators in their environment and execute appropriate behaviors to increase survival. We investigated *Caenorhabditis elegans*’ responses to a nematode predator, *Pristionchus pacificus*. Upon detecting *P. pacificus* excretions, *C. elegans* responded with behaviors on multiple timescales - an instantaneous escape behavior and a prolonged reduction in egg laying. In collaboration with the Schroeder lab (Cornell University), we used an activity guided fractionation approach to identify the relevant signals. We found that *P. pacificus* specifically produces a family of novel sulfated lipids that drive both *C. elegans* responses. Using cell ablations and calcium imaging, we found that a third of the *C. elegans* amphid sense organ (ASI, ASJ, ASH and ADL neurons) is involved in detecting predator excretions. Exploiting genetic mutants, we show that cyclic nucleotide gated and transient receptor potential potential channels function in distinct subsets of neurons to drive *C. elegans* responses. Finally, we show that a human anti-anxiety drug, Zoloft attenuates *C. elegans* responses to the predator. These studies show that signaling pathways for processing external threats are likely conserved from worms to mammals. Animals discover predators in their environment and execute appropriate behaviors to increase survival.

6 Dissecting the foundations of commensalism using *C. elegans* and its natural microbiome. F. Zhang1, J.L Weckhorst1, C.A Ayoub1, M.A. Félix2, B.S. Samuel1 1) Alkek Center for Metagenomics and Microbiome Research, Baylor College of Medicine, Houston, TX; 2) École Normale Supérieure (ENS), Institut de biologie de l’ENS (IBENS), Paris, FRANCE.

Partnerships between animals and microbes are ancient and coevolved relationships are common and born out of mutual benefit. Proper establishment and maintenance of these relationships relies on strong lines of host-microbial communication. Our goal is to develop a comprehensive understanding of the host-microbial signaling pathways that regulate these processes. To do this, we employ the genetically tractable, high-throughput amenable and microbially ‘tuned’ nematode *C. elegans* as it harbors a simple community of microbes in the wild (its ‘microbiome’). Through collaborative meta-analyses, we defined our core microbiome that is largely distinct from that of corresponding substrates— *Enterobacteriaceae, Pseudomonadaceae, Xanthomonadaceae, Sphingomonadaceae*, three *Bacteroidetes* families and several less numerous families.

To comprehensively examine host selection of its microbiome, we defined a community of 68 bacterial isolates with >98% identify to meta-analyses from our collection (>550 microbes) and fed this model microbiome to wild *C. elegans* strains (42) for comparison. Using a high-throughput gut colonization method that we developed, we observed host strain specific colonization levels over a 30 fold range with N2 at an extreme. As in the wild, sequencing of animals over time demonstrated strong host role in selecting its microbiome distinct from the lawns. This process appears to be deterministic, as specific wild strains (42) for *cis*-regulatory differences. Using a high-throughput gut colonization method that we developed, we observed host strain specific colonization levels over a 30 fold range with N2 at an extreme. As in the wild, sequencing of animals over time demonstrated strong host role in selecting its microbiome distinct from the lawns. This process appears to be deterministic, as specific wild strains when challenged with the *Chryseobacterium*. The majority of the RNAi clones further enhanced JU1218 resistance, suggesting partial loss of functions in the wild strain, but also identified 27 promising candidates in signaling pathways (e.g., a GPCR and several transcription factors) influence *Chryseobacterium* impact on N2. Thus, through these and additional studies, we hope to leverage *C. elegans* as a robust natural genetic system to catalogue the pathways that are important managing microbiome form and function.

7 Evolutionary insights into the *C. elegans* biology from the morphology, ecology and genome of the sister species *Caenorhabditis sp.* 34. T. Kikuchi1, I. Tsai2, R. Tanaka1, G. Woodruff2, J. Wang2, M. Berriman1, P. Sternberg5, A. Sugimoto6, N. Kanzaki3 1) Faculty of Medicine, Miyazaki, JP; 2) Biodiversity Research Center, Academia Sinica, Taiwan; 3) Forestry and...
The C. elegans natural diversity resource. Daniel Cook, Joshua Roberts, Stefan Zdrajlic, Erik Andersen  Molecular Biosciences, Northwestern University, Evanston, IL.

Caenorhabditis elegans is used as a model in over 1,100 labs around the world and has enabled biological discoveries in many diverse fields. However, the majority of C. elegans research focuses on the laboratory-domesticated N2 strain, neglecting potential insights gleaned from natural populations. Studies of C. elegans natural variation can identify the genetic factors underlying biomedically relevant traits and genome evolution. To address the need for resources to study natural variation, we developed the Caenorhabditis elegans Natural Diversity Resource (CeNDR) — available at www.elegansvariation.org. The web-based CeNDR platform includes three areas: (1) a central repository for the deposition, organization, and dissemination of wild C. elegans strains, including detailed information for each strain (e.g. collection date, GPS location, substrate, and elevation); (2) a data portal for dissemination of whole-genome sequence data in BAM or CRAM formats and variant data in VCF format for each of the 383 wild isolates, including a powerful interactive genome browser that can be used to interrogate genetic variation across the population for genes or regions of interest; (3) a genome-wide association mapping portal to enable mappings of quantitative traits measured using wild C. elegans strains, including a comprehensive report of significance, variation, measures of selection, etc. We believe that CeNDR will become an indispensable tool within the C. elegans toolkit to enable researchers to examine natural populations and identify interesting new biological phenomena.

The C. elegans multiparent experimental evolution mapping panel. Luke Noble1, Ivo Chelo2, Thiago Guzella2, Bruno Afonso3, David Riccardi1, Patrick Ammerman1, Adel Dayarian2, Sara Cavalho2, Anna Crist3, Ania Pino-Querido2, Boris Shraiman4,5, Matthew Rockman3, Henrique Teotonio3 1) Center for Genomics and Systems Biology, New York University, NY, USA; 2) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 3) Institut de Biologie, École Normale Supérieure, Paris, France; 4) Kavli Institute for Theoretical Physics, University of California, Santa Barbara, CA, USA; 5) Department of Physics, University of California, Santa Barbara, CA, USA.

We report a recombinant inbred line (RIL) quantitative trait locus (QTL) mapping panel for the hermaphroditic nematode Caenorhabditis elegans, the C. elegans multiparental experimental evolution (CeMEE) panel. The CeMEE panel, comprising 507 RILs, was created by hybridization of 16 wild isolates, experimental evolution at moderate population sizes for 140-190 discrete generations, and inbreeding by selfing for 13-16 generations. The panel contains around 22% of single nucleotide polymorphisms known to segregate in natural populations, and complements existing mapping resources for C. elegans by providing high nucleotide diversity across >95% of the genome, and fine-scale mapping resolution.


While in toto imaging and image analysis methods have advanced the study of multicellular phenomena in development at single-cell resolution, not much progress has been made in the design of tools to perturb complex tissues with comparable spatial and temporal resolution. Mutagenesis and RNAi have proven to be powerful tools for dissecting the genetic basis of developmental phenomena, yet neither offers single cell specificity nor the temporal control required for dissecting the roles played by individual cells in coordinating development. Both classical single cell perturbation techniques such as laser cell ablation and newer technologies built around photoactivatable reagents and proteins offer significant promise in filling this need. Their use to-date, however, remains limited by the challenges associated with reliably identifying specific target cells for perturbation and with systematically monitoring experimental outcomes and off-target effects.

We developed ShootingStar, a platform for the real-time detection and tracking of single cells in 3D tissues to enable reproducible single cell perturbations at high throughput and without a need for cell-specific markers. ShootingStar is able track thousands of cells in real-time with sufficient accuracy to reconstruct cell lineages over many rounds of mitosis. Target cells are
automatically identified using a user-defined set of criteria which can draw from direct and quantitative measurements of cell behavior, gene expression and lineage history. These same measurements can be used to systematically and quantitatively validate experimental results and to detect subtle artifacts caused by off-target effects. ShootingStar can target multiple cells for perturbation, in multiple specimens in parallel, enabling complex experiments to be carried out with relatively high throughput. Furthermore, it can be adapted to different modalities of perturbation and to samples derived from different organisms. We used ShootingStar to automate laser ablations in the embryo of Caenorhabditis elegans and the larva of Danio rerio to probe the role of the GLR's in the morphogenesis of the nerve ring and the establishment of hair cell polarity in the lateral line, respectively. Additionally, we used ShootingStar to automate cell labeling by selective photoconversion in the C. elegans embryo to capture the dynamics of process outgrowth in a single neuron. ShootingStar simplifies the conduct of a broad set of challenging experiments while simultaneously enabling their systematic validation and review.

11 Model organism Encyclopedia of Regulatory Networks (modERN). V Reinke1, M Kudron1, M Han1, S Samanta1, A Higdon2, L Gewirtzman2, J Patton3, D Vafeados4, A Victorsen1, L Ma5, J Xu6, KK Yian6, WW Fisher6, AS Hammonds4, R Weiszmann4, M Gerstein1, SE Celniker4, KP White3, RH Waterston2 1) Yale University, New Haven, CT; 2) University of Washington, Seattle, WA; 3) University of Chicago, Chicago, IL; 4) Lawrence Berkeley National Laboratory, Berkeley, CA.

The goal of the modERN consortium is to create comprehensive maps of transcription factor (TF) binding sites in C. elegans and D. melanogaster. We have developed an effective high throughput pipeline to create strains of flies and worms with GFP-tagged TFs, which we use to perform ChIP-seq experiments. We are targeting 691 sequence-specific TF genes in the worm and 708 TF genes in the fly. The modERN project has generated 420 worm strains and 330 fly strains to date. A subset of worm strains had tagged TFs that localized to the cytoplasm (54), were poorly expressed (23), or did not pass quality control measures for ChIP (37). Thus, including data generated previously for the modENCODE project, we have completed ~900 ChIP-seq experiments for 235 factors (219 TFs and 16 other regulatory proteins) in the worm and 287 TFs in the fly. We processed all data sets with a uniform peak-calling pipeline for worm and fly. The resultant inferred binding sites can be useful to predict gene expression, particularly using combinations of TF binding profiles. In the worm, for example, combinatorial TF binding patterns can suggest expression in muscle, intestine, hypodermis, pharynx and neurons. For a limited number of transcription factors, we are conducting RNA-seq of strains with knock down of specific transcription factors by deletion or RNAi to identify potential target genes of the given TF and to validate targets identified by the ChIP-seq experiments. To date, we have collected RNA-seq data from 30 worm TF knockdown lines and 12 TF fly knockdown lines. RNA-seq data and ChIP-seq data are deposited to the ENCODE DCC for access by the research community through the ENCODE DCC (https://www.encodeproject.org), as well as through the UCSC genome browser. A dedicated track hub for the worm data at UCSC is available as well. TF tagged lines are available through the Caenorhabditis Genetics Center and Bloomington Drosophila Stock Center. Here, we will summarize progress, present future plans, and demonstrate how to access and use the data.

12 De-completing the C. elegans genome. E.M. Schwarz1, J. Yoshimura2, K. Ichikawa2, M. Shoura3, K.L. Artiles3, I. Gabdank4, L. Wahba3, C.L. Smith3,4, M.L. Edgley3, A.E. Rougvie5, A.Z. Fire3,4, S. Morishita6 1) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853, USA; 2) Department of Pathology, Stanford University, Stanford, CA 94305, USA; 3) Department of Genetics, Stanford University, Stanford, CA 94305, USA; 4) Department of Zoology and Michael Smith Laboratories, University of British Columbia, Vancouver, British Columbia, Canada; 6) Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, Minnesota 55454, USA.

By 2005, the C. elegans genome was sequenced to apparent completion from a mixture of divergent laboratory strains, all derivatives of Brenner's original "N2". The lack of a single worm strain matching this sequence creates challenges for genomics and systems biology. We thus chose to produce a updated reference genome for C. elegans from the strain VC2010, which was derived from a clonal isolate of N2. After sequencing to a depth of 115x coverage with trimmed, error-corrected PacBio and Oxford Nanopore reads having an average length of 11.8 kb, we assembled a updated reference genome with near-chromosomal contiguity. Half of the assembly falls into 10 blocks of continuous sequence (contigs) ranging from 3.6 Mb to 8.4 Mb in size, and the entire assembly is contained in only 82 contigs. Our assembly is very close to the previous reference assembly's quality (97.6% full-length CEGMA hits, versus 98.4% in previous N2). It is also slightly larger than the previous reference assembly (102.0 Mb versus 100.3 Mb in previous N2, a difference of 1.7%). We have closed some remaining gaps in the updated reference genome assembly with PacBio and Oxford Nanopore single reads, and used Hi-C to estimate gap sizes. This revealed 26 unlinked regions in the updated reference genome of ~130 kb, whose flanks contain highly repetitive DNA. In the official N2 genome, these regions have relatively few copies of repetitive DNA, and are ungapped. We suspect that the large gaps are real, but were lost during propagation and sequencing of cosmids clones during the original N2 genome project, making them a kind of genomic "dark matter". In other words, we think that we have de-completed the C. elegans genome. The apparent 1.7% increase in the updated reference's genome size may reflect other overlooked repeats that exist biologically in N2. Sequences in N2 that are missing from the official genome were recently described by Zhao et. al. (PubMed 26039588), with our observations expanding the discrepancy from 40 kb to 1.7 Mb. Such "dark matter" regions probably exist in other nematode genomes and make them harder to assemble. Since they have persisted in C. elegans, they may have biological functions; possibilities for such functions include generation of ncRNAs and aiding chromosomal segregation. Our consensus VC2010 genome assembly will be made available in Wormbase, with CGC distributing a VC2010-derived strain (PD1074) after July 2017.
Balancer chromosomes are critical tools for genetic research. In C. elegans, chromosomal rearrangements including reciprocal translocations, inversions and free duplications have been used to maintain lethal and sterile mutations in heterozygotes. We recently established a CRISPR/Cas9-mediated method for generation of artificial chromosomal rearrangements, namely crossover suppressors, which are composed of integrated and sequential inversions and prevent recombination between homologous chromosomes. The key advance of the crossover suppressors is an absence of aneuploidy, compared with chromosomal translocations. Thus, the crossover suppressor chromosomes make more chances to obtain chromosomally intact animals. However, only four classical crossover suppressor alleles (qC1, sC1, sC4 and mnC1) have been established to date, totally covering approximately only 15% C. elegans genome. Here, we generated a new set of crossover suppressor chromosomes. Our balancer chromosome toolkit includes 15 crossover suppressors and totally covers approximately 83% C. elegans genome together with the classical crossover suppressors. We also labeled with fluorescent markers on the new balancers that we newly created. In addition, we generated transgenic insertions of fluorescent marker in the pairing center where chromosomal inversion is hard to be constructed. The toolkit will provide more efficient experiments in which the lethal and sterile mutants will be constructed or analyzed.


The goal of the Caenorhabditis Genetics Center (CGC) is to promote research on the small metazoan Caenorhabditis elegans by curating important, genetically characterized nematode stocks and distributing them upon request to researchers and science educators throughout the world. The CGC is housed at the University of Minnesota and is supported by the National Institutes of Health - Office of Research Infrastructure Programs (NIH-ORIP) and nominal user fees. There are now nearly 20,000 different strains in the collection. We strive to have at least one allele of every published gene and all useful chromosome rearrangements, duplications and deficiencies. Selected multiple-mutant stocks and transgenic strains are also available, such as strains that express various fluorescent protein reporter fusions. A small research component has enhanced the genetic toolkit available to C. elegans researchers, in part by labeling existing balancer chromosomes with fluorescent markers. A searchable list of strains, including information about CGC stocks, is accessible through the CGC website (www.cbs.umn.edu/CGC/) and WormBase. Requests for strains should be made on-line through our website, using credit cards for payments whenever possible. We provide yearly reports to the NIH with statistics that reflect our services to the worm community. A key tracked parameter is the number of published papers that acknowledge the CGC for providing strains. Please help us retain our funding by remembering to acknowledge the CGC in your publications!


WormBase (www.wormbase.org) has been serving the scientific community for 17 years as the central repository of genomic and genetic information for C. elegans and other nematodes. We continue to enrich the catalogue of data, develop the suite of tools and displays for exploring it on the website, and improve the back-end database and infrastructure to allow us to capture more data and serve it faster. Recent developments include: an updated version of our data-mining platform (Wormine), with new data-types; a new query tool (SimpleMine) for performing common queries quickly and intuitively; a new analysis tool for discovering genes that have enriched expression in certain tissues or confer particular phenotypes (TEA and PEA); a new graph display of phenotype annotations (SOBAn); and an expansion of the of parasitic nematode genomic data in WormBase ParaSite (parasite.wormbase.org). As the C. elegans research community, continue to innovate, WormBase is faced with curating, storing and serving data that is constantly growing in size and complexity. While we continue to improve our efficiency of extracting information from papers, we need your help in getting your data into searchable and computable form. We thus have a number of tools and user-friendly forms that allow you to submit your data directly to us in a structured way, making it faster to incorporate into WormBase. For certain data types, submissions can also result in citable credits in the form of Micropublications (www.micropublicationbiology.org). Finally, we have joined forces with a number of other Model Organism Databases to form the Alliance of Genome Resources (www.alliancegenome.org). This Alliance will pool knowledge and expertise across the member resources to develop shared curation methods, data models, and infrastructure, with the aim of delivering model organism resources that are more comprehensive, efficient, scalable, and sustainable.
complexity of this tissue is that *C. elegans* germ cells are connected to a shared cytoplasmic core via cytoplasmic bridges, called ring channels. Here we mapped the positions of ring channels in three dimensions and report two unexpected features of germ line architecture. First, we find that germ cells isolate themselves from the rest of the germ line during mitosis. Ring channels close during mitosis, preventing the contents of mitotic germ cells from leaking into neighboring cells. This finding provides a mechanism by which germ cells divide asynchronously even in a syncytium. Second, we find that germ cells in adult hermaphrodites move through the distal germ line along a sinuous, folded path. The shape of this path is caused by the distal germ line being highly folded and sometimes coiled. These folds begin during L4, are dynamic over time, and bring together regions of the germ line that are topologically far apart, often creating the illusion of abrupt changes in the levels of germ cell regulators, such as GLD-1. GLD-1 levels are low in distal-most germ cells and increase gradually—not abruptly—as cells move proximally along the folded path. This pattern suggests that germ cells differentiate gradually in the distal germ line, and that differentiation is best gauged by a cell’s position along the folded path, rather than its position along a strict distal-to-proximal axis. Our work provides a new understanding of how germ cells in *C. elegans* divide and differentiate across a folded epithelium.

17 A Non-Cell-Autonomous Role of BEC-1/BECN1/Beclin1 in Coordinating Cell-Cycle Progression and Stem Cell Proliferation during Germline Development. Kristina Ames1,2, Dayse Da Cunha1,4, Brenda Gonzalez4, Marina Konta3, Feng Lin1, Gabriel Shechter1, Lev Starikov1, Sara Wong1, Hannes Bülow1,5, Alicia Meléndez1,2,3 1) Biology, Queens College, City University of NY, Flushing, NY; 2) Biochemistry, The Graduate Center of the City University of NY, NY; 3) Biology, The Graduate Center of the City University of NY, NY; 4) Department of Genetics, Albert Einstein College of Medicine, Bronx, NY; 5) Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY.

Autophagy is a conserved cellular recycling process crucial for cellular homeostasis. In a multistep process, cellular material destined for degradation is enclosed in an organelle with a double membrane, the autophagosome, which in turn fuses with the lysosome. BEC-1, the *C. elegans* ortholog of Beclin1/BECN1 in mammals, was shown to be a haploinsufficient tumor suppressor protein in mammals, crucial for the initial nucleation step of autophagosome formation. In previous studies we showed that BEC-1 serves important functions during development, and longevity of multicellular organisms. In addition, we demonstrated a role for BEC-1 in endocytosis, including in retromer transport from endosome to Golgi, and lipid homeostasis. We now describe a novel role for BEC-1 and autophagy in germ line stem cell homeostasis. The decision of a stem cell to proliferate or differentiate is finely controlled, and several pathways, such as GLP-1/Notch, DAF-2 insulin IGF-1 receptor (IIR), and DAF-7/TGFβ signaling have been shown to be required for the proper number of germ line progenitors. We found that BEC-1, and several other autophagy proteins, such as ATG-18 (in mammals WIPI1/2), ATG-16.2 (in mammals ATG16L) and ATG-7 (in mammals ATG7) are required for the late larval expansion of germ line stem cell progenitors during development. Interestingly, BEC-1, ATG-18, and ATG-16.2 act independently of the GLP-1/Notch or DAF-7/TGFβ pathways, but upstream of the DAF-2 insulin IGF-1 receptor (IIR) signaling pathway, to promote germ line stem cell proliferation. BEC-1, ATG-18, and ATG-16.2, all promote cell cycle progression and are negatively regulated by DAF-18/PTEN, similar to DAF-2/IIR. However, whereas BEC-1 acts through SKN-1/Nrf1, ATG-18 and ATG-16.2 act through the DAF-16/FOXO transcription factor. In contrast, ATG-7 functions together with the DAF-7/TGFβ pathway, to promote germ line proliferation, and is not required for cell cycle progression. Interestingly, BEC-1/Beclin1 functions cell non-autonomously to facilitate cell cycle progression and stem cell proliferation. Thus, our findings demonstrate a novel, non-autonomous role for BEC-1 in the control of stem cell proliferation, and cell cycle progression.

18 Regulation of germline stem cell maintenance by S6-Kinase. Debasmita Roy, E. Jane Hubbard New York University School of Medicine, Skirball Institute of Biomolecular Medicine, New York, NY.

Accumulation and maintenance of stem cells is crucial for proper organogenesis and tissue homeostasis. We previously showed that the conserved Target of Rapamycin (TOR) pathway (*let-363/TOR, daf-15/RAPTOR*) and its targets *rsks-1/p70-S6-Kinase (S6K)* and *ife-1/eIF4E* promote the accumulation of germ line stem cells (GSCs) during larval germ line development. Among these, only *rsks-1/S6K* displays a synergistic interaction with *glp-1/Notch* that implies its role in GSC maintenance: loss of S6K enhances and suppresses sterility associated with reduced and elevated *glp-1* activity, respectively. In this GSC maintenance role, S6K acts germ-line-autonomously and requires a conserved TOR phosphorylation site. The mechanism(s) underlying a role for the highly conserved S6K in cell fate regulation *in vivo* are unclear. To determine how *rsks-1/S6K* maintains GSCs, we used both candidate and unbiased approaches. Similar to loss of *rsks-1/*, inhibiting Cyclin-E/CDK-2 or MAPK pathway activity enhances loss of GSCs in a mutant with reduced *glp-1* activity. We tested these and found that neither acts in a simple linear fashion with S6K to maintain GSCs. We undertook a genome-wide RNAi screen to identify genes that when knocked down by RNAi would phenocopy the genetic interaction of *rsks-1/S6K* with *glp-1/Notch*. We screened the Ahringer library in the *e2141* mutant at a semi-permissive temperature and scored for enhancement of sterility. Sterility was strictly defined as =1 embryo in the uterus, and RNAi feeding was started at the L1 larval stage to bypass embryonic lethality. The strain also carried *nrf-1(pk1417)* to restrict RNAi primarily to the germ line and markers (pharyngeal mCherry, GFP embryos) to facilitate semi-automated scoring.

We found 139 genes that, when depleted by RNAi, caused highly reproducible and penetrant sterility in *glp-1/e2141*. These include regulators of replication, transcription, RNA splicing, translation, ribosome biogenesis, cell cycle, development, and metabolism. Similar to loss of *rsks-1*, RNAi targeting 62 of these genes caused penetrant sterility in the *glp-1* mutant but not in the wild type. These results considerably extend the set of known enhancers of *glp-1* sterility. Further, 20 of these 62 genes caused obvious defects in GSC maintenance in the *glp-1* background. Among these 20 are several genes that may act in a linear pathway with *rsks-1/S6K* to promote GSC maintenance since depletion of these genes by RNAi did not further enhance the penetrance of GSC loss in the *glp-1/e2141* *rsks-1/null* double mutant.
Unexpectedly, our screen results and subsequent analysis implicate Hedgehog-like signaling in GSC maintenance, in a linear pathway with S6K.

19 Development of germline tumors after extended starvation during L1 arrest. James M. Jordan¹, Amy K. Webster², Rojin Chitrakar¹, Ryan Guzman¹, E. Jane Albert Hubbard³, L. Ryan Baugh¹ ² ¹) Department of Biology, Duke University, Durham, NC; ²) University Program in Genetic and Genomics, Duke University, Durham, NC; ³) Helen and Martin Kimmel Center for Stem Cell Biology, Skirball Institute of Biomolecular Medicine, Department of Pathology, New York University School of Medicine, New York, New York.

Environmental stress during development can have persistent effects on adult physiology. C. elegans has a variety of attributes that make it an ideal model for such developmental origins of adult disease. We found that extended starvation during L1 arrest results in the formation of germline tumors in adults. We performed RNAseq at egg-laying onset, and our analysis suggested a role for glp-1/Notch signaling, germline apoptosis, insulin-like signaling (IIS) and fatty acid metabolism in tumor formation. We found that disruption of gld-1/Quaking, car-1/LSM14, cgh-1/DDX6 and cep-1/p53 significantly enhance tumor formation after L1 starvation and that glp-1/Notch is epistatic to L1 starvation. SYTO12 staining also suggests reduced germline apoptosis in tumorous gonads. Together these results are consistent with disruption of germ cell differentiation and apoptosis following L1 starvation, resulting in tumor formation. We hypothesize extended L1 starvation causes a "latent niche" to form due to aberrant germ cell-gonadal sheath communication, which increases glp-1/Notch signaling activity and drives the observed effects on germ cell differentiation and apoptosis. We also found that reducing IIS in the soma during recovery from starvation suppresses tumor formation in daf-16/FoxO-dependent fashion, confirming a role of IIS as suggested by RNA-seq. RNA-seq suggests reduced IIS alters regulation of fatty acid metabolism, including down-regulation of enzymes involved in fatty acid synthesis, desaturation, oxidation in peroxisomes and mitochondria, and storage as triacylglycerides. We found that supplementation with the monoensaturated fatty acid oleic acid suppresses tumor formation. Furthermore, RNAi of acs-17, an IIS-regulated acyl-CoA synthetase, enhances tumor formation alone and prevents tumor suppression by reduced IIS. Taken together, this suggests IIS-mediated fatty acid metabolism is tumorigenic following L1 starvation, and acs-17 and oleic acid act downstream of IIS to promote a less oncogenic mode of fatty acid metabolism. We are working to more specifically define the effects of L1 starvation and IIS on fatty acid metabolism, and to link those effects to glp-1/Notch signaling in order to explain how early-life nutrient stress causes adult germine pathology.

20 Analysis of DLC-1 mediated regulation of the tumor suppressor protein GLD-1. M. Ellenbecker, E. Osterli, E. Voronina  Division of Biological Sciences, University of Montana, Missoula, MT.

Dynein light chain (DLC-1) is a component of the dynein motor complex involved in transport of molecules and organelles along microtubules. Additionally, DLC-1 interacts with a diversity of cellular proteins and we propose that it functions as an allosteric regulator in ribonucleoprotein complexes. We previously found that DLC-1 promotes the activity of the RNA-binding protein FBF-2 in the stem cell region of the C. elegans germline. Since DLC-1 interacts with many cellular proteins, we hypothesized that DLC-1 may facilitate GLD-1, a component of the regulatory network that controls decision between stem cell proliferation and differentiation. GLD-1 is an RNA-binding protein that promotes germ cell differentiation during animal development by binding to and repressing translation of target mRNAs. This RNA regulatory function is essential for preventing ectopic proliferation in germ cells. Our genetic studies show that disruption of the DLC-1/GLD-1 interaction using dlc-1 RNA interference predisposes the nematode to tumor formation and this phenotype is also observed when the DLC-1 binding site on GLD-1 is mutated. We found that knockdown of DLC-1 does not affect GLD-1 levels. DLC-1 helps regulate translation of a subset of GLD-1 target mRNAs and this regulation is not dependent upon the dynein motor related function of DLC-1. Biochemical assays show that DLC-1 binds a conserved peptide motif located on the unstructured N-terminal domain of GLD-1 (YSQTP). By contrast, mutation of a conserved phosphorylated residue on the N-terminal domain (S22A) does not inhibit DLC-1 binding GLD-1. Transgenic worm experiments suggest that the DLC-1/GLD-1 binding interaction enables GLD-1 function in vivo. GLD-1 mutant worms unable to bind DLC-1 exhibit disorganized germline phenotype and the SQT to AAA mutation is associated with an increase in sterility. Together, these data support the hypothesis that DLC-1 facilitates the RNA regulatory and tumor suppressor functions of GLD-1 through a direct protein interaction. Mammalian Quaking proteins are GLD-1 orthologs and function as potent glioblastoma multiforme tumor suppressors by regulating the TGFβ signaling network that inhibits DLC-1 binding GLD-1. Together these results are consistent with disruption of germ cell differentiation and apoptosis following L1 starvation, resulting in tumor formation. We hypothesize extended L1 starvation causes a "latent niche" to form due to aberrant germ cell-gonadal sheath communication, which increases glp-1/Notch signaling activity and drives the observed effects on germ cell differentiation and apoptosis. We also found that reducing IIS in the soma during recovery from starvation suppresses tumor formation in daf-16/FoxO-dependent fashion, confirming a role of IIS as suggested by RNA-seq. RNA-seq suggests reduced IIS alters regulation of fatty acid metabolism, including down-regulation of enzymes involved in fatty acid synthesis, desaturation, oxidation in peroxisomes and mitochondria, and storage as triacylglycerides. We found that supplementation with the monoensaturated fatty acid oleic acid suppresses tumor formation. Furthermore, RNAi of acs-17, an IIS-regulated acyl-CoA synthetase, enhances tumor formation alone and prevents tumor suppression by reduced IIS. Taken together, this suggests IIS-mediated fatty acid metabolism is tumorigenic following L1 starvation, and acs-17 and oleic acid act downstream of IIS to promote a less oncogenic mode of fatty acid metabolism. We are working to more specifically define the effects of L1 starvation and IIS on fatty acid metabolism, and to link those effects to glp-1/Notch signaling in order to explain how early-life nutrient stress causes adult germine pathology.

21 SCFPROM-1 acts in parallel to the gld-1 & gld-2 pathways to promote initiation of meiotic development. A. Mohd¹, K. V. Broek², A. Daryabegi³, V. Jantsch³, D. Hansen⁴, T. Schedl¹ ¹) Washington University, St. Louis, MO; ²) University of Calgary, Calgary, Canada; ³) Max F. Perutz Laboratories, University of Vienna, Vienna, Austria.

SCF (Skp, Cullin, F-box) E3 ubiquitin ligase complexes are key players in controlling mitotic cell cycle transitions. We found SCFPROM-1 functions in the transition of mitotic cycling progenitor zone cells to meiotic development. Previous work identified a regulatory network where glp-1 Notch signaling promotes the stem cell fate by inhibiting the gld-1 and gld-2 translational regulatory pathways that function redundantly to promote meiotic development. However, genetic analysis indicated that activities in addition to the gld-1 and gld-2 pathways are also necessary for the transition.

We found that skr-2 (Skp), cul-1 (Cullin) and prom-1 (F-box) are required for downregulation of CYE-1 (cyclin E), WAPL-1 (cohesin chaperone), KNL-2 (kinetochore assembly) and pCDC-6 (DNA replication), that occurs at the boundary of the progenitor zone and leptotene/zygotene. We showed that PROM-1 induces polyubiquitination of CYE-1 in a cell culture based assay. Together, these results indicate that PROM-1 is acting as a specificity subunit for skr-2 and cul-1 (SCFPROM-1) in the
degradation of CYE-1 at meiotic entry, and by extension, the degradation of WAPL-1 and KNL-2. We also found that SCEPTPROM-1 is necessary for homologous chromosome pairing, acting upstream or in parallel to CHK-2, a key regulator of the initiation of meiotic chromosome pairing.

While SCEPTPROM-1 mutant hermaphrodites display ectopic mitotic proteins (CYE-1, etc) proximal to the progenitor zone, these cells are not mitotically cycling and express meiotic proteins HIM-3 (axis protein) and COH-3/-4 (meiotic cohesin). By contrast in SCEPTPROM-1 mutant males, cells proximal to the progenitor zone that express mitotic proteins are mitotically cycling, although they express meiotic proteins HIM-3 and COH-3/-4. These results indicate that SCEPTPROM-1 functions to down regulate mitotic activity at meiotic entry, as well as promote meiotic chromosome pairing. As these two activities are important for initiation of meiotic development, then SCEPTPROM-1 may act in parallel to the gld-1 and gld-2 pathways to promote the switch to meiotic development. In support of this hypothesis we find that (a) SCEPTPROM-1 double mutants with gld-1 pathway genes show ectopic proliferation; (b) SCEPTPROM-1 double mutants with gld-2 pathway genes show ectopic proliferation; (c) the ectopic proliferation found in SCEPTPROM-1 double mutants with gld-1 or gld-2 pathway genes is epistatic to the premature meiotic entry phenotype of glp-1 null; and (d) SCEPTPROM-1 mutants enhance the meiotic entry defect (overproliferation) observed in gld-1 gld-2 pathway double mutants. Thus C. elegans employs three posttranscriptional pathways, SCEPTPROM-1 mediated protein degradation, GLD-1 mediated translational repression and GLD-2 mediated translational activation to promote the initiation of meiotic development.

22 Analyzing DNA organization during meiotic chromosome pairing. S. Ramakrishnan, A. Woglar, K. Lasker, A. Villeneuve 1,2, 1) Department of Developmental Biology, Stanford School of Medicine, Stanford, CA; 2) Department of Genetics, Stanford School of Medicine, Stanford, CA.

The ability of homologous chromosomes to locate, recognize and align with their correct pairing partners is a hallmark feature of meiosis, yet the nature of interactions between homologous DNA sequences is still poorly understood. In order to better understand the DNA organization within the chromosomal structures that we observe cytologically, we have developed and validated a robust experimental pipeline for Hi-C analysis of C. elegans meiotic prophase germ cell nuclei. Our overall goals are to understand: 1) how DNA is organized in the context of fully aligned homologs, and 2) how DNA is organized within chromosomes during the process of homolog pairing. Several features of C. elegans make it well-suited for using Hi-C technology to investigate chromosome organization during meiotic prophase, including a high abundance of germ line nuclei in adult worms and robust tools for cytological validation of Hi-C data.

To enrich for germ cell nuclei, we subject frozen worms to mild mechanical disruption followed by several filtering steps. Cytological analysis of the filtered nuclei shows that over 90% of the nuclei in our sample are germ cell nuclei; moreover, 80% of the nuclei are in meiotic prophase. Following crosslinking, nuclear lysis, restriction digestion, DNA ligation, junction capturing and high-throughput sequencing, computational analysis of the data yields Hi-C heatmaps of DNA interactions showing characteristic signatures of individual chromosomes. We were able to identify the chromosomal fusion points in worms carrying the two-chromosome fusion mntT12(1V;X), and we further observed that DNA interactions between the fused chromosomes spread far beyond the fusion points especially on the X-chromosomes. We also identified the translocation breakpoints in the reciprocal translocation nT1(IV;V), and our analysis further revealed additional cryptic chromosomal rearrangements in these balancer chromosomes.

Moreover, we detected robust interactions between hetero-synapsed segments of chromosomes IV and V in worms heterozygous for nT1(IV;V), providing strong proof-of-principle that our experimental pipeline is indeed capable of detecting chromosomal interactions that depend on meiotic synopsis. These experiments also provided evidence of synaptic adjustment, i.e. the ability to achieve apparent lengthwise synapsis between chromosomes with different lengths. We are currently comparing chromosomal organization between meiotic germ cells and tumorous mitotic germ cells. Finally, we are investigating interactions between homologous chromosomes using worms that are heterozygous for two distinct haplotypes.

23 Meiotic chromosome axis assembly requires active DNA Damage Response (DDR) during C. elegans meiosis. Z. Yu, A.F. Dernburg 1,2,3 1) Department of Molecular and Cell Biology, University of California, Berkeley; 2) Howard Hughes Medical Institute; 3) California Institute for Quantitative Biosciences, Berkeley.

The DNA Damage Response (DDR), which is orchestrated by evolutionarily conserved PI3K family kinases ATM and ATR (ATM-1 and ATL-1 in C. elegans), safeguards genome integrity during a variety of developmental processes. Studies in many organisms have revealed central roles for DDR kinases in meiosis, but the roles of ATM-1 and ATL-1 are not well-defined in C. elegans meiosis. Efforts to characterize ATM/ATR function have been complicated by the essential roles of ATL-1 during cell proliferation, including in the premeiotic germline. To circumvent these issues to enable investigation of the roles of ATM/ATR during meiosis, we generated alleles of ATM-1 and ATL-1 that can be depleted using the auxin-inducible degradation (AID) system. Depletion of ATM-1, ATL-1, and both kinases together, indicates that these kinases have strongly overlapping meiotic functions. We find that signaling by ATM/ATR is strongly induced during early meiosis and declines at mid-prophase. While this coincides with activation and inactivation of the meiosis-specific CHK-2 kinase, we find that CHK-2 is activated independently of ATM/ATR activity. Conversely, CHK-2 does not appear to activate ATM/ATR, although they are induced in response to meiotic double-strand breaks (DSBs), which do require CHK-2. Codepletion of ATM-1 and ATL-1 causes a severe defect in meiotic cohesion; COH-3/-4-containing cohesin complexes are strongly reduced along meiotic chromosomes and REC-8 is not detected, HORMA-domain proteins fail to be recruited to the axes, and synopsis is therefore abrogated. We further identified WAPL-1, a negative regulator of cohesion, as a downstream effector of the meiotic DDR pathway. Co-depletion of ATM-1/ATL-1 or CHK-2 results in mislocalization of WAPL-1 throughout the germline. Through our ongoing analysis, we plan to systematically define the targets of ATM/ATR signaling in C. elegans and to investigate how these kinases contribute to germline maintenance, fertility,
24 Trisomy correction occurs during both meiosis I and meiosis II. Elizabeth Vargas, Karen McNally, Jacob Friedman, David Wang, Ian Korf, Francis McNally Dept Molec & Cellular Biol, Univ California, Davis, Davis, CA.

The vast majority of eukaryotes have exactly two copies of each chromosome and reproduce sexually through the process of meiosis. Accurate chromosome segregation requires the physical attachment of exactly two homologous chromosomes by a crossover so that each homologous chromosome can form attachments to opposite poles of a bipolar spindle. Thus a third copy of a chromosome might be expected to segregate randomly, resulting in 50% of progeny inheriting the extra chromosome. Hodgkin et al. (Genetics 91:67) reported that C. elegans with a third copy of the X chromosome have far fewer than 50% trisomic progeny. Cortes et al. (Elife 4:e06056) found that the preferential elimination of the extra X occurs during anaphase I of female meiosis. To test whether extra copies of all C. elegans chromosomes are preferentially eliminated, we scored the inheritance of three different DNA polymorphisms for each autosome among the progeny of triploid hermaphrodites mated with diploid males. For all 5 autosomes, the frequency of trisomic offspring was significantly less than expected from random segregation. Preferential elimination of the extra copy of chromosome V was confirmed by FISH. We also generated a trisomy IV worm strain and when trisomy IV hermaphrodites were mated to diploid males, the frequency of triplo IV progeny was again much lower than expected from random segregation. Cortes et al. (2015) found that univalent X chromosomes do not lose cohesion during anaphase I and thus lag before being preferentially captured by the polar body. Counting of mCherry:histone-labeled chromosomes in triploids revealed that chromosome number decreased between metaphase I and metaphase II and decreased again between metaphase II and diakinesis in the adult progeny. Data from live imaging of anaphase chromosome segregation and REC-8 staining of fixed meiotic spindles support a model in which some univalents remain intact during anaphase I whereas other univalents segregate apart at anaphase I. The resulting single chromatids then lag during anaphase II and are preferentially captured by the second polar body. These data demonstrate that asymmetric female meiotic divisions can correct triploidy and trisomy in C. elegans.


Mechanotransduction, converting a mechanical signal into a biochemical response, is vital for proper development, tissue function and homeostasis. Defects in a cell’s ability to sense and respond to external stimuli can lead to complications in development and diseases such as asthma. The C. elegans spermatheca, the site of fertilization, provides an ideal in vivo model to study this biological process. Stretch of the incoming oocyte is converted into waves of calcium potentiated by PLC-1 that culminates in acto-myosin contractility and expulsion of fertilized embryos into the uterus. What remains unknown however, is what activates PLC-1. Loss of functional PLC-1 results in trapping of embryos within the spermatheca due to a lack of calcium signaling within the tissue. To identify potential activators of PLC-1, we conducted a candidate RNAi screen and identified two heterotrimeric G protein alpha subunits, GOA-1 (Gi/Go class) and GSA-1 (Gs class) as essential for proper oocyte transit through the spermatheca. Depletion of goa-1 via RNAi and a loss of function allele result in a significant increase in the number of embryos occupying spermathecae compared to wild type animals. Further analysis revealed that this increase in occupied spermathecae coincides with abnormal calcium signaling and extremely long embryo transits through the spermatheca, resulting in a significant reduction in broodsize. Depletion of gsa-1 via RNAi results in trapping of embryos in the spermatheca due to a lack of calcium signaling in the spermathecal bag comparable to the loss of plc-1. These results provide novel evidence that heterotrimeric G protein signaling may play an essential role in the stimulation of calcium signaling perhaps through the regulation of phospholipases like PLC-1. Given the conservation of PLC-1, GOA-1, GSA-1 and the pathway that regulates C. elegans ovulation, this work should provide insights into stretch activation of phospholipase signaling in vivo.

26 A translational repression-to-activation switch controls oocyte meiotic maturation and the oocyte-to-embryo transition in Caenorhabditis elegans. T. Tsukamoto1, M. D. Gearhart1, C. A. Spike1, G. Huelgas-Morales1, M. Mews1, P. R. Boag2, T. H. Beilharz2, D. Greenstein1 1) Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, MN USA; 2) Department of Biochemistry and Molecular Biology, Monash University, Clayton 3800, Australia.

An extended meiotic prophase is a hallmark of oogenesis. Hormonal signaling activates the CDK1/cyclin B kinase to promote meiotic maturation, which involves the nuclear maturation events of nuclear envelope breakdown, meiotic spindle assembly, and chromosome segregation. Cytoplasmic maturation involves major changes in oocyte protein translation and cytoplasmic organelles and is less well understood. In the nematode Caenorhabditis elegans, sperm release the major sperm protein (DSP) hormone to promote oocyte growth and meiotic maturation. A large translational regulatory ribonucleoprotein (RNP) complex containing the RNA-binding proteins OMA-1, OMA-2, and LIN-41 regulates meiotic maturation downstream of MSP signaling. To understand the control of protein translation during meiotic maturation, we purified LIN-41-containing RNPs and characterized their protein and RNA components. Protein constituents of LIN-41 RNPs include essential RNA-binding proteins, the GLD-2 cytoplasmic polyadenylase, the CCR4-NOT deadenylase complex, and translation initiation factors. RNA sequencing defined mRNAs associated with LIN-41 and OMA-1, as well as sets of mRNAs associated with either LIN-41 or OMA-1. As a class, transcripts that selectively associate with LIN-41 exhibit shortened poly(A) tails in gld-2 mutants, suggesting that GLD-2 might stimulate their efficient translation. We analyzed the translational regulation of two transcripts specifically associated with LIN-41 that encode the RNA regulators, SPN-4 and MEG-1. We found that LIN-41 represses translation of spn-4 and meg-1; whereas, OMA-1 and OMA-2 promote their expression. Upon their synthesis, SPN-4 and MEG-1 assemble into LIN-41 RNPs in the oocyte prior to their functions in germline development in the embryo. Genetic results indicate that LIN-41 functions in oocytes to
promote the activity of SPN-4 needed for the proper development of the germline of the next generation. This study defines a translational repression-to-activation switch as a key element of cytoplasmic maturation.

27 Sex, Secretion, Muscle, and Males. Daniela Chavez, Gillian Stanfield Human Genetics, University of Utah, Salt Lake City, UT.

To successfully complete the journey to fertilize an oocyte, sperm rely on extracellular cues to direct their development and migration. In the final stages of maturation, during a process called sperm activation, sperm become polarized and develop a pseudopod used to crawl towards oocytes. Establishment of an extracellular signaling environment that spatially and temporally regulates activation is critical to fertility, as males with prematurely activated sperm are infertile. Key components of this regulation are the serine protease TRY-5 and a protease inhibitor, SWM-1. TRY-5 is a seminal fluid protein that is stored in the vas deferens and transferred to hermaphrodites, where it activates sperm. In the absence of SWM-1, TRY-5 spreads into the seminal vesicle, where sperm are stored, resulting in premature activation. We are investigating how TRY-5 is regulated by SWM-1 to ensure that sperm gain motility at the right place and time.

We have discovered that somatic tissues create the sperm signaling environment that precisely controls the location and timing of activation. By analyzing a swm-1 transcriptional reporter and a SWM-1::mCherry knock-in, we determined that swm-1 is expressed by body wall muscle cells and cuboidal cells in the vas deferens while SWM-1 protein is present not only in the vas deferens but also in the seminal vesicle, surrounding sperm. SWM-1 is also present in the pseudocoelom, where it is taken up into coelomocytes. Surprisingly, tissue-specific expression suggests that muscle-derived SWM-1 is sufficient to rescue the premature activation of swm-1 mutants, but that vas deferens-derived SWM-1 fails to rescue. Our data suggest a model in which SWM-1 is secreted from muscle into the pseudocoelom and then taken up by the gonad where it attenuates TRY-5. Indeed, removing the secretion signal from SWM-1 caused it to accumulate in muscle, where it no longer rescues activation. These data show that we have identified a soma-to-germline signal critical to fertility and have determined that muscle is a key regulator of sperm cell success. Interestingly, uptake of proteins from the pseudocoelom is not specific to SWM-1, as extragonadal sources of mCherry and GFP also enter the gonad. Furthermore, vas deferens-specific mCherry is found in the pseudocoelom, thus, exchange of proteins into and out of the gonad is apparently not limited to SWM-1, and could be a general phenomenon.

In ongoing work, we seek to determine whether swm-1 plays a second role within the hermaphrodite to promote reproductive success. SWM-1 is present in hermaphrodites, where it has a small but measurable effect on sperm activation. Additional SWM-1 is transferred in seminal fluid, but its role in this context is unknown. We are analyzing whether absence of SWM-1, in either the hermaphrodite or seminal fluid, affects sperm functions including migration, fertilization, or competition.

28 The ZIPT-7.1 transporter mediates zinc signaling to promote sperm activation in C. elegans. Chieh-Hsiang Tan1, Yanmei Zhao2, Andrea Scharf1, Nicholas Dietrich1, Kurt Warnhoff1, Ronald E. Ellis2, Kerry Kornfeld1 1) Department of Developmental Biology, Washington University in St. Louis School of Medicine, St. Louis, MO; 2) Department of Molecular Biology, Rowan University SOM, Stratford, NJ; 3) Lab of Noncoding RNA, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China.

Sperm activation involves the rapid transition of a round, immotile spermatid into a polarized, motile sperm capable of fertilization. This transition in cell fate does not involve transcriptional changes, because the nucleus is not accessible. Although a large number of genes involved in sperm activation have been characterized in C. elegans, the signaling pathway that mediates this transition is not well-defined. We identified the zipt-7.1 gene, which encodes a ZIP (Zrt/ Irt-like protein), using a reverse genetic approach. A deletion of the gene caused a partially penetrant sterile phenotype in hermaphrodites and reduced the fertility of males. The sterile hermaphrodites laid unfertilized oocytes and had mostly inactive sperm. Since the sterile phenotype of the hermaphrodites could be rescued by crossing with wild-type males, it must have been caused by a sperm defect. A similar phenotype was observed in mutants of the related species C. tropicalis. Finally, WT spermatids could be activated in vitro with pronase, trypsin or zinc, but zipt-7.1(II) spermatids were less responsive to pronase and trypsin and had almost no response to zinc. Consistent with these results, RNAi showed that ZIPT-7.1 functions in the germ line, and GFP::ZIPT-7.1 was expressed in spermatocytes. Sperm activation in C. elegans depends on two genetic pathways - the spe-8 pathway and the try-5 protease pathway. Epistasis studies suggest that zipt-7.1 functions downstream of spe-8 in the spe-8 pathway. To understand the mechanism of zipt-7.1-dependent sperm activation, we used molecular approaches. Transcription of zipt-7.1 was regulated by environmental zinc levels, and zipt-7.1 mutant sperm displayed reduced amounts of labile zinc, suggesting that it regulates zinc levels in sperm. Expression of ZIPT-7.1 in mammalian cells promoted zinc uptake, showing that it can transport zinc. Homologues of ZIPT-7.1 in other species localize to ER-Golgi, and ZIPT-7.1 expressed in human cell lines localized to the Golgi and lysosomes. Based on these results, we propose that ZIPT-7.1 is localized to vesicles in spermatids, and that a sperm activation signal results in the activation of its transporter activity and the release of zinc stores into the cytoplasm. This cytoplasmic zinc constitutes a second messenger that promotes activation. Our results provide the first identification of a zinc transporter that mediates sperm activation, and may have broad relevance, since zinc has been implicated in sperm function in a variety of animals.
29  **Eukaryotic *de novo* NAD\(^+\) biosynthesis from tryptophan in the absence of a QPRTase homolog.**  
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NAD\(^+\) is found in all living cells, and is an essential coenzyme, which impacts the entire metabolome. Hence, NAD\(^+\) biosynthesis has proven to be an attractive and promising therapeutic target for influencing health-span and obesity-related phenotypes as well as tumor growth. A wide range of animals and yeast synthesize NAD\(^+\) *via de novo* synthesis from the degradation of tryptophan (Trp), via the kynurenine pathway. The *C. elegans* genome encodes all the enzymes involved in the kynurenine pathway. However, the genome lacks the critical quinolinic acid phosphoribosyltransferase (QPRTase) that converts QA into NaMN for biosynthesis of NAD\(^+\). Because *C. elegans* lack an apparent QPRTase homolog, it’s been assumed that this species lacks active NAD\(^+\) *de novo* synthesis. We’ve uncovered evidence that suggests NAD\(^+\) *de novo* synthesis is active and contributing to NAD\(^+\) biosynthetic capacity in *C. elegans*. We previously found that phenotypes that are dependent on NAD\(^+\) levels could be reversed upon supplementation with QA and other kynurenine metabolites, suggesting that the kynurenine pathway may contribute to NAD\(^+\) biosynthesis. We have directly demonstrated that isotopic label from Trp is incorporated into NAD\(^+\). Furthermore, we show that loss of kynurenine pathway activity decreases global NAD\(^+\) levels and prevents incorporation of isotopic label supplied from Trp into NAD\(^+\), supporting the presence of *de novo* synthesis in *C. elegans*. Using genetics and isotope tracing experiments, we determined that the enzyme UMP-S1, (uridine monophosphate synthetase), is required for NAD\(^+\) biosynthesis from Trp in this organism. Finally, we connected the kynurenine pathway to normal fecundity. This evidence demonstrates that NAD\(^+\) *de novo* synthesis is active and contributes to NAD\(^+\) biosynthetic capacity and homeostasis in *C. elegans*. Intriguingly, a conserved enzyme is substituting for the missing QPRTase, raising questions about the relevance of similar underground metabolic activity in higher organisms.

30  **Discovery and biosynthesis of hybrid polyketide-nonribosomal peptides in nematodes.**  
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Polyketides and nonribosomal peptides are well-known for their antibiotic, antifungal, immunosuppressant, anti-parasitic, and anti-cancer activities. These structurally complex natural products are biosynthesized by polyketide synthases (PKSs) and nonribosomal synthetases (NRPSs), modular megasynthases that function in either an assembly-line or iterative manner. Although polyketides and nonribosomal peptides are produced by many species of bacteria, fungi, and plants, they are extremely rare in metazoans. The genome of the nematode *Caenorhabditis elegans* is predicted to encode a huge, multi-module, hybrid PKS/NRPS (PKS-1), as well as a multi-module NRPS (NRPS-1), with unknown functions. Using comparative metabolomics and NMR spectroscopy, we identified and elucidated the chemical structure of nemamide, a hybrid polyketide-nonribosomal peptide that is biosynthesized by PKS-1/NRPS-1 using both iterative and assembly-line mechanisms. In recent work, we used CRISPR-Cas9 to make specific mutations in *pks-1* and *nrps-1* in order to investigate the roles of specific enzymatic domains in the biosynthesis of nemamide. We showed that nemamide promotes survival during starvation-induced larval arrest and facilitates recovery from arrest in response to food. Nemamide is produced in the CAN neurons under starvation conditions and downregulates insulin/insulin-like growth factor-1 (IGF-1) signaling. Our results uncover a novel mechanism by which animals respond to nutrient fluctuations to extend survival. Homologs of PKS-1 and NRPS-1 are present in most nematode species, including parasitic ones, and thus, nemamide likely plays an evolutionarily conserved role across nematode species.

31  **PEGCs: novel glycosphingolipids that mobilize cholesterol in *Caenorhabditis elegans*.**  
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*Caenorhabditis elegans* cannot synthesize cholesterol *de novo*, and requires exogenous cholesterol to progress through its four larval stages. Previously, we showed that worms grown for 2 generations in the absence of cholesterol arrest early in development. This suggests that maternal contributions of sterols are exploitable only in the first generation, but sterol reservoirs are either depleted or inaccessible by the second generation. Here, we present a novel class of phosphorylated glycosphingolipids, which we coined phosphoethanolamine glucosylceramides (PEGCs), that can overcome the sterol deprivation-induced larval arrest. However, they are not direct substitutes for cholesterol because they rescue larval arrest in only one additional generation. Instead, we propose a new model where larval arrest in the second generation of continuous sterol deprivation is due not to depletion of internal reservoirs, but a failure to mobilize those reservoirs for promoting growth/development through PEGCs-induced mobilization of internal sterol pools. More precisely, we found that NPC1 and DAF-7 mutants, which display a Daf-c phenotype due to an impaired sterol transport to places of DA synthesis, are rescued by feeding PEGC. Moreover, the biosynthesis of PEGC depends on functional NPC1 and TGF-β, indicating that these proteins control larval development at least partly through promoting increases in PEGC. Furthermore, glucosylceramide deficiency dramatically reduced PEGC amounts; however, the resulting developmental arrest could be rescued by over-saturation of food with cholesterol. This indicates that PEGC is a major regulator of cholesterol utilization in *C. elegans* and, thus, of development. The remarkable similarity in sterol trafficking between *C. elegans* and other metazoans, including mammals, suggests PEGCs...
might be conserved regulators of sterol transport.

32 **Does C. elegans benefit from physical exercise? Effects of acute and long-term swim training.** R. Laranjeiro1, G. Harinath1, M. A. Royal1, J. E. Hewitt1, M. Rahman1, J. H. Hartman1, J. N. Meyer1, S. Vanapalli1, B. P. Braeckman1, M. Driscoll1 1) Rutgers University, NJ, USA; 2) Texas Tech University, TX, USA; 3) Duke University, NC, USA; 4) Ghent University, Belgium.

Physical exercise is the most efficient and accessible intervention that can promote healthy aging in humans. In fact, exercise has been reported to prevent, or mitigate consequences of, a wide range of conditions such as diabetes, cancer, sarcopenia, cardiovascular disease, and neurodegenerative diseases. However, the molecular mechanisms by which exercise can confer systemic health benefits remain poorly understood.

We used microcalorimetry to show that C. elegans swimming has a greater energy cost than crawling. Animals that swim continuously for 90 min specifically consume muscle fat supplies and exhibit post-swim locomotory fatigue, with both muscle fat depletion and fatigue indicators recovering within one hour of exercise cessation. qPCR transcript analyses also suggest an increase in fat metabolism during the swim followed by downregulation of specific carbohydrate metabolism transcripts in the hours post-exercise. During a 90 min swim, muscle mitochondrial matrix environments become more oxidized as visualized by a localized mito-roGFP reporter. qPCR data support specific transcriptional changes in oxidative stress defense genes during and immediately after a swim. Consistent with potential antioxidant defense induction, we find that a single swim session suffices to confer protection against juglone-induced oxidative stress inflicted 4 hours post-exercise.

Exercise adaptation occurs after long-term training. Therefore, we tested different swimming regimens in C. elegans and found that multiple swims per day over several days lead to upregulation of muscle structural genes. Once again, these results are consistent with exercise adaptation described in mammals. Importantly, and taking advantage of the unique characteristics of C. elegans, we show that our training regimen not only leads to changes in body wall muscles but also in neurons. Specifically, mitochondria in touch neurons of exercised worms exhibit a lower oxidation level and an increased turnover rate, both indicators of mitochondrial health. Moreover, long-term swim training in C. elegans delays the functional decline of touch neurons in a polyglutamine (polyQ) aggregation model. These results suggest that physical exercise can promote physiological changes in multiple tissues of C. elegans and open the door to the genetic dissection of exercise systemic health benefits.

33 **Exercise in Caenorhabditis elegans promotes mitochondrial maintenance and protects against chemical-induced mitotoxicity.** J.H. Hartman1, K.L. Gordon1, D.R. Sherwood1, R. Laranjeiro2, M. Driscoll2, J.N. Meyer1 1) Duke University, Durham, NC; 2) Rutgers University, New Brunswick, NJ.

Health, disease, and aging are determined by genetic factors, environment, and lifestyle. In humans, environmental contributions to long-term health arise such as environmental chemical exposures and sun exposure, while lifestyle impacts health through mental health and stress, diet, drug usage, and exercise. In laboratory animals, the impact of environment and lifestyle are minimized through carefully controlled experimental conditions, and can therefore be modulated to study the effects of these factors. The effect of exercise on general health has been reported: positive impacts on cognitive function, maintenance of skeletal muscle, and protection from age-related diseases are increasingly recognized. However, molecular mechanisms underlying those protections are not well understood. Furthermore, it is unknown what impacts regular exercise training may have on other health-modifying factors such as toxic exposures from the environment. In this study, we used C. elegans to study the impact of regular exercise training on mitochondrial health and chemical toxicity. For exercise experiments, beginning at L4 stage, animals were transferred to unseeded agar plates without (control) or with liquid (causing worms to swim/thrash) for 90 minutes twice daily. This regimen was carried out for six days, and mitochondrial and toxicity outcomes were tested following exercise on adult day 6 and adult day 10. Preliminary results show that mitochondrial morphology is not significantly different between control and exercise groups at adult day 6 (p=0.64); however, on day 10, control animals have highly fragmented and disorganized mitochondria, while exercised animals exhibit significantly healthier mitochondrial morphology (p=0.0065). Furthermore, mitochondrial respiration significantly differed in spare capacity (p<0.001) on adult day 6, with exercised animals showing increased spare capacity compared to controls. Respiration experiments with day 10 adults are underway; total ATP, mitochondrial DNA copy number, and mitochondrial DNA lesions are also being investigated. Preliminary toxicity experiments showed that exercise-induced changes in mitochondrial health were accompanied by a 30-50% reduction in lethality induced by the mitochondrial toxicants arsenite and rotenone. Together, these data demonstrate that changes in physical activity result in altered mitochondrial health, which extends to protection against chemical toxicants known to damage mitochondria. Ongoing and future experiments will further explore the biochemical and metabolic changes underlying this phenomenon.

34 **Microfluidics-based evaluation of neuromuscular healthspan in C.elegans.** Mizanur Rahman1, Hunter Edwards1, Frank Van-Bussel3, Jerzy Blawzdziewicz3, Nathaniel Szewczyk3, Monica Driscoll2, Siva Vanapalli1 1) Department of Chemical Engineering, Texas Tech University, Lubbock, TX; 2) Department of Molecular Biology and Biochemistry, Rutgers University; 3) Department of Mechanical Engineering, Texas Tech University; 4) MRC/Arthritis Research UK Centre for Musculoskeletal Ageing Research, University of Nottingham, UK.

Aging studies in C.elegans based on functional metrics and molecular markers are essential to identify genetic and environmental determinants of healthspan. Although functional measures based on locomotion, pharyngeal pumping and oxidative stress have been used to interrogate healthspan of C.elegans, our understanding on maintenance and deterioration of the neuromuscular system with age is far from complete. Here, we propose muscle strength and stimulated reversals as two novel functional measures to report on the neuromuscular health of C.elegans. These measures are possible due to two
technological advances made in our laboratory: NemaFlex – an image-based force-sensing micropillars that can quantify muscle strength and NemaLife – a microfluidics-based culturing apparatus that enables longitudinal aging experiments without the need for drug-induced blocking of progeny.

We profiled strength across the lifespan of a wild-type population, and our results show that strength increases 5-fold from young adult to mid-life followed by a sharp decline – providing the first direct evidence of muscle strength loss due to age in C. elegans. With respect to stimulated reversals, we find that worms maintain a high reversal speed during their reproductive period and then declines at a rate that is nearly identical to the animal mortality rate. Interestingly, the decline in reversal speed precedes the onset of strength decline by about 3 days indicating that reversal speed is an early predictor of mortality. Moreover, since reversal speed is an indicator of motor activity, the early decline in reversal speed suggests that the presynaptic loss of function occurs early in life, consistent with prior electrophysiological studies.

The stochastic nature of aging produces a wide distribution of lifespans in isogenic C. elegans populations. We observed that long lived worms generally maintain muscle strength long after median lifespan although their motor activity significantly declines. We observed four major classes of strength profiles among individual worms, each of which has a unique healthspan and motor activity pattern. For example, individuals starting with high reversal speed do not necessarily have long lifespan but all strong and long lived worms maintain very high motor activity during the late reproductive period. In summary, maintenance of strength together with motor activity is a possible route for increasing locomotory healthspan.

35 Mitochondrial accumulation of saccharopine, an intermediate of lysine metabolism, induces mitochondria damage in C. elegans. JX. Zhou1,3, X. Wang2, RF. Tang1,3, Q. Zhang1,3, FY. Wang1,3, LY. Zhao1,3, CL. Yang1,2
1) State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences; 2) Center for Life Sciences, School of Life Sciences, Yunnan University, Kunming 650091, China; 3) University of Chinese Academy of Sciences.

Mitochondria are not only the “power house” of the cell, but also home to a vast array of metabolic and signaling processes. Metabolic disorders may disrupt mitochondrial homeostasis and lead to a wide spectrum of human diseases. Nevertheless, it is not well understood how mitochondrial homeostasis is maintained in response to metabolism in a living organism. In the hypodermis of C. elegans, mitochondria form networks and undergo dynamic change between spherical and tubular morphologies. From an EMS screen we identified two mutants, yq170 and yq211, that altered mitochondrial morphology. These two mutants affected the same gene and contain greatly enlarged spherical mitochondria in hypodermal cells and gonad sheath cells. Transmission electron microscopy analysis revealed that the cristae were disappeared and membranes were broken in these abnormal mitochondria. Consistent with this, the ATP levels were strongly reduced in the hypodermis in these mutant animals. Using SNP mapping and sequencing, we determined that yq170 and yq211 affect aass-1, which encodes an ortholog of human alpha-aminoadipate semialdehyde synthase (AASS). AASS-1 is a mitochondrial protein that contains 2 enzymes, the N-terminal lysine-ketoglutarate reductase (LOR), which converts lysine and a-ketoglutarate to saccharopine, and the C-terminal saccharopine dehydrogenase (SDH), which oxidizes saccharopine to generate glutamate and a-aminoadipate semialdehyde. By genetic analysis, we demonstrated that only disruption of SDH activity led to formation of huge abnormal mitochondria. In agreement with this, aass-1 SDH mutants had much higher levels of saccharopine than the wild type. This suggests that mitochondrial accumulation of saccharopine induced abnormal mitochondrial enlargement and damage. We further performed suppressor screens and identified genes and pathways that when mutated suppressed the mitochondrial defects in aass-1 SDH mutants. Given that mutations in human AASS lead to 2 metabolic disorders, hyperlysinemia and sacchropinuria, our findings provide important insight into the pathological mechanisms of these genetic metabolic diseases.

36 Metabolic defects caused by hypomorphic gut specification are mediated by an NHR gene. M.F. Maduro, Gina Broitman-Maduro, Hailey Choi, Kollan Doan Dept Biol, University of California, Riverside, Riverside, CA.

Perturbations that compromise specification generally result in developmental arrest, making it difficult to address questions of whether or not differentiation gene networks can correct for these in later development. We are using "hypomorphic gut specification" (HGS) strains in which activation of the gut specification factors end-1 and/or end-3 in the E (gut) lineage has been compromised. In HGS strains, development of gut becomes stochastic, manifested as a delay in activation of elt-2, a gene whose product maintains the intestinal fate, and changes in the E lineage. Surviving HGS adults have normal levels of ELT-2, but they variably have excess numbers of gut cells (hyperplasia) and store excess fat, as detected by the presence of abnormally large lipid storage granules revealed by Oil Red O staining and localization of ATGL-1::GFP. Other pleiotropic effects, including a shorter life span, variable sterility and morphological changes, suggest that animals are undernourished. We do not find hyperlipidemia in cdc-25.1(n31) adults, which contain an even greater number of gut cells than HGS animals, suggesting that hyperplasia is not the cause of hyperlipidemia. We observe an increase in expression and nuclear localization of DAF-16, a primary regulator of lipid storage. However, a daf-16 null mutant background only partially reduces lipid accumulation in HGS animals, suggesting that other genes are the primary cause of the hyperlipidemia. To identify such genes, we performed tissue-specific RNA-Seq on isolated intestines from control and HGS adults. Only a small set of intestinal genes appears to be differentially expressed. One of these, a previously uncharacterized nuclear hormone receptor (NHR) gene, is strongly activated in the anterior gut in controls but downregulated in HGS animals. A null mutant in the gene induced by CRISPR/Cas9 results in adults that retain abnormally high amounts of lipids, suggesting that a major component of the fat accumulation in HGS strains is lack of expression of this NHR. Our results suggest two models by which HGS leads to hyperlipidemia and changed metabolism. First, compromised activation of specification through END-1,3 may prevent robust establishment of normal metabolism due to a failure to activate some early factors other than elt-2. In a second model, the anterior cells of the gut may undergo a transformation in fate to a more posterior type of gut cell, resulting in a failure to adequately digest bacteria arriving from the pharynx. This might cause a decrease in digestive capacity of the gut, resulting in starvation-like defects. Together, our
results connect progenitor specification with function of the differentiated organ, showing that a failure of robust early gene expression can result in abnormalities that are not corrected by activation of a terminal organ identity gene.

37 Function and regulation of the elongation factor kinase efk-1/eEF2K in starvation response. F. Bhanshali 1, J. Watts 2, A. Jan 1, A. An 1, P. Sorensen 1, S. Taubert 1 1) University of British Columbia, Vancouver, BC, CA; 2) School of Molecular Biosciences, Washington State University, Pullman, WA, US.

Protein synthesis is a highly energy consuming process. EFK-1/eEF2K is an evolutionarily conserved serine/threonine kinase that protects cells from acute nutrient deprivation by inactivating the translation elongation factor, eEF2, thus blocking mRNA translation elongation to preserve cell energy. In C. elegans, the eEF2K ortholog efk-1 is transcriptionally upregulated by starvation and hypoxia, and is required for normal life span and for L1 starvation survival. However, the pathways that induce efk-1 during starvation as well as its downstream targets and processes that enable starvation survival are unknown. To delineate efk-1 induction, we used qPCR analysis to quantify efk-1 mRNA expression in fed and starved wild-type worms and in worms carrying mutations in various transcription factors (TFs) known to regulate starvation responses. We found that transcription factors HLH-30/TFE3 and DAF-16/FOXO are required to upregulate efk-1 mRNA upon starvation in L1 and L4 stage worms. We further found that upregulation of efk-1 is specific to starvation, as it was not induced by oxidative stress. To characterize how efk-1 promotes starvation survival, we performed GC-MS analysis and found that efk-1 mutants have defects in lipid metabolism. Specifically, whereas WT worms catabolize TAGs in starvation, efk-1 mutants do not show a significant change in TAG or PC levels upon starvation. Moreover, unsaturated cardiolipin is significantly lower in starved efk-1 mutants, suggesting defects in mitochondrial function. Besides studying lipid metabolism, we also explored parallels between the starvation response and the response to pathogen virulence factors such as Shiga toxin or Pseudomonas ToxA; like starvation, these toxins induce translational arrest by acting on EEF-2. In these scenarios, translational arrest activates downstream transcriptional programs driven by the TFs ZIP-2 and CEBP-2/CEBP gamma. Thus, we asked whether these TFs might also be activated by starvation-driven translational arrest and whether mutants in these TFs are sensitive to starvation. We found that, like efk-1 mutants, zip-2 and cebp-2 mutants were sensitive to starvation, suggesting that these TFs may act downstream of EFK-1 in the starvation response. In the future, we plan to perform gene expression profiling by RNA-seq on WT and mutants of efk-1, cebp-2 and zip-2 in fed and starved worms to identify the additional genes regulated by them. Overall our study has uncovered the new players in the EFK-1 pathway for the control of mRNA translation elongation and survival in nutrient scarce conditions.

38 Specific tissues coordinate changes in organismal growth, longevity and resilience to stress in response to reduced mRNA translation in C. elegans. S. Snow 1, A. Howard 2, J. Rollins 3, P. Kapahi 3, A.N. Rogers 3 1) Davis Center for Regenerative Biology and Medicine, MDI Biological Laboratory, Salisbury Cove, ME; 2) Natural Lab Science, University of Maine at Augusta, Augusta, ME; 3) Buck Institute for Research on Aging, Novato, CA.

Background: Different forms of physiological stress result in adaptive changes in gene expression in order to maintain homeostatic balance within cells and between tissues. However, the ability to maintain and recover from perturbations in homeostasis declines with age and contributes to age-related disease. An inability to efficiently regulate protein homeostasis, or proteostasis, is considered a causative factor in protein conformational diseases like Alzheimer’s and Parkinson’s. An environmental intervention with the potential to slow age-related decline involves adaptation to low nutrient availability, which induces changes in gene expression that include the restriction and redirection of mRNA translation. Reducing mRNA translation genetically has been shown to ameliorate stress caused by unfolded proteins. We recently discovered that it also protects cells from acute nutrient deprivation by inactivating the translation elongation factor, eEF2, thus blocking mRNA translation elongation to preserve cell energy. In C. elegans, the eEF2K ortholog efk-1 is transcriptionally upregulated by starvation and hypoxia, and is required for normal life span and for L1 starvation survival. However, the pathways that induce efk-1 during starvation as well as its downstream targets and processes that enable starvation survival are unknown. To delineate efk-1 induction, we used qPCR analysis to quantify efk-1 mRNA expression in fed and starved wild-type worms and in worms carrying mutations in various transcription factors (TFs) known to regulate starvation responses. We found that transcription factors HLH-30/TFE3 and DAF-16/FOXO are required to upregulate efk-1 mRNA upon starvation in L1 and L4 stage worms. We further found that upregulation of efk-1 is specific to starvation, as it was not induced by oxidative stress. To characterize how efk-1 promotes starvation survival, we performed GC-MS analysis and found that efk-1 mutants have defects in lipid metabolism. Specifically, whereas WT worms catabolize TAGs in starvation, efk-1 mutants do not show a significant change in TAG or PC levels upon starvation. Moreover, unsaturated cardiolipin is significantly lower in starved efk-1 mutants, suggesting defects in mitochondrial function. Besides studying lipid metabolism, we also explored parallels between the starvation response and the response to pathogen virulence factors such as Shiga toxin or Pseudomonas ToxA; like starvation, these toxins induce translational arrest by acting on EEF-2. In these scenarios, translational arrest activates downstream transcriptional programs driven by the TFs ZIP-2 and CEBP-2/CEBP gamma. Thus, we asked whether these TFs might also be activated by starvation-driven translational arrest and whether mutants in these TFs are sensitive to starvation. We found that, like efk-1 mutants, zip-2 and cebp-2 mutants were sensitive to starvation, suggesting that these TFs may act downstream of EFK-1 in the starvation response. In the future, we plan to perform gene expression profiling by RNA-seq on WT and mutants of efk-1, cebp-2 and zip-2 in fed and starved worms to identify the additional genes regulated by them. Overall our study has uncovered the new players in the EFK-1 pathway for the control of mRNA translation elongation and survival in nutrient scarce conditions.

39 Longevity from dietary restriction and reduced insulin/IGF-1 signaling requires modulation of p38/ATF-7 innate immunity. Ziyun Wu 1,2, Natalie Moroz 1,2, Metem Isik 1,2, Michael Steinbaugh 1,2, Keith Blackwell 1,2 1) Research Division, Joslin Diabetes Center, Boston, MA; 2) Department of Genetics and Harvard Stem Cell Institute, Harvard Medical School, Boston, MA.

Dietary restriction (DR) and reduced insulin/IGF-1 signaling (rIIS) are the most robust environmental and genetic interventions, respectively, that to extend healthy lifespan in diverse organisms. In mammals, through unknown mechanisms DR reduces chronic inflammation, a cause of aging-related disease. Here we show that in C. elegans, DR and rIIS promote longevity by modulating innate immunity. For DR and rIIS lifespan extension, the conserved innate immunity pathway TIR-1(SARM)–NSY-1(ASK1)–SEK-1(MKK3)–PMK-1(p38)–ATF-7(ATF2) must be intact. However, in contrast to other protective mechanisms, p38/ATF-7 immune activity is reduced to a basal level by DR and rIIS, which mobilize other pathogen resistance programs. Elevated p38/ATF-7 immunity accelerates aging, but can be reversed by DR or rIIS to allow lifespan extension. We conclude that innate
immunity modulation is critical for longevity assurance, and that regulation of immunity by anabolic signals may have contributed to immune system evolution.

40  **C. elegans** neurons package neurodegenerative disease-associated toxic peptides into bioactive, chaperone-filled exosomes that are secreted into the external environment.  

*J. Russell*, S Mehta, S Pabustan, A Mendenhall, M Kaeberlein  

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Extracellular vesicle (ECV) signaling is an ancient and ubiquitous cell signaling modality in which cells secrete small (50-150 nm) vesicles into the extracellular space to transfer proteins, lipids, and RNA (e.g., mRNAs & miRNAs) throughout the body. This regulated secretion and targeted migration of ECVs to distant cells is a fundamental aspect of cell biology that affects development, cell-cell & animal-animal communication & disease. Recent work has revealed extracellular vesicle signaling pathways that result in the neuronal secretion of extracellular vesicles outside the body of the animal. We have optimized a method for obtaining highly-enriched homogeneous populations of extracellular vesicles secreted by *C. elegans*. We have visualized our size-exclusion-column-purified exosomes and extracellular vesicles by transmission electron microscopy (TEM), which revealed numerous vesicles between 50 and 150nm with no apparent cell debris of any kind. The vesicle morphologies look similar to other micrographs reported in the literature and show no membrane structures with sizes >150nm. Vital dye flow cytometry measurements indicate the vesicles bioactive. Vesicles were also pulled-down by anti-CD63 immunoprecipitation beads. CD63 affinity is the gold standard for specifically marking human exosomes. This suggests that at least some of the ECVs are exosomes and there is a strong degree of conservation of key exosome trafficking machinery between humans and *C. elegans*. To complement these antibody-affinity experiments we also examined whether the *C. elegans* ortholog to CD63, encoded by *tps-7*, was required for alae formation; alae require functional exosomes to be formed. Indeed, we found pronounced alae defects on mutant lines missing either 150 or 350bp of *tps-7* sequence. Initial mass spectrometry analysis of the enriched vesicle population indicates the presence of several hundred *C. elegans* proteins in the exosomes, including many conserved proteins corresponding to exosome markers in human studies. We also identified many canonical membrane trafficking proteins (RABs, vATPases, GPI-anchored proteins) that have recently been shown to be necessary for epidermal exosome secretion in nematodes. Intriguingly, some of the most-abundant proteins we found were chaperones, some of which are also frequently found in human exosomes (e.g. orthologs of HSP90 and HSPA8). We found that animals that express human toxic neurodegenerative disease associated peptides in their neurons package these peptides into exosomes and secrete them from their bodies. Given the recent discovery that exosome pathways contribute to the cellular spreading of toxic proteins, *C. elegans* might be a useful model for studying the genetic pathways that are necessary for the cell non-autonomous spreading of neurodegenerative stress signals or toxic peptides in exosomes.

41  **High-throughput screening technology identifies dronedarone HCl as proteostasis regulator in C. elegans polyglutamine aggregation models.**  

*S. Mondal*, E. Hegarty, C. Martin, S.K. Gökçe, N. Ghorashian, A. Ben-Yakar  

1) Mechanical Engineering, University of Texas at Austin, Austin, TX; 2) Biomedical Engineering, University of Texas at Austin, Austin, TX; 3) Electrical and Computer Engineering, University of Texas at Austin, Austin, TX.

The nematode Caenorhabditis elegans has been proved to be a useful in vivo model for next generation drug screening to identify new hits and optimize novel leads. Current in vivo assays can operate either at low throughput with high resolution or with low resolution at high throughput. To enable both high-throughput and high-resolution imaging of *C. elegans* models labeled with different fluorescent reporters, we developed an automated microfluidic platform. The platform includes a large-scale microfluidic chip with 96-well designed in standard microtiter plate format and densely packed trapping channels in each well. The channels are uniquely designed to immobilize approximately 4,000 animals simultaneously in 3 min in a lateral orientation. The automated imaging platform can image 15 z-stack images of all trapped animals, capturing their whole volume with a micron resolution in less than 16 min. An automated graphical user interface (GUI) loads all the images, identifies the single animal and perform image processing steps to identify the aggregates present in the body wall muscle cells of our *C. elegans* polyglutamine aggregation models. Using this platform, we screened ~100,000 animals of the polyglutamine aggregation model, with 35 glutamine repeats, using a total number of 25 chips operated with a Z'-factor of 0.8. We tested the efficacy of ~1,000 FDA approved drugs in improving the aggregation phenotype of the model and identified 4 confirmed hits, one of which has a strong dose-response. The confirmed hit was verified on a different strain with 40 polyglutamine repeat length, showing a protein aggregation in the much earlier stage of the development, using our microfluidic platform in a dose-dependent manner. This robust platform now enables high-content screening of various *C. elegans* disease models at the speed and cost of in vitro cell-based assays.

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Thursday, June 22 8:30 AM – 11:30 AM

Carnesale Palisades Ballroom

**Neuronal Development and Cell Biology**

Co-chairs: Yingchuan (Billy) Qi, Hangzhou Normal University, and Meital Oren-Suissa, Weizmann Institute of Science

42  **A DM domain gene and the Ubiquitin-Proteasome System control the balance between sex-specific synapse maintenance and pruning.**  

*M. Oren-Suissa*, O. Hobert  

1) Neurobiology, Weizmann Institute of Science, Rehovot, IL; 2)
Department of Biological Sciences, Columbia University, New York, NY; 3) Howard Hughes Medical Institute.

A comparison of the connectome of the hermaphrodite and male nervous systems reveals the existence of sexually dimorphic synaptic connections between sex-shared neurons (Jarrell et al., 2012). We recently discovered that many neurons initially form synapses in a non-discriminatory manner in both the male and hermaphrodite pattern before sexual maturation, but sex-specific pruning events result in the sex-specific maintenance of subsets of the connections (Oren-Suissa et al., 2016). Synapse pruning is a widely used mechanism to shape neuronal circuits during development, but it has not been previously implicated in the context of generating sex-specific neuronal circuits. Proteasome-mediated protein degradation is a potent biochemical pathway used to control protein stability in various cellular processes. Since the proteasome has been shown to be involved in synapse remodeling, we decided to test whether there is active degradation of synapses in a sex specific manner. We found that inhibiting protein degradation by growing animals on Bortezomib-containing plates prevented synapse pruning and resulted in non-dimorphic adult synaptic connections. The E1 ubiquitin-activating enzyme lies at the heart of the biochemical degradation reaction and is necessary for all subsequent steps. As in other eukaryotes, C. elegans has a single E1 protein, UBA-1 (Kulkarni et al., 2008). Using a temperature sensitive allele of uba-1, it129, we show that in uba-1 mutants elimination of synapses doesn’t occur and the adult neuronal state is non-dimorphic. uba-1-mediated protein degradation is temporally restricted, as a temperature shift in late larval stages is not sufficient to prevent synapse elimination.

At the genetic level, we have linked the globally-acting sex determination system and synaptic pruning, and showed that dmd-5, a phylogenetically conserved transcription factor of the Doublesex/MAB-3 family is dimorphically expressed in the male AVG shared neuron. Using transsynaptic synapse labeling of male and hermaphrodite specific AVG connections, we dissected the interplay between dmd-5 and the protein degradation machinery. We have previously shown that dmd-5 is required for maintenance of the male specific PHB to AVG synapses. PHA to AVG synapses are hermaphrodite specific: male PHA to AVG synapses are pruned during development. Blocking the protein degradation machinery resulted in maintenance of PHA-AVG male synapses. Similarly, in dmd-5 mutant males PHA-AVG synapses were not pruned. Blocking protein degradation in dmd-5 mutant males resulted in maintenance of PHA-AVG synapses. Taken together, our results suggest that synapse elimination by protein degradation is balanced by active maintenance of synapses, and both processes are required for generating the adult dimorphic circuitry.

43 Conserved cell adhesion protein interactions mediate neural wiring of a sensory circuit in the C. elegans male. **B. Kim**, S.W. Emmons 1,2

1) Department of Genetics, Albert Einstein College of Medicine, Bronx, NY; 2) Dominick P. Purpura Department of Neurosciences, Albert Einstein College of Medicine, Bronx, NY.

The function of the nervous system relies on precise synaptic connections. A number of cell adhesion proteins are implicated in the cell recognition of synaptic partners, but how these proteins determine the synaptic connectivity in vivo is poorly understood. Here, we show that two pairs of interacting cell adhesion proteins regulate the neural wiring of a male-specific sensory circuit in C. elegans. This circuit is generated during the L4-adult transition: a pair of sex-shared PHC neurons extends processes that follow and make synapses with the axon of the sex-shared interneuron AVG; later, a male-specific sensory neuron HOA adds its axon to the PHC-AVG bundle, making a circuit composed of three neurons with extensive synaptic connections. Using expression, mutant analysis, cell-specific rescue experiments, and protein binding studies, we identify CASY-1/calsyntenin and RIG-6/contactin as postsynaptic factors acting in AVG for axon fasciculation between synaptic partners (see abstract by Kim, Ivashkiv, and Emmons). In the presynaptic cells, BAM-2/neurexin-related is expressed in HOA and is a binding partner of CASY-1 while SAX-7/L1CAM is expressed in PHC and is a binding partner of RIG-6. Axon fasciculation defects of casy-1 and rig-6 mutants are distinguishable: in mutants lacking casy-1, the HOA axon was frequently detached from the PHC-AVG axon bundle, whereas in rig-6 mutants, both HOA and PHC axons were detached from AVG, but the HOA-PHC fasciculation was intact. Cell ablation studies revealed that HOA-AVG axon fasciculation is dependent on PHC, raising the possibility that in rig-6 mutants the HOA axon may follow the PHC process separated from AVG. Structure-function analyses reveal that the LNS (Laminin, Neurexin, Sex hormone-binding globulin) domain, but not the cadherin domains, of CASY-1 and the immunoglobulin domains of RIG-6 are responsible for the axon fasciculation. Mutations in all of these cell adhesion proteins disrupt male vulva location behavior during mating, consistent with the functions of these neurons. Our findings suggest that cell-cell recognition via phylogenetically conserved protein interactions of neural cell adhesion proteins is a crucial component of neural circuit assembly.

44 The degenerins and mechanosensory experience determine dendritic arborization and behavior. **S. Inberg**, B. Podbielnicz

Technion- Israel Institute of Technology, Haifa, IL.

Sensory experience affects the structure and function of the nervous system by modifying its epigenetic, molecular, synaptic, and network properties. Little is known about how neuronal activity and sensory experience influence dendritic trees during adulthood. In particular, how environmental signals re-organize the structure and function of complex arborized neurons. To study whether mechanosensory experience affects the architecture of stereotypic dendritic trees of the polymodal PVD neuron and behaviour, we use natural mechanical stimulation induced by physical contacts between C. elegans individuals1. We found that in animals grown in isolation there is a decrease in response to harsh touch (PVD-dependant function) and a change in proprioception-dependent posture, compared to worms grown in crowded, mechanosensory enriched conditions. We then asked whether the stereotypic morphology of the PVD is also affected by mechanical isolation. We focused on the number, the shape and the self-avoidance properties of the repetitive candelabra-like dendritic trees2. We found that isolation of both larvae and adult worms significantly affect the structure and function of the mature PVD. We hypothesized that voltage independent epithelial sodium channels (ENaCs) degenerins are involved in the differential response to mechanical stimulation in isolated and crowded worms. To this end we tested whether animals mutated in different degenerins have PVD morphological and behavioural phenotypes. We found that loss-of-function mutations in the degenerins

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1 Department of Genetics, Albert Einstein College of Medicine, Bronx, NY; 2) Dominick P. Purpura Department of Neurosciences, Albert Einstein College of Medicine, Bronx, NY.

2 Conserved cell adhesion protein interactions mediate neural wiring of a sensory circuit in the C. elegans male. **B. Kim**, S.W. Emmons 1,2

1) Department of Genetics, Albert Einstein College of Medicine, Bronx, NY; 2) Dominick P. Purpura Department of Neurosciences, Albert Einstein College of Medicine, Bronx, NY.

3 The degenerins and mechanosensory experience determine dendritic arborization and behavior. **S. Inberg**, B. Podbielnicz

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Sensory experience affects the structure and function of the nervous system by modifying its epigenetic, molecular, synaptic, and network properties. Little is known about how neuronal activity and sensory experience influence dendritic trees during adulthood. In particular, how environmental signals re-organize the structure and function of complex arborized neurons. To study whether mechanosensory experience affects the architecture of stereotypic dendritic trees of the polymodal PVD neuron and behaviour, we use natural mechanical stimulation induced by physical contacts between C. elegans individuals. We found that in animals grown in isolation there is a decrease in response to harsh touch (PVD-dependant function) and a change in proprioception-dependent posture, compared to worms grown in crowded, mechanosensory enriched conditions. We then asked whether the stereotypic morphology of the PVD is also affected by mechanical isolation. We focused on the number, the shape and the self-avoidance properties of the repetitive candelabra-like dendritic trees. We found that isolation of both larvae and adult worms significantly affect the structure and function of the mature PVD. We hypothesized that voltage independent epithelial sodium channels (ENaCs)/degenerins are involved in the differential response to mechanical stimulation in isolated and crowded worms. To this end we tested whether animals mutated in different degenerins have PVD morphological and behavioural phenotypes. We found that loss-of-function mutations in the degenerins
asic-1 and mec-10, but not degt-1, failed to show differential change in harsh touch response following isolation. Moreover, the structural plasticity of the PVD following mechanosensory deprivation depends on the activities of asic-1, mec-10, and degt-1 in a complex combinatorial way. Thus, degenerins control both structural and behavioural plasticity. We will discuss models explaining how environmental mechanosensory signals sensed by degenerins, modify PVD architecture and behavioural outputs.

45  HPO-30/Claudin forms a co-receptor complex with DMA-1 to promote dendritic branching in *C. elegans* PVD neurons. Wei Zou¹, Xintong Dong¹, Ao Shen², Timothy Broederdorf², Xing Liang³, Kevin Xiang³, Baoyu Chen³, Kang Shen¹,⁴ ¹) Department of Biology, Stanford University, Stanford, CA; 2) Department of Pharmacology, University of California, Davis, Davis, CA; 3) Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, IA; 4) National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China.

Dendrite morphogenesis is a critical step for neural circuit assembly. However, the underlying molecular mechanisms are still not fully understood. Previous studies by others and us have identified a receptor-ligand complex, which includes DMA-1, SAX-7, MNR-1 and LECT-2, guides the branching and growth of *C. elegans* PVD dendrites. Here we found that HPO-30, a claudin-like protein, forms a co-receptor complex with DMA-1 in PVD dendrites to regulate dendritic branching. Genetically hpo-30 functions in a same pathway with *dma-1*, and *dma-1* over-expression can partially bypass the requirement of HPO-30. HPO-30 interacts with DMA-1 both *in vitro* and *in vivo*. Using single-molecule pull-down assays, we found that HPO-30 forms a multi-protein complex with DMA-1, SAX-7, MNR-1 and LECT-2, but is not required for the interaction between DMA-1 and the ligand importantly, the cytosolic domain of HPO-30 interacts with the WAVE regulatory complex to promote dendritic branching. Meanwhile, the cytosolic domain of DMA-1 interacts with TIAM-1/RacGEF and is required for the formation of dendritic branches. Together, our study revealed that HPO-30 and DMA-1 form a novel co-receptor complex to promote robust dendritic branch formation by regulating local actin assembly.

46  MIG-14/Wntless Regulates Dendrite Self-Avoidance via Actin Asssembly and Independent of Wnt. Chien-Po Liao¹, Hsu Li¹, Hsiu-Hsiang Lee¹, Cheng-Ting Chien¹, Chun-Liang Pan¹ ¹) Institute of Molecular Medicine, National Taiwan University College of Medicine, Taipei 10002, Taiwan; 2) Institute of Molecular Biology, Academia Sinica, Nankang, Taipei 11529, Taiwan.

The number of molecules controlling dendrite self-avoidance between sister branches from the same neuron is still limited. Here we report a novel role of the Wnt-secretory factor MIG-14/Wntless in mediating dendrite self-avoidance in the *C. elegans* multidendritic PVD neuron. This function of MIG-14 is likely independent of Wnts, as none of the Wnts or Frizzled receptors mutants show PVD self-avoidance defects, and MIG-14 acts cell-autonomously in the PVD, but not in Wnt-expressing tissues, to regulate dendrite self-avoidance. Homophilic interaction of MIG-14 between neighboring dendrites is sufficient to trigger repulsion when ectopically expressed. We map a leucine-rich motif in one of the extracellular loops of MIG-14 that is critical for dendrite self-avoidance but dispensable for Wnt secretion, indicating that these two functions of MIG-14 are genetically separable. We further show that MIG-14 genetically acted through WASP-dependent actin assembly, whereas a mutation in actin depolymerizing factor unc-60 suppressed dendrite self-avoidance defects of the *mig-14* mutant. Self-avoidance defects could be found in *Drosophila* class IV da neuron of Wntless RNAi or mutants, indicating that dendrite self-avoidance is a conserved MIG-14/Wntless function. Our findings expand the repertoire of self-avoidance molecules and uncover a previously unknown, Wnt-independent function of MIG-14/Wntless.

47  The synaptic organizer neurexin coordinates cholinergic synaptic connectivity with GABAergic motor neurons. A. Philbrook¹, M. Lemons¹, S. Ramachandran¹, D. Oliver¹, C. Lambert¹, M.M. Francis¹ ¹) UMASS Medical School, Worcester, MA; 2) Assumption College, Worcester, MA.

In the central nervous system, individual neurons often project divergent synaptic connections to multiple postsynaptic partners. However, we have only a limited understanding of how synaptic specificity across postsynaptic partners is established, and the mechanisms that govern differential patterns of synaptic connectivity. Dyadic excitatory synapses in the *C. elegans* motor circuit provide a useful model for elucidating the molecular basis of synaptic specificity. Excitatory cholinergic motor neurons synapse onto both inhibitory GABAergic neurons and body wall muscles. While genes required for muscle cholinergic synaptic assembly have been well defined, excitatory synaptic connections onto GABAergic neurons appear to require a distinct molecular scaffold. We found that the cell adhesion molecule *nrx-1* is specifically required for the assembly of synaptic inputs onto GABAergic neurons. In the absence of *nrx-1*, both cholinergic receptor localization and the formation of synapse-associated morphological features of GABAergic dendrites are disrupted. As a result, excitatory transmission onto GABAergic neurons is severely impaired. *nrx-1* expression in cholinergic neurons is coregulated with cholinergic neuron identity, and is required for proper synaptic connectivity with GABAergic neurons. Surprisingly, synaptic development is not affected by mutation of neurulin, neurexin’s most well characterized binding partner. We therefore propose that neurexin expression in cholinergic neurons organizes synaptic connectivity with GABAergic neurons through a neurulin-independent trans-synaptic signaling pathway. Our findings provide evidence that cholinergic neurons utilize distinct molecular signals to govern the establishment of synaptic connectivity with GABAergic motor neurons and body wall muscles, offering a novel view into molecular mechanisms responsible for generating divergent patterns of synaptic connectivity.

48  Local inhibition of microtubule dynamics by dynein is required for neuronal cargo distribution. Shaul Yogev, Celine Maeder, Kang Shen Dept Biology, Stanford, Stanford, CA.
Microtubules (MTs) are the main cytoskeletal substrate on which axonal transport progresses. How the architecture and dynamics of the MT network affect the transport of neuronal cargo is poorly understood. Furthermore, molecular motors not only carry cargoes but also regulate MT behaviour, and the mechanisms underlying this regulation, and their role in vivo, are not clear. We developed a rapid, fluorescence-based method for analyzing neuronal MT length, abundance and dynamics in C. elegans, and used it to explore how neuronal MTs are patterned and how these patterns affect cargo transport. We identified a surprising function for dynein heavy chain (DHC-1), in setting polymer distribution and dynamics in the DA9 dendrite. In disease-mimicking dhc-1 alleles, MT length and abundance were normal, but polymers were shifted distally, and excessive growth and collapse occurred at the dendrite tip, resulting in the formation of aberrant MT loops. dhc-1 mutants also accumulated neuronal cargo at the tip of the dendrite, leading to severely reduced availability of mitochondria and synaptic vesicle precursors in normal locations. Strikingly, cargo mis-localization is not due to defects in dynein’s function as a motor in cargo transport, but to its role in locally suppressing MT dynamics. Unstable looping MTs acted as cargo traps, and cargo could be restored to its endogenous sites by suppressing MT dynamics. Live imaging of axonal transport, mutant analysis and single molecule in-vitro assays suggest a model wherein an anchored dynein pool at the tip of the dendrite interacts with plus-end-out MTs to stabilize them and allow efficient retrograde transport. These results identify functional significance for neuronal MT stability and suggest a mechanism for cellular dysfunction in dynein-linked disease.

49 RHO-1 and the activator RHGF-1/RhoGEF are required for EBP-2 distribution in growth cones. Mahepta Gujar, Aubrie Stricker, Erik Lundquist. Molecular Biosciences, University of Kansas, Lawrence, KS.

Previous studies showed that UNC-6/Netrin and its receptors UNC-40/DCC and UNC-5 control axon guidance by regulating protrusion of growth cone lamellipodia and filopodia (Norris et al., 2011; Norris et al., 2014). UNC-6 stimulates protrusion in growth cones attracted to UNC-6 via the UNC-40 homodimeric receptor, and inhibits protrusion in growth cones repelled from UNC-6 via the UNC-5/UNC-40 heterodimeric receptor. Protrusion via UNC-40 requires CDC-42, the Rac GTPases MIG-2 and CED-10, the Rac GEF TIA-M, and the actin modulating molecules Arp2/3 and UNC-115/abLIM. This results in polarized distribution to the leading edge of the growth cone. We delineated a pathway downstream of UNC-5/UNC-40 required to inhibit protrusion that also involves the Rac GTPases and a distinct GEF, UNC-73/Trio, as well as the microtubule-interacting protein UNC-33, similar to Collapsin response mediating protein (CRMP).

We used EBP-2::GFP expression in VD neurons to monitor MT + ends in VD growth cones. We found that mutations in components of the UNC-5, UNC-33/CRMP pathway result in excess EBP-2::GFP puncta in VD growth cones, suggesting that this pathway might normally restrict MT + end entry into the growth cones to inhibit growth cone protrusion. Interestingly, a mutation affecting specifically the Rac GEF domain of unc-73 did not affect EBP-2::GFP distribution in growth cones despite having excessively protrusive growth cones. F-actin distribution was affected, indicating that inhibition of protrusion can act through both actin and MTs. We find that the MT-dependent mechanism involves RHO-1 and its upstream activator RHGF-1/RhoGEF. rhg-1 mutants show large protrusive growth cones similar to those seen in unc-5 and unc-33 mutants and displayed greatly increased numbers of EBP-2::GFP puncta in VD growth cones and protrusions. The dominant negative form of RHO-1, rho-1(T19N) also resembles unc-5, with excess lamellipodial and filopodial protrusions and show increased EBP-2::GFP puncta. In sum, these studies indicate that growth cone protrusion can be inhibited by UNC-6/Netrin signaling by restricting MT + ends from growth cones, and that ectopic MT + ends in growth cones drive ectopic protrusion.

50 Distinct effects of tubulin isotype mutations on neurite growth in Caenorhabditis elegans. Chaogu Zheng, Margaretie Diaz-Cuadros, Susan Jao, Ken Nguyen, David Hall, Martin Chalfie. 1) Department of Biological Science, Columbia University, New York, NY, 10027; 2) Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY, 10461.

Tubulins, the building block of microtubules (MTs), play a critical role in both supporting and regulating neurite growth. Eukaryotic genomes contain multiple a and b tubulin isotypes, and their dominant missense mutations cause a wide range of neurodevelopmental defects in humans. Thus, identifying the specific functions of each tubulin isotype and evaluating how particular mutations would change those functions during neuronal morphogenesis is fundamental for an understanding of the role of MTs in the nervous system. Using the C. elegans touch receptor neurons (TRNs) as a model system, we have analyzed the effects of 67 missense mutations in the mec-12/a-tubulin and mec-7/b-tubulin genes on neurite growth. Three types of mutations emerged based on their phenotypes: 1) loss-of-function (lf) mutations, which are found throughout the molecule and cause the specific shortening of posterior neurites; 2) antimorphic (anti) mutations, which mapped to the GTP binding site and intradimer and interdimer interaction interfaces and significantly reduced MT stability causing severe defects in the growth of all TRN neurites; and 3) neomorphic (neo) mutations, which mapped to the exterior surface of the tubulin heterodimer and increased MT stability, causing ectopic neurite growth. Importantly, we were able to engineer several disease-associated human tubulin mutations into the C. elegans mec-7 gene and examine their impact on neuronal development at cellular level. We also discovered a MT-destabilizing a-tubulin isotype TBA-7, whose loss led to the formation hyperstable MTs and the generation of ectopic neurites in vivo; the lack of potential sites for polyamination and polyglutamination on TBA-7 may be responsible for this destabilization. These results support the “multi-tubulin” hypothesis that distinct tubulin isotypes have specific functions and posttranslational modification is the key for the functional difference.

51 Myrf ER-bound transcription factors drive C. elegans synaptic plasticity via cleavage-dependent nuclear translocation. Jun Meng, Xiaoxia Ma, Huaping Tao, Xia Jin, Daniel Witvliet, James Michell, Ming Zhu, Meng-Qiu Dong, Mei Zhen, Yishi Jin, Yingchuan Qi. 1) College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou, Zhejiang, CN; 2) Department of Physiology, University of Toronto, Toronto, Ontario, Canada; 3) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 4) Department of Physics, Harvard University, Cambridge,
Our results identify rules governing the transcriptional regulatory code of a critically important neuronal type in two species closely resembles that of mouse 5HT neurons, which reveals deep homology.

Separated by over 700 million years.

Finally, Principal Coordinates Analysis suggests that, among counterparts. Over-expression of active forms of MYRF is sufficient to accelerate synaptic rewiring. MYRF-1 and MYRF-2 are the first genes identified to be indispensable for promoting synaptic rewiring in *C. elegans*. These findings reveal a novel molecular mechanism underlying synaptic rewiring and developmental circuit plasticity.

52  An importin protein controls asymmetric olfactory neuron differentiation by mediating nuclear transport of a homoeodomain transcription factor for sox-2 expression. A. Alqadah1, Y.-W. Hsieh1, B.J. Lesch2, C.-F. Chuang1 1) Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL, USA; 2) Howard Hughes Medical Institute, Laboratory of Neural Circuits and Behavior, The Rockefeller University, New York, NY 10065, USA.

Defects in brain laterality are associated with several neurological diseases. Left-right asymmetry in the nervous system is observed in many species; however, the mechanisms used to establish brain lateralization are not well understood. The pair of AWC olfactory neurons displays molecular and functional left-right asymmetry in the nematode *C. elegans*. The neurons are differentiated into the AWC<sup>OFF</sup> and AWC<sup>ON</sup> subtypes that express different sets of genes and have unique functions. The AWC neurons display stochastic asymmetry, such that either subtype has equal probability of residing on the left or right side of the animal. This asymmetry is established using a calcium-triggered signaling pathway consisting of voltage-gated calcium channels, CaMKII, TIR-1 (Sarm1) adaptor protein, and a MAP kinase cascade to promote the default AWC<sup>OFF</sup> identity. The contralateral neuron represses the calcium signaling pathway through NSY-5 gap junctions and downstream redundant SLO-1/SLO-2 BK potassium channels to induce the AWC<sup>ON</sup> cell identity. It is unknown what molecules function downstream of the SLO-1 and SLO-2 potassium channels to induce AWC<sup>ON</sup>. To address this question, we performed a forward genetic screen to discover suppressors of slo-1(gf) mutants that act as modifiers of K<sup>+</sup> channels (mok genes). Out of 6,000 genomes screened, we identified 16 mok mutants. Using whole genome sequencing (kindly performed by Oliver Hobert’s lab), we found that the mok-5 gene encodes a highly conserved importin protein. A *vy10* missense mutation in the mok-5 importin gene results in a 2AWC<sup>OFF</sup> phenotype, suggesting an essential role of mok-5 in promoting the AWC<sup>ON</sup> subtype. Double mutant analysis places mok-5 downstream of the calcium-triggered MAP kinase cascade to promote the AWC<sup>ON</sup> subtype. Consistent with the genetic data, mok-5 importin is asymmetrically expressed in the AWC<sup>ON</sup> neuron and acts cell autonomously to specify the AWC<sup>ON</sup> subtype. Furthermore, we show that mok-5 importin functions to mediate transport of a homeodomain transcription factor required for AWC<sup>ON</sup> subtype specification into the nucleus of AWC neurons. Lastly, we show that the homeodomain transcription factor regulates the expression of HMG transcription factor sox-2 by direct binding to the sox-2 promoter to promote the AWC<sup>ON</sup> identity. To the best of our knowledge, this is the first study to implicate an importin protein in the development of left-right asymmetry in the nervous system.

53  Conserved transcriptional regulatory rules define the serotonergic-neuron identity. Carla Lloret-Fernández1, Miren Maicas1, Carlos Mora1, Alejandro Artacho2, Ángela Jimeno1, Laura Chirivella1, Peter Weinberg3, Nuria Flames1 1) Developmental Neurobiology Unit, Instituto de Biomedicina de Valencia IBV-CSIC, Valencia, ; 2) Department of Genomics and Health, Centro Superior de Investigación en Salud Pública, FISABIO, Valencia; ; 3) Department of Biochemistry and Molecular Biophysics, Howard Hughes Medical Institute, Columbia University Medical Center, NY, USA.

Cell differentiation is controlled by individual transcription factors (TFs) that together activate a selection of enhancers in specific cell types. How these combinations of TFs identify and activate their target sequences remains unknown. Here, we identify the cis-regulatory transcriptional code that controls the differentiation of serotonergic (5HT) HSN neurons in *C. elegans*. Loss of function mutant and cis-regulatory analyses reveal that direct activation of the HSN transcriptome is orchestrated by a collective of six TFs. This TF code is composed by AST-1 (ETS TF family), UNC-86 (POU TF family), SEM-4 (SPALT TF family), HLF-3 (bHLH TF family), EGL-46 (INSM TF family) and EGL-18 (GATA TF family). Bioinformatically identified binding site clusters for these six TFs are enriched in known HSN expressed genes compared to a random set of genes. Through *in vivo* reporter analysis, we demonstrate that the clustering of TF collective binding sites constitutes a regulatory signature that is sufficient for *de novo* identification of HSN neuron functional enhancers. This regulatory signature contains certain syntactic constraints that further improve the prediction of enhancer expression in the cell. Mouse orthologs of most members of this TF collective are known regulators of mammalian 5HT differentiation programs and can functionally substitute for their worm counterparts. Finally, Principal Coordinates Analysis suggests that, among *C. elegans* neurons, the HSN transcriptome most closely resembles that of mouse 5HT neurons, which reveals deep homology.

Our results identify rules governing the transcriptional regulatory code of a critically important neuronal type in two species separated by over 700 million years.
Biogenesis and Function of Social Extracellular Vesicles (EVs).  Juan Wang, Malan Silva, Maureen Barr Human Genetics Institute of NJ and Genetics Department, Rutgers University, Piscataway, NJ.

Extracellular vesicles are emerging as an important aspect of intercellular communication by delivering a parcel of proteins, lipids even nucleic acids to specific target cells over short or long distances (Maas 2017). A subset of C. elegans ciliated neurons release EVs to the environment and elicit changes in male behaviors in a cargo-dependent manner (Wang 2014, Silva 2017). Our studies raise many questions regarding these social communicating EV devices. Why is the cilium the donor site? What mechanisms control cilary EV biogenesis? How are bioactive functions encoded within EVs?

EV detection is a challenge and obstacle because of their small size (100nm). However, we possess the first and only system to visualize and monitor GFP-tagged EVs in living animals in real time. We are using several approaches to define the properties of an EV-releasing neuron (EVN) and to decipher the biology of cilary-released EVs. To identify mechanisms regulating biogenesis, release, and function of ciliary EVs we took an unbiased transcriptome approach by isolating EVNs from adult worms and performing RNA-seq. We identified 335 significantly upregulated genes, of which 61 were validated by GFP reporters as expressed in EVNs (Wang 2015). By characterizing components of this EVN parts list, we discovered new components and pathways controlling EV biogenesis, EV shedding and retention in the cephalic lumen, and EV environmental release. We also identified cell-specific regulators of EVN ciliogenesis and are currently exploring mechanisms regulating EV cargo sorting. Our genetically tractable model can make inroads where other systems have not, and advance frontiers of EV knowledge where little is known.


54  A role for RNA processing proteins in maintaining genome stability.  J.A. Kamp1, B.B. Lemmens2, R. van Schendel1, M. Tijsterman1 1) Department of Human Genetics, LUMC, Leiden, NL; 2) Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, SE.

Multiple pathways exist to repair DNA double strand breaks (DSBs), and most of the components of these pathways are conserved across species. Yet, which DSB repair pathway acts to repair a DSB depends on the cellular and genomic context and the type of break, and is highly regulated. While Homologous Recombination (HR) and polymerase Theta-Mediated End Joining (TMEJ) are the major DSB repair routes in the germline, the classical Non-Homologous End Joining (cNHEJ) pathway is the main DSB repair pathway in somatic tissues.

To identify and characterise genetic factors involved in NHEJ and its regulation, we performed an unbiased forward genetic screen in nematodes carrying a transgenic NHEJ reporter. We isolated seven bona fide NHEJ mutants, three of these contained mutations in the well-known cNHEJ factors cku-70 and cku-80. The other four mutants carried mutations in genes of the THO ribonucleoprotein complex (thoc-2, thoc-5 and thoc-7) and in pnn-1. Both the THO complex and PNN play a role in RNA processing and are conserved in humans. We found that deficiency of PNN and the THO complex also leads to sensitivity to ionizing radiation in somatic tissues, but not in the germline, which is similar to the response of animals defective in cku-70 and cku-80, and points towards a role for the THO complex and PNN in cNHEJ. Transcriptome analysis by RNA sequencing identified a subset of transcripts to be differentially expressed and/or spliced in THO mutants but cNHEJ factors.

To identify the mechanism by which the THO complex influences cNHEJ efficiency, a suppressor screen was performed in a thoc-5 deficient background. We found that mutated smg-1 rescues the NHEJ defect in THO complex mutants, but surprisingly this was independent of SMG-1’s well-established role in nonsense-mediated decay. These findings classify the phosphatidylinositol 3-kinase-related kinase SMG-1 as a suppressor of NHEJ. To address the hypothesis that SMG-1 is hyperactive in THO complex deficient backgrounds we are currently performing phosphoproteomic experiments. Interestingly, smg-1 mutants are hypersensitive to ionizing radiation-induced DSBs in the germline, similar to HR and TMEJ mutants. This hypersensitivity could suggest that SMG-1 stimulates repair of germline DSBs via HR or TMEJ. We postulate that SMG-1 regulates the repair of DNA double strand breaks by inhibiting NHEJ and promoting other DSB repair pathways.

55  SMRC-1, a putative annealing helicase, links chromatin regulation and DNA repair in the C. elegans germ line.  B. Yang1, X. Xu1, M. Sullenberger1, L. Russell2, J. Yanowitz2, E. Maine3 1) Department of Biology, Syracuse University, Syracuse, NY; 2) Magee-Womens Research Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA.

Histone post-translational modifications are implicated in transcriptional control and chromosomal events. One modification, histone H3 lysine 9 dimethylation (H3K9me2), accumulates at certain classes of repetitive sequence across the C. elegans genome and is enriched on unsynapsed chromosomes in meiosis (Guo et al. 2015, Zeller et al. 2016, McMurchy et al. 2017, Kelly et al. 2002). MET-2 is the histone methyltransferase responsible for H3K9me2 (Andersen & Horvitz 2007, Bessier et al.

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Thursday, June 22  8:30 AM – 11:30 AM
De Neve Auditorium
Genome Stability, Gene Expression and Technologies

Co-chairs:  Sam Gu, Rutgers University, and Anna Zinovyeva, Kansas State University
Using CRISPR-Cas9 methods, we flag-tagged the endogenous smrc-1 gene and generated smrc-1 loss-of-function mutations. We confirmed the interaction with MET-2 by co-IP with 3xFLAG::SMRC-1. We detect 3xFLAG::SMRC-1 in mitotic and meiotic nuclei in XX and XO germ lines. Consistent with the proposed role of SMARCAL1 in repairing DNA damage at the replication fork, smrc-1 mutant animals have an impaired response to DNA damage. E.g., they are hypersensitive to replication stress introduced by hydroxyurea and have elevated germline apoptosis that is dependent on the DNA damage checkpoint protein, CEP-1. In addition, smrc-1 mutants display developmental defects, including reduced fertility and embryonic lethality. Interestingly, meiotic H3K9me2 is variably reduced in smrc-1 mutants after passage for multiple generations, suggesting that SMRC-1 might promote MET-2 activity in the germline. Moreover, meiotic recombination is elevated in smrc-1 mutants. We observe enhanced sensitivity to DNA damage and highly penetrant sterility in the met-2 smrc-1 double mutant. We propose that MET-2 and SMRC-1 activities function in parallel to limit DNA damage and recombination at repetitive regions.

57 Polymerase Θ is a key driver of genome evolution, of CRISPR/Cas9-mediated mutagenesis and of mutagen-induced deletion allele formation. R. van Schendel, M. Tijsterman Human Genetics, Leiden University Medical Center, Leiden, Zuid-Holland, NL.

For more than half a century, genotoxic agents have been used to induce mutations in the genome of model organisms to establish genotype-phenotype relationships. We have recently demonstrated for two of the most widely used mutagens in C. elegans, i.e. ethyl methanesulfonate (EMS) and photo-activated trimethylpsoralen (UV/TMP), that deletion mutagenesis is the result of polymerase Theta (POLQ)-mediated end joining (TMEJ) of double strand breaks (DSBs) 1, 2. This discovery allowed us to survey many thousands of available deletion alleles, which reveals a step-wise and versatile model for the in vivo mechanism of TMEJ, explaining the molecular nature of mutagen-induced deletion alleles 3. We also found that CRISPR/Cas9-induced genomic changes are exclusively generated through POLQ action, refuting a previously assumed requirement for NHEJ in their formation; Unlike somatic cells, which use NHEJ to repair DNA breaks, germ cells use an alternative route 4. Finally, through whole-genome sequencing of propagated populations, we show that only POLQ-proficient animals accumulate genomic scars that are abundantly present in genomes of wild C. elegans, pointing towards POLQ as a major driver of genome diversification. New insights into the mechanism of genome engineering, genome maintenance and evolution will be discussed.


58 Use of the Million Mutation Project to study transposons and genome biology. Stephen Frenk1, Shawn Ahmed1,2 1) Department of Genetics, University of North Carolina, Chapel Hill, NC; 2) Department of Biology, University of North Carolina, Chapel Hill, NC.

Transposons are abundant selfish genetic elements that are capable of moving to new locations in the genome. Uncontrolled movement of transposons could have catastrophic effects on the genome, but transposition also promotes genomic variation in a manner that is useful for the crucible of evolution. We used a recently published resource, the Million Mutation Project (MMP) (Thompson et al. 2013), to investigate genetic pathways that repress transposons. The MMP consists of around 2000 isogenic C. elegans strains isolated from a chemical mutagenesis screen. Each strain was selfed for ten generations before genomic DNA was prepared and subjected to whole genome sequencing. Using this resource, we identify a subset of “Mutator” strains defined as having novel transposon insertions, likely revealing all transposon types that are capable of moving in a metazoan. This subset of Mutator strains is highly enriched for mutations in genes that are known to repress transposition of Tc DNA transposons. Helitron transposons represent a recently discovered class of DNA transposon that moves via a rolling circle replication mechanism. Data from cluster analysis suggests that Helitrons are repressed by a mechanism that is distinct from that used to repress almost all other transposons. This implies that Helitron transposons are resistant to canonical transposon silencing systems. We are studying mutations in genes of MMP strains with novel Helitron insertions in an effort to define how Helitrons are repressed. More broadly, our work demonstrates the power of forward genetics combined with whole genome sequencing as an unbiased method to identify factors that influence genome evolution.

59 EMR-1/emerin is involved in tissue-specific anchoring of chromatin to the nuclear envelope and neuromuscular junction activity. C. Muñoz Jiménez, C. Ayuso, P. Askjaer Andalusian Center for Developmental Biology (CABD), CSIC/JA/Universidad Pablo de Olavide, Seville, Spain.

The nuclear envelope (NE) regulates transport of macromolecules between the nucleus and the cytoplasm and plays critical roles in nuclear organization and gene expression. Reflecting the importance of its functions, several human diseases are
attributed to alterations in NE structure. Most notably are the laminopathies, with one example being Emery-Dreifuss muscular dystrophy (EDMD), which is caused by mutations in the inner nuclear membrane protein emerin or in the nuclear lamina protein lamin A.

We have found recently that EMR-1, the Caenorhabditis elegans ortholog of emerin, associates with genes implicated in muscle and nervous system function and regulate their expression. Interestingly, deletion of emr-1 causes local changes in nuclear architecture and hypersensitivity to the cholinesterase inhibitor aldicarb, indicating altered activity at neuromuscular junctions. Although many NE proteins are ubiquitously expressed, laminopathies often affect a single tissue. It is hypothesized that tissue-specific alterations in nuclear organization might be responsible for particular symptoms of laminopathies. For this reason, we have developed novel tools to study changes in interactions between NE proteins and chromatin in a tissue-specific manner in intact animals. We report here the results of DamID experiments based on FLP-mediated recombination to dissect the function of EMR-1 in tissue-specific nuclear organization. Interestingly, EMR-1 interaction profiles in muscles vs. neurons reveal both many common domains and local changes in chromatin organization, with central part of chromosomes interacting more frequently with EMR-1 in neurons than in muscles. We are currently addressing the role of EMR-1 in regulation of gene expression in a tissue-specific way to understand the contribution of this protein to the neuromuscular junction activity and potentially to unravel the etiology of EDMD.

It has been reported that NE morphology is prone to suffer from changes during normal aging and in progeria patients. Again, it has been proposed that alterations in nuclear organization through development and aging may have an impact on the aging-associated disorders. By performing FLP-mediated temporal DamID in young vs. old animals we have detected that chromatin accessibility changes dramatically in aged worms. Furthermore, we have identified local changes in EMR-1 interaction profiles in aged vs. young animals, with depletion of EMR-1 in central part of chromosomes in old animals.

60 Developmental regulation of H3K9me2 and chromatin compaction by C. elegans synMuv B proteins. Meghan E Fealey1, Andreas Rechsteiner2, Thea Egelhofer2, Ethan Doerger1, Susan Strome2, Lisa N Petrella1 1) Biological Sciences, Marquette University, Milwaukee, WI; 2) MCD Biology, UC Santa Cruz, Santa Cruz, CA.

A central question in development is how chromatin is organized and regulated to ensure proper gene expression and cell fate. During early embryo development, before gene expression is globally upregulated, chromatin is found in an open state. As development proceeds and cells differentiate, the genome compacts and becomes organized into open and closed domains. Genes required for a particular fate remain open and poised for transcription factor binding, while genes not needed for that fate are further compacted and sequestered to the nuclear periphery. Although this process is highly regulated, many of the proteins involved in this process are unknown. Loss of C. elegans synMuv B proteins causes changes in the developmental regulation of chromatin and gene expression. Many synMuv B mutants, including lin-15B, show ectopic expression of germline genes in somatic cells and a high temperature larval arrest (HTA) phenotype. The HTA phenotype is rescued by knockdown of chromatin modifiers, suggesting that synMuv B proteins regulate gene expression programs epigenetically. To investigate ways in which synMuv B proteins may affect chromatin, we performed ChIP-seq analysis of several histone modifications in wild type and synMuv B mutants at 20°C and 26°C. Intriguingly, lin-15B mutants lose promoter localized H3K9me2 over germline genes in somatic tissues in a temperature independent manner. Typical enrichment of H3K9me2 over gene bodies is not disrupted in these mutants. Promoter localized H3K9me2 is an unstudied pattern for this mark in C. elegans, and the mechanism by which it regulates gene expression is unknown. We investigated if synMuv B proteins function to regulate developmental chromatin compaction. synMuv B mutants display a developmental delay in chromatin compaction that is sensitive to temperature. This delay results in open chromatin during the window when ectopic expression of germline genes in somatic tissues begins. Using temperature shift assays, we found that the crucial developmental time period for the HTA phenotype is the same as the time period when synMuv B mutants display open chromatin. Open chromatin during this period may allow germline genes to be poised for ectopic expression in somatic tissues of synMuv B mutants. Interestingly, the last cells to divide in the intestine are the last cells to adopt compact chromatin, suggesting that the transition to organized chromatin is coupled with differentiation. Understanding synMuv B regulation of promoter enrichment of H3K9me2 and chromatin compaction will help elucidate pathways used to achieve proper gene expression and correct development.

61 Programmed DNA Elimination in Nematodes. J. Wang, Y. Kang, S. Gao, M. Zagoskin, A. Neff, R.E. Davis  Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Aurora, CO.

Maintenance of genome integrity is essential. In the parasitic nematode Ascaris, programmed DNA elimination removes specific DNA sequences from the genome during early development in somatic cells (4-16 cell stage), leaving the germline genome intact. We found that ~13% of the genome is eliminated during DNA elimination. The eliminated DNA consists of repetitive and unique sequences, including ~1000 genes (5% of all genes). The same DNA is eliminated independently in five different pre-somatic cells that give rise to different cell lineages. The eliminated genes are primarily expressed in the germline, suggesting that DNA elimination in Ascaris is an irreversible mechanism for silencing a subset of germline-expressed genes in somatic tissues. We identified ~40 sites where chromosome breaks occur and are healed by telomere addition. We sequenced the genomes of a related horse parasitic nematode Parascaris, that also undergoes DNA elimination, to determine how conserved DNA elimination is in nematodes. The DNA breaks, eliminated genes, and the expression pattern of the eliminated genes are largely conserved between Ascaris and Parascaris indicating that DNA elimination is a specific, conserved and highly regulated process. We further show that Ascaris has holocentric chromosomes in the germline. Prior to DNA elimination in the four-cell embryo, CENP-A, the epigenetic mark of centromeres, is significantly diminished in chromosome regions that will be lost. This leads to the absence of kinetochores and microtubule attachment sites necessary for chromosome segregation,
resulting in loss of these chromosome regions during mitosis. These data suggest that CENP-A localization contributes to the identification of regions to be retained and lost playing a regulatory and mechanistic role in DNA elimination. Finally, we identified two worm specific Argonautes (WAGO) associated with condensed chromosomes during DNA elimination. One WAGO preferentially associates with retained DNA only during a DNA elimination mitosis. The other WAGO is enriched on DNA that will be eliminated. Thus, these WAGOs, their associated small RNAs and/or proteins may play a role in nematode DNA elimination.

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62 Long-distance cooperation and hierarchy between recruitment sites specify the X chromosome for dosage compensation. Sarah Albritton, Anna-Lena Kranz, Lara Winterkorn, Lena Street, Sevinc Ercan Department of Biology and Center for Genomics and Systems Biology, New York University, New York, NY.

Dosage compensation mechanisms specifically target gene regulatory complexes to the X chromosomes for transcriptional regulation. However, it remains unclear how X is specified, as the DNA sequence motifs shown to be important for binding of dosage compensation machineries are themselves not X-specific. Here, we analyzed binding of the C. elegans dosage compensation complex (DCC) through a series of experiments that include deletion and ectopic insertion of recruitment site sequences. Our data suggest that DCC recruitment is initiated by a small number of primary recruitment sites characterized by clusters of the 12-bp recruitment motif and overlap with high occupancy transcription factor target (HOT) regions, two features that fully explain X-specificity. SDC-2, the protein essential for hermaphrodite-specific recruitment of the DCC during early embryogenesis, is required to maintain open chromatin specifically at the primary recruitment sites whose DNA sequence encodes for high intrinsic nucleosome occupancy. Along the X, the primary recruitment sites are interspersed by secondary, weaker DCC recruitment sites. While insertion of a secondary recruitment element on an autosome failed to fully recruit the DCC, the same element was capable of recruitment at an ectopic locus on the X, suggesting that the function of the weaker recruitment sites are X-dependent. On the autosome, insertion of multiple recruitment elements in tandem or at a distance (>30 kb) increased DCC recruitment, demonstrating that recruitment sites cooperate over long distances. On the X, deletion of single recruitment sites resulted in reduced DCC binding across several megabases flanked by topologically associating domain (TAD) boundaries, suggesting that DCC recruitment and spreading occurs within defined X chromosomal domains. Our work illustrates a fundamental strategy for specifically targeting large chromosomal domains for co-regulation, which involves hierarchy and long-distance cooperativity between functional genomic elements.

63 Expression profiling of synchronized cell-type populations during embryonic development. Adam Warner, Louis Gevirtzman, Robert Waterston Genome Sciences, University of Washington, Seattle, WA.

As a C. elegans embryo develops from one cell to 558 cells, the transcriptional profile of each cell undergoes unique and dramatic changes to drive differentiation into distinct cell-types and tissues. While gene expression has been measured in isolated cell-types (Meissner et al, 2009; Spencer et al, 2011; Burdick et al, 2016), the data was primarily generated from single point time samples. Conversely, a batch whole embryo time series (Boeck et al, 2016) provides excellent temporal data from early embryo to late, but is a blend of the gene expression from all cell types present in the embryo, thus lacking cell-type specificity. We have created a more comprehensive dataset with gene expression levels for all genes, not only in distinct isolated cell-types from synchronized populations of those cells, but at discrete time points during embryonic development using high depth RNA-seq analysis. We used FACs to isolate fluorescent and non fluorescent cells from synchronized embryos containing early and highly cell-type specific tissue/lineage markers – determined by cell lineageing using 4-D microscopy as part of the Expression Patterns in Caenorhabditis (EPiC) project (http://epic.gs.washington.edu/Epic2) (Murray et al, 2012). To cover major lineages and cell types, we have created RNA-seq libraries with highly correlated replicates for the ABa sublineage (tbx-37), muscle (hlh-1), intestine (end-1), pharynx (pha-4, excluding end-1), hypoderm (nhr-25), neurons (cnd-1), and head neurons (ceh-32). Each 5 point time series begins at the earliest expression of the marker gene with time points every 90 min. We observe dramatic differences in gene expression both between cell-types, and over time within the same cell population including differential splice isoform usage. We have determined which genes have broad expression, which have biased expression in only one or a few cell-types, and the order of transcription factor (TF) activation in each cell-type. We then used this approach to assay expression changes after TF loss by focusing on the muscle specific and essential TF hlh-1. We crossed a phlh-1::mCherry marker into both WT and mutant hlh-1(ts) strains, synchronized populations, and shifted to non-permissive temperature at the YA stage. Using FACs, we isolated a time series of only hlh-1 expressing embryonic cells (~10% of all cells), eliminating noise from non-muscle cells (~90% of all cells) unaffected by loss of hlh-1. In the hlh-1 mutant hundreds of genes have significantly reduced expression, most of which normally have muscle enriched expression in WT. When the most affected genes are compared with HLH-1 targets identified by ChIP-seq (Araya et al, 2014), a majority have ChIP-seq peaks indicating direct regulatory relationships. We continue to assay additional genes in this manner in an effort to gain insight into the transcriptional regulatory relationships in the major cell lineages.

64 Targeted, high-throughput interrogation of regulatory elements in C. elegans. Jonathan Froehlich, Margareta Herzog, Nikolaus Rajewsky Systems Biology of Gene Regulatory Elements, Berlin Institute for Medical Systems Biology, Max Delbrueck Center for Molecular Medicine, Berlin, DE.

Gene regulatory elements determine when, where and how much a protein is expressed. C. elegans allows tracking of cell-, tissue- or developmental stage specific expression in vivo. Furthermore it is possible to evaluate viability or other phenotypes and to observe several generations in a short time. Regulatory regions controlling gene expression have been studied in C. elegans by cloning different regions and injection of each construct ("bashing"). Few elements have been isolated in forward
Here we present our approach for unbiased interrogation of regulatory regions for a given locus, with CRISPR/Cas9 based systematic mutation. Using strains with inducible Cas9, staged embryos, and pools of sgRNAs, we are able to obtain thousands of independent mutant F1s in parallel, requiring very few injections. We have set up a targeted sequencing approach using large 3-6 kb amplicons to analyze indels with deep sequencing. As a proof of concept we applied this method to the *his-72* locus. We focused on the 3' untranslated region, also targeting promoter-, coding-, and intron sequences at different developmental stages. In addition, we cover the *lin-41* 3'UTR with different sets of sgRNAs and analyze the depletion of genotypes over several generations. We will report about these ongoing experiments.

65  **Meta analysis of RNAseq datasets provides quantitative measurement of alternative exons usage in C. elegans.** Denis Dupuy, Jonathan Millet, Nicolas Tourasse  Laboratoire ARNA, Inserm U1212, CNRS UMR5320, Institut Européen de Chimie et Biologie (IECB), 2, rue Robert Escarpit, 33607 Pessac, France.

Almost twenty years after the completion of the *C. elegans* genome sequence, gene structure annotation is still an ongoing process with new evidence for gene variant still being regularly uncovered by more in-depth transcriptome studies. Alternative splice forms, allow a single gene to encode several protein variants, called isoforms, with altered stability, localization, specificity or activity. Here we generated a compendium of ~1,700 *C. elegans* RNAseq datasets to expand the dynamic range of detection of RNA isoforms and obtain robust measurement of the relative abundance of each splicing event. We detected ~700,000 exon-exon junctions and over 5,000,000 transsplicing sites, but most of these sites are only supported by a very low number of reads: 98% of splicing reads come from ~60,000 high confidence-splice junctions and 88% of transspliced reads come from ~36,000 robust transsplice sites. Our analysis indicates that the mechanism of transsplicing generates more specific species than cis-splicing, but it also highlights that putative erroneous splicing only represent a minor proportion of detectable spliced RNA species.

We found that rarely used splice sites within coding genes are significantly less conserved in other nematode genomes than splice sites with a higher usage frequency. We generated updated gene models including previously unreported transcription start and splice sites and including quantitative exon usage information for the entire *C. elegans* genome to allow users to visualize at a glance the relative expression of each isoform of their gene of interest.

66  **Cell type-specific transcriptome profiling using the Translating Ribosome Affinity Purification technique.** Xicotencatl Gracida1,2, Yun Zhang2,3, John A. Calarco1,4 1) FAS Center for Systems Biology; 2) Department of Organismal and Evolutionary Biology; 3) Center for Brain Science, Harvard University, Cambridge, MA, 02138; 4) Department of Cell and Systems Biology, University of Toronto, Toronto, Canada, MSS 3G5.

Tissues and defined cell types execute specialized functions in multicellular organisms, largely through tailored gene expression programs. Thus, profiling the transcriptomes of specific cell and tissue types remains an important tool for understanding how cells become specialized. Classical methods to detect gene expression differences have utilized samples from whole-animals, dissected tissues, or sorted cells. Despite these advances, there is still a challenge and a need in most laboratories to implement less invasive yet powerful cell-type specific transcriptome profiling methods. We adapted the Translating-Ribosome Affinity Purification (TRAP) method for *C. elegans* to detect cell type-specific gene expression signatures. In TRAP, the ribosomal protein RPL-1 is fused to GFP and is expressed under cell-type specific promoters to mark genetically defined cell types in vivo. Affinity purification of GFP in lysates of animals expressing the tag enriches for ribosome-associated mRNAs of the targeted tissue. The purified mRNA is then used for making cDNA libraries subjected to high-throughput sequencing to obtain genome-wide snapshots of the translating mRNAs in a given cell type.

We obtained translational profiles from L4 larvae across three major cell types: neurons, intestinal cells, and body wall muscle cells, and validated the reproducibility and specificity of our procedure. Furthermore, we adapted TRAP and obtained translational profiles of a smaller subset of cells corresponding to two major neuromodulatory cell types, the dopaminergic and serotoninergic neurons. We have identified hundreds of tissue-enriched mRNAs in these tissues and cell types, and found a strong agreement between our TRAP data and previously validated patterns. Additionally, we have found genes that were not known to be expressed these tissues or cell-types. We validated and functionally characterized the case of a subunit of an E3 ubiquitin ligase complex, the Elongin C ortholog *elc-2* that we found specifically expressed in a subset of serotoninergic sensory neurons.

In addition to detection of relative transcript levels across samples, with TRAP we have also obtained global patterns of alternative mRNA splicing in a tissue and cell-type specific manner. Taken together, the ease of exposing *C. elegans* to diverse stimuli, coupled with available cell type specific promoters, makes TRAP a useful approach to be adapted in other laboratories to enable the discovery of molecular components in response to external or genetic perturbations.

67  **MIP-MAP: Targeted sequencing for high-throughput mapping of C. elegans mutant TS alleles.** C.A Mok1, O.A Thompson1, V Au2, M Edgley2, L Gevirtzman1, J Yochem1, J Lowry2, M Wallenfang3, D Rasoloson4, B Bowerman5, R Schnabel5, G Seydoux5, D.G. Mooreman4, R.H. Waterston1 1) Genome Sciences, University of Washington, Seattle, WA, USA; 2) Department of Zoology, University of British Columbia, Vancouver, BC, Canada; 3) Institute of Molecular Biology, University of
Temperature sensitive (TS) alleles are valuable tools in elucidating the function of essential genes, potentially allowing the control of gene function in a tissue- and temporal-specific manner. To this end, we have produced a collection of mutagenized, fully sequenced TS strains complementary to the Million Mutation Project (MMP). Our collection is composed of 173 TS strains from three separate screens representing a range of embryonic lethal and maternal sterile phenotypes. In toto, this collection contains 56,803 single nucleotide variants (SNVs) with missense mutations in 7,972 unique genes. Per strain there are an average of 71 relevant coding mutations or <1 coding change per Mb of genome. We developed a targeted sequencing approach using a modified version of molecular inversion probes (MIPs, Hiatt et al., 2013) to map these conditional alleles with a technique we call MIP-MAP. We identified a polymorphic strain from the MMP with minimal deleterious mutations and no obvious phenotypes, and designed 89 probes spaced at <2 Mb intervals across the genome that target SNVs specific to this mapping strain. We tested the efficacy of this method with the bulk-segregant mapping of a sma-9(tm572) mutation and by developing a competitive fitness assay to map a TS embryonic-lethal allele of hlh-1(cc561). We then sampled sixteen strains from our TS collection and successfully used MIP-MAP to identify a TS-associated genomic interval in each of them. Our results identified candidate TS alleles for a novel sperm-specific gene, the mitochondria-specific gene cts-1, and TS alleles for an additional 5 essential genes for which no previous TS alleles have been reported. Our method was also able to identify two independent TS loci within the same TS strain. Rather than resequencing DNA from multiple F2 genomes to a high depth, our targeted method achieves similar resolution with far fewer reads. The MIP-MAP protocol is capable of multiplexing at least 96 samples using no more than 20M reads total, while still sequencing target loci to an average depth of more than 1000X. The ability to multiplex MIP libraries facilitates the mapping of large collections in a high-throughput manner. This approach can be used to identify alleles from other mutagenesis experiments by using a combination of MIP-MAP and WGS. Furthermore, investigations into phenotypes of the MMP or similar collections can be quickly mapped to relatively small intervals containing, on average, only a few coding SNVs. We will share the MIP-MAP technique and MIP oligos with members of the C. elegans community to augment their own mapping efforts.
not enter the ring until later, we estimate that about half of the total 140 embryonic nerve ring neurons enter the ring by the onset of muscle movement, and that the 6 distinguishable groups account for half of these.

70 Making a brain: glia and pioneer neurons initiate *C. elegans* nerve-ring assembly through non-canonical Chimaerin/Furin-dependent axon guidance.  
*Georgia Rapti*, Shai Shaham  The Rockefeller University, New York, NY.

Brain assembly is thought to begin when pioneer axons extend over non-neuronal cells to form tracts guiding follower axons. Yet, identities of early pioneer cells, their guidance substrates, and their interactions remain uncharacterized. Glia direct axon navigation, yet molecular mechanisms governing this are also not well understood. To determine if and how neurons and glia cooperate during brain formation, we studied assembly of the *C. elegans* nerve ring (NR), a brain-like neuropil of ~180 axons enveloped by astrocyte-like CEPsh glia. How the NR is assembled has been a long-standing mystery, and although hypotheses have been put forth, they have not been experimentally tested.

Using time-lapse embryonic imaging, electron microscopy, genetics, cell ablations, and functional studies, we uncovered the early events of NR assembly. Assembly proceeds in an orderly manner, with CEPsh glia initiating neurit formation. We identify pioneer neurons, with unique growth and anatomical properties. These neurons are guided by CEPsh glia and cooperate with glia to guide diverse follower axons into the NR. Importantly, CEPsh glia guide pioneer and follower axons using distinct molecular pathways we identify.

Through genetic screens and candidate gene approaches, we identified a repertoire of factors directing NR assembly. We isolated a novel mutant that severely and specifically disrupts NR axon guidance, while sparing neuron outgrowth initiation and navigation elsewhere in the animal. Two conserved factors, a GTPase regulator and a pro-hormone convertase, both defective in this mutant, function non-cell-autonomously in glia and pioneer-neurons to regulate guidance-cue trafficking. The GTPase regulator functions through CDC-42. The pro-hormone convertase likely acts by cleaving guidance cues at conserved furin motifs. While double mutants exhibit 70% NR axon entry defects, each lesion alone results in only mild NR defects (5-15%). We used this synergy to study redundancies in NR formation, a problem plaguing genetic analyses of the process. We identified conserved guidance cues acting from glia and pioneer neurons for pioneer and follower axon guidance. Moreover, we isolated 20 redundant axon-guidance mutants, several of which appear to disrupt previously unknown genes.

Our studies suggest a pivotal role for glia in brain assembly initiation and open the door to uncovering new axon guidance genes. Embryonic CEPsh glia are reminiscent of vertebrate radial glia, whose molecular biology is largely uncharacterized. Furthermore, the genes we identified have mammalian homologs with known axon guidance roles, yet their glial activities remain unappreciated. Our studies, therefore, likely reveal conserved mechanisms promoting brain assembly.

71 Investigating the structure and sexual dimorphisms of the *C. elegans* connectome.  
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We present the first whole-animal maps of synaptic connectivity, including anatomical connection strength, of both adult sexes of a species. Our results are based on analyses of legacy and new serial section electron micrographs (EMs) of the *C. elegans* nervous system and the tissues it innervates. In a graph representation of connectivity, the pathways of information flow can be arranged hierarchically, revealing a largely feed forward structure of shallow (1-5 synapses) depth. Our reconstruction has revealed that muscles and other end-organs are more extensively cross-connected than previously reported. Sensory information converges and diverges widely throughout the fully-connected neural network. The sexes differ not only by the addition of sex-specific neurons and muscles, but also at numerous points in the connectivity of shared neurons. Differences between the hermaphrodite and male reconstructions could be either inter-individual differences or differences due to genetic sex. To distinguish between these possibilities, we examined a subset of 7 synaptic connections that were respectively stronger in the male reconstruction, 4 that were stronger in the hermaphrodite reconstruction, and 4 that were similar, using in vivo trans-synaptic labeling. In each instance, the differences seen in the reconstructions was confirmed in multiple animals. Extrapolating these results to the number of statistically significant differences in the reconstructions, we conclude that there is an unexpectedly large number of sexually dimorphic connections. These connections were mainly located in the nerve ring, and embedded within the connectome at least one synapse away from any sex-specific neuron. Our results showed that AVA receives sex-specific input from ADL, ASH, and AVF in the hermaphrodite, while RIB receives sex-specific input from IL1, IL2, and RIA in the male. AIC, which has been reported to change its neurotransmitter from glutamate in the hermaphrodite to acetylcholine in the male, makes a strong male-specific connection to AIB. These hubs of sex-specific connectivity also maintained the majority of their sex-shared output. Our results suggest that the genetic sex of the nervous system allows for diverse synaptic patterns in a relatively small nervous system.

72 The *C. elegans* connectome consist of invariant, stochastic, and developmentally regulated synapses.  
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When animals are born, neuronal networks are already in place to integrate sensory cues and effect appropriate behavioral or homeostatic responses. During the course of postnatal development, synapses and circuits undergo refinement and remodeling, updating or adapting sensorimotor behaviors. Rules for developmental remodeling are poorly characterized, because a circuit-level analyses at multiple time points, in multiple animals are missing.

We use an isogenic C. elegans N2 population to examine how the neural circuit changes during development. At birth, C. elegans has 218 neurons (excluding CAN); 82 new neurons are incorporated into the nervous system before the end of larval development. These include sensory, motor, and interneurons that are located throughout the body, suggesting a system-wide modification of the juvenile circuit during post-embryonic development. Using serial-section electron microscopy, we mapped the synaptic connectivity in the head and tail ganglia for five animals, from hatching to late larval development. These datasets allow us to separate the connectome into core connections, synapses that are present throughout the lifetime, transient connections, synapses that are present only during early or late development, and variable connections, synapses that are not conserved between animals. We propose that the core connections may drive hard-wired behaviors, and developmentally regulated connections exert stage-specific roles, while variable connections may confer individual variability and/or be stochastic in nature.

Our current data analyses lead to two notions: First, developmentally regulated connections are enriched for synapses between sensory neurons, and with neuromodulatory neurons, while connections between interneurons are remarkably stable. This implies that for C. elegans, core circuits for decision-making are already established at birth, but their modulation and multisensory integration are refined or shaped during development. Second, variable connections, comprising about half of the connection edges in the published adult dataset, are prominent. Stochastic or experience dependent variability in connections may contribute to variability of behaviors set by hard-wired core connections.

73 Inhibition of axon regeneration: unexpected roles of piRNA and NAD⁺ pathways. K. Kim1, A. Chisholm1, Y. Jin1,2 1) Division of Biological Sciences, University of California, San Diego, La Jolla, CA; 2) Howard Hughes Medical Institute.

Axon regeneration after nerve injury is a conserved biological process in many animals. However, the molecular mechanisms underlying the ability of axons to regrow after injury remain poorly understood. By systematic genetic screening of >1200 genes following laser axotomy we have identified novel axon regeneration pathways (Chen et al., 2011 and this work). Here we report two pathways regulating PLM sensory axon regrowth. One involves the piwi-interacting RNA (piRNA) pathway. piRNA is the largest class of small non-coding RNAs and plays an essential role in maintaining genomic integrity. We found that loss of function in a cohort of genes involved in the generation and regulation of piRNAs resulted in enhanced PLM axon regrowth. We show that two essential regulators for piRNA expression, PRDE-1 and PRG-1, act independent of their roles in germline. While this finding is surprising because both proteins are reported to be almost exclusively expressed in the C. elegans germline, it is consistent with emerging reports of piRNA’s somatic effects in other organisms such as memory formation in Aplysia neurons and stem cell maintenance in planarian neoblasts. The second inhibitory pathway involves the nicotinamide mononucleotide adenyllytransferase (Nmnat) enzyme, which catalyzes a vital step in nicotinamide adenine dinucleotide (NAD⁺) salvage biosynthetic pathway and confers neuroprotection in many organisms including C. elegans, flies, and mice. We found that Nmnat/NMAT inhibits PLM axon regeneration dependent on its enzymatic activity for NAD⁺ production. Taken together, our genetic screening uncovers unexpected roles of piRNA and NAD⁺ pathways in neurons and provides new insights into the molecular network of the intrinsic regulators for axon regeneration.


74 Phosphatidylserine ‘save-me’ signals drive functional recovery of severed axons. Z.C. Abay1, M.A. Hilliard2, B. Neumann3 1) Monash Biomedicine Discovery Institute, Monash University, Melbourne VIC, Australia; 2) Queensland Brain Institute, The University of Queensland, Brisbane QLD, Australia.

Functional regeneration after axonal injury requires transected axons to regrow and re-establish connection with their original target tissue. The spontaneous regenerative mechanism known as axonal fusion provides a highly efficient means of achieving targeted reconnection, as a regrowing axon is able to recognize and fuse with its own detached axon segment, thereby rapidly re-establishing the original axonal tract. Here we use behavioral assays and fluorescent reporters to demonstrate that axonal fusion enables full recovery of function following axotomy of Caenorhabditis elegans mechanosensory neurons. Furthermore, we reveal that the phospholipid phosphatidylserine, which becomes exposed on the damaged axon to function as a ‘save-me’ signal, defines the rate of axonal fusion. We also show that successful axonal fusion correlates with the regrowth potential and branching of the proximal fragment, and with the retraction length and degeneration of the separated segment. Finally, we identify discrete axonal domains that vary in their propensity to regrow through fusion, and demonstrate that the rate of axonal fusion can be genetically modulated. Taken together, our results reveal that axonal fusion restores full function to injured neurons, is dependent on exposure of phospholipid signals, and is achieved through the balance between regenerative potential and rate of degeneration.

75 GSA Edward Novitski Prize. J. Hodgkin  University of Oxford.

no abstract submitted

76 Multi-neuronal imaging of C. elegans males during courtship and mating. V. Susoy1, V. Venkatachalam1, M. Wu2, W. Hung2, M. Zhen2, A. Samuel1 1) Harvard, Physics, Cambridge, MA; 2) University of Toronto, Mount Sinai Hospital,
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The nematode Caenorhabditis elegans is an ideal model for systems neuroscience because it offers the possibility of in vivo recording of the activity of the entire brain at a single-neuron resolution in unrestrained animals. With the help of customized imaging setups built in our laboratory, and using newly optimized C. elegans strains that express both a nuclear-localized sensitive calcium indicator GCaMP6 for visualization of neuronal activity and red fluorescent protein for neuronal tracking and segmentation, we have been able to make extended recordings of the multi-neuronal activity and motor behavior in individual freely moving C. elegans across a range of ecologically-relevant conditions. In particular, we have been focusing on the neuronal bases of male mating behavior, probably the most complex behavior in C. elegans. During mating, C. elegans male needs to constantly modify his motor output in response to multiple sensory stimuli to succeed in achieving his goal. We have been able to make recordings of activity of all neurons in the male tail during the entire sequence of the courtship and mating behaviors, which included navigation towards the hermaphrodite, response to contact, turning, vulva location, spicule insertion, sperm release, and refractory period. Using these recordings of unprecedented detail, completeness, and length, we can now follow the progression of neuronal responses from known sensory inputs to motor outputs and identify relationships between different parts of neuronal networks that result in behavioral transitions. In addition to providing insights into orchestration of this complex behavior, our “top-down” approach helps to understand better the behavioral repertoire and biology of this model organism, specifically, recognition of conspecifics and chemical communication.


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Animals must be able to halt locomotion, e.g. to follow for certain events or to abruptly change directionality. In vertebrates, descending signals from the brain stem can induce a stop of locomotion, but allow the animal to maintain muscle tone. We suggest that the peptidergic/GABAergic interneuron RIS, well-known for its role in developmentally timed sleep (DTS) [1], attains the role of a stop neuron in adult behavior. In animals expressing Channelrhodopsin-2 (ChR2) solely in the RIS neuron, photo-depolarization led to acute and persistent locomotion and pharyngeal pumping inhibition with no effect on the defecation cycle. We showed that the photo-evoked phenotype required neuropeptides, but not GABA. Interestingly, in contrast to photodepolarization of all GABAergic neurons, evoking general muscle relaxation, RIS::ChR2 photoactivation allowed the animal to maintain its bodily posture. This was confirmed by Ca²⁺ imaging as well as recording of body wall muscle currents. However, RIS::ChR2 photoactivation reduced muscle tone in the head region, causing slight anterior body elongation.

To identify peptides required for RIS’ effects, we analyzed its L4 transcriptome. We characterized several enriched neuropeptides and found that FLP-11, which is also implicated in DTS regulation, is required for the optogenetically induced stop phenotype. Since this optogenetically evoked phenotype may be non-physiological, we assessed RIS’ activity during spontaneous locomotion. Combining Ca²⁺ imaging and animal tracking [2] with custom-written software, we analyzed Ca²⁺ dynamics specifically in the RIS process, despite deformations caused by locomotion. Ca²⁺ signals in the RIS neural process preceded the cell body, indicating that they were due to synaptic activity. Temporal analysis of Ca²⁺ dynamics and locomotion speed suggested RIS as being instructive for locomotion stop before initiation of reverse locomotion. We are currently dissecting the role of individual RIS-expressed neuropeptides in the sequence of events associated with reversals.

In sum, RIS, exploiting the molecular mechanisms present in DTS, acquires or maintains a role in adulthood to coordinate movement inhibition and reversal initiation. Hence, RIS spontaneous activity and its signaling physiology suggest a role in inhibiting a central unit of locomotion initiation and/or maintenance, without directly controlling ventral cord motor neurons.

Refs:
1 Turek et al. Curr Biol 2013
2 Faumont et al. PLoS ONE 2011

78 Integration of plasticity mechanisms within a single sensory neuron of C. elegans actuates a memory. J.D. Hawk, A.C. Calvo, A. Almoril-Porras, A. Aljobeh, M.L. Torruella-Suárez, I. Ren, N. Cook, J. Greenwood, L. Luo, A.D.T. Samuel, D.A. Colón-Ramos

1) Program in Cellular Neuroscience, Neurodegeneration, and Repair, Department of Cell Biology, Yale University, New Haven, CT; 2) Department of Physics and Center for Brain Science, Harvard University, Cambridge, MA; 3) Key Laboratory of Modern Acoustics, Ministry of Education, Department of Physics, Nanjing University, Nanjing; 4) Instituto de Neurobiología, Recinto de Ciencias Médicas, Universidad de Puerto Rico, 201 Blvd del Valle, San Juan, Puerto Rico.

Neural plasticity—the ability of a neuron to change its cellular properties in response to past experiences—underpins the nervous system’s capacity to form memories and actuate behaviors. How different plasticity mechanisms act together in vivo and at a cellular level to transform sensory information into behavior is not well understood. Here we show that in the nematode C. elegans two plasticity mechanisms—sensory adaptation and presynaptic plasticity—act within a single cell to encode thermosensory information and actuate a temperature-preference memory. Sensory adaptation enables the primary thermosensory neuron, AFD, to adjust the temperature range of its sensitivity to the local environment, thereby optimizing its ability to detect temperature fluctuations associated with migration. Presynaptic plasticity transforms this thermosensory information into a behavioral preference by gating synaptic communication between sensory neuron AFD and its postsynaptic
Bypassing or altering AFD presynaptic plasticity predictably changes the learned behavioral preferences without affecting sensory responses. Our findings indicate that two distinct and modular neuroplasticity mechanisms function together within a single sensory neuron to encode multiple components of information required to enact thermotactic behavior. The integration of these plasticity mechanisms result in a single-cell logic system that can both represent sensory stimuli and guide memory-based behavioral preference.

79  **Ordered arrangement of dendrites within the C. elegans amphid bundle.**  Z.C. Yeo$^{1,2,3}$, M.G. Heiman$^{1,2}$  1) Division of Genetics and Genomics, Boston Children’s Hospital, Boston, MA; 2) Department of Genetics, Harvard Medical School, Boston, MA; 3) Program in Neuroscience, Harvard Medical School, Boston, MA.

How cells are arranged determines their function – the location of each cell dictates its interacting partners and local environment. Yet, specifying the order of cells within an organ is a non-trivial problem because the number of available genes is dwarfed by the number of cells. What are the rules behind specifying cell arrangements? To study this question, we turned to the C. elegans amphid sense organ, which consists of 12 amphid neurons that each extend a single dendrite into a well-organized dendrite bundle. Classical EM studies from the 1970s suggested that each dendrite occupies a stereotyped position within the bundle, a remarkable level of organization. These findings had not been pursued because the observations required painstaking EM. We revisited these observations, and developed a fluorescent imaging-based approach combined with computational methods to visualize single dendrites within the bundle and to quantify their order relative to each other. We showed that dendrite order within the bundle is indeed highly stereotyped: dendrites change position along the length of the bundle, but these patterns are reproducible. Moreover, time-point analysis showed that amphid dendrites remain ordered even as animals move and grow. We considered the hypothesis that dendrite order might be determined by cell adhesion molecules (CAMs) expressed in different but partially overlapping sets of amphid neurons. To test this hypothesis, we conducted a candidate screen for CAMs required for dendrite order. We identified two CAMs, SAX-7/L1CAM and PTP-3/LAR, that seem to act in partially overlapping subsets of amphid neurons to specify dendrite order within the bundle. Interestingly, sax-7 mutants not only show an altered order of amphid dendrites, but this order deteriorates with time, suggesting sax-7 plays separate roles in development and maintenance of amphid dendrite order. To determine whether SAX-7 acts permissively or instructively to specify dendrite order, we misexpressed SAX-7 in all amphid neurons in a wild-type background and found that amphid dendrites took on a new order, suggesting that SAX-7 plays an instructive role in establishing order. Altogether, our results suggest that differential expression of CAMs can organize dendrites in a way that is robust and replicable, without using a large number of molecules.

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Friday, June 23  8:30 AM – 11:30 AM  
Northwest Auditorium  
**Evolution and Ecology**  

Co-chairs:  **Erik Andersen**, Northwestern University, and  **en-Ping Hsueh**, Academia Sinica

80  **Evidence of small RNA involvement in nematode benzimidazole resistance.**  M. Zamanian$^1$, Daniel Cook$^2$, Daehan Lee$^3$, Shannon Brady$^2$, Stefan Zdraljic$^2$, Robyn Tanny$^2$, Briana Rodriguez$^2$, Junho Lee$^3$, Erik Andersen$^2$  1) Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI; 2) Department of Molecular Biosciences, Northwestern University, Evanston, IL; 3) Seoul National University, Seoul, South Korea.

Parasitic nematodes impose a debilitating health and economic burden across much of the world. The morbidity and mortality inflicted by these pathogens are partly curtailed by mass drug administration programs that depend on the continued efficacy of a limited portfolio of anthelmintic drugs. Benzimidazoles are WHO-designated "Essential Medicines" and an indispensable component of this limited arsenal. Nematode resistance to benzimidazole chemotherapy threatens parasite control efforts in both human and veterinary medicine. Despite this threat, the genetic landscape of potential resistance mechanisms to this critical drug class remains largely unexplored. There is an urgent and recognized need to identify molecular mechanisms and genetic markers that cause benzimidazole resistance. In order to identify conserved nematode drug responses, we explored natural variation in two model roundworms, *Caenorhabditis elegans* and *Caenorhabditis briggsae*, to discover quantitative trait loci (QTL) that control benzimidazole sensitivity. Surprisingly, we found that resistance to benzimidazoles mapped to syntenic piRNA-enriched regions of the genome with few protein-coding genes in both *Caenorhabditis* species. We used near isogenic lines (NILs) to narrow the major-effect benzimidazole QTL in *C. elegans* to a smaller region of the genome and demonstrate that the benzimidazole-resistance phenotype results from piRNA variation that is dependent on the function of the piRNA-associated argonaute *prg-1*. We identified candidate piRNAs causal to the resistance phenotype and putative genes targeted for silencing by downstream 22G RNAs. Our results indicate that small RNAs require consideration in drug resistance mechanisms in nematodes, because the piRNA pathway and related small RNA pathways are conserved in many medically and agriculturally important parasitic nematodes. Importantly, this resistance mechanism could be mediated through the heritable trans-generational effects of small RNAs. This finding has significant implications for parasite control and the management of drug resistance in other phyla and systems.
81 Some C. elegans wild isolates display a heat-triggered mortal germ line phenotype. Lise Frézal1, Emilie Demoinet2, Gaotian Zhang1, Christian Braendle6, Eric Miska3, Marie-Anne Félix1 1) Institut de Biologie de l’ENS (IBENS), Paris, FR; 2) Institut Biologie Valrose, Nice, FR; 3) Gurdon Institute, Cambridge, UK.

The mortal germ line phenotype was defined by Ahmed and Hodgkin as a multigenerational phenotype, whereby a selfing C. elegans line becomes sterile after several generations (1). The N2 reference strain does not display this phenotype, but Ahmed and Hodgkin could isolate mutants that are mortal. Some of these mutations are temperature-sensitive and affect small RNA and chromatin modification pathways, which may explain the multigenerational nature of the phenotype (e.g. 2,3). We discovered serendipitously that many C. elegans wild isolates display a strong mortal Germ Line (Mrt) phenotype: chronic exposure to high temperature, e.g. 25°C, progressively leads to sterility within several generations. Here we discuss this surprising finding.

We first assayed a reference panel of 97 C. elegans wild isolates (4) for their Mrt phenotype, scoring the number of generations at 25°C after which full sterility occurred. Wild isolates showed strong, reproducible differences. We found no association between the severity of the Mrt phenotype and the climatic origin of C. elegans isolates. In the generations before full sterility, we observed a progressive brood size decrease, germ line differentiation defects, chromosomal aberrations at diakinesis and DNA damage. The mortal germline phenotype was however fully reversed by switching back from 25°C to 15°C before the last generation. Most surprisingly, germline immortality of some isolates was rescued by artificial infection with Nematocida microsporidia, natural intracellular C. elegans pathogens infecting intestinal cells, which suggests signaling from soma to germline. We suggest that the Mrt phenotype observed under laboratory conditions is likely not commonly displayed under natural conditions, yet provides an exciting model to test whether and how epigenetic inheritance systems are modulated by natural genetic variation.

In order to pinpoint the molecular inheritance system underlying this mortal phenotype, we focused on determining the genetic basis of the quantitative variation among the C. elegans wild isolates, using both association mapping and laboratory crosses. The two approaches yielded three candidate regions, which we confirmed through introgression experiments. Thus, although the multigenerational nature of the Mrt phenotype may stem from inheritance of small RNAs and chromatin marks that are temperature-sensitive, phenotypic variation in the Mrt phenotype among C. elegans wild isolates is due to genetic variation.


82 CRISPR/Cas9-based dissection of a genetically complex, pleiotropic chromatin-remodeling gene under selection in laboratory growth conditions. W. Xu, P. McGrath Georgia Tech, Atlanta, GA.

There is general interest in understanding the genetic basis of adaptation to new environmental conditions. In the process of studying adaptation of LSJ2, a sister strain of N2 that grew in liquid axenic media for over 50 years, we identified a 60bp deletion in nurf-1, which encodes a subunit of the NURF nucleosome remodeling factor complex. This mutation seems to change life history strategy of the worms towards K-mode type growth: animals live longer and grow slower but at the cost of later reproductive timing and reduced reproductive rate. Interestingly, the nurf-1 gene seems to be abnormally complex – it is predicted to encode 16 different isoforms, suggesting that nurf-1 was targeted by evolution due to its genetic complexity. These predicted isoforms include a full-length version containing a DDT domain necessary for interaction with ISWI ATPase, domains necessary for interactions with transcription factors, and two PHD finger domains and a bromodomain that recognize histone marks H3K4me3 and H4K16Ac. However, other predicted isoforms contain subsets of these domains. In order to study and validate the nurf-1 isoforms, we first validated 6 independent transcriptional start sites using promoter::GFP fusions. We then introduced stop codons in 8 exons using CRISPR/Cas9 genome editing to knock out isoforms of interest. For isoforms with unique exons, we used these mutations to evaluate their role in growth and reproduction. For isoforms without unique exons, we used compound heterozygotes to create strains lacking specific isoforms. Surprisingly, the long form of nurf-1 (the a isoform) that contains all functional domains and is the primary isoform in vertebrates seems to have no effect on our measured biological traits. But three short isoforms – b, d and o forms – had independent effects on growth and egg laying. This is unexpected because d and o isoforms lack the DDT domain necessary for interaction with ISWI, which is conventionally thought to be an essential nurf-1 partner. This suggests additional potential functions for nurf-1 besides the regulation of histone occupancy through interaction with ISWI. Using nurf-1.d cDNA extrachromosomal array rescue we demonstrated that we could independently rescue the growth phenotype, indicating these traits are independently regulated by different cells. In conclusion, we demonstrated nurf-1 functions in isoform and cell-specific manner and propose that it might be involved in cellular processes other than through its conventional role in the NURF complex. Our work also demonstrates how CRISPR/Cas9 can be used to dissect a complex gene.

83 Distinct genetic mechanisms underlie natural variation in left-right arrangement of the major organs in males and hermaphrodites of C. elegans. Agustin Lopez Santos1,2, Melissa Alcorn2, Davon Callander2, Joel Rothman2 1) School of Biological Sciences, University of Auckland, Auckland, NZ; 2) Molecular, Cellular and Developmental Biology, UC Santa Barbara, Santa Barbara, CA, USA.

Establishing the proper arrangement of internal organs is critically important during development, and defects in this process can lead to serious and even lethal birth defects. While the left-right (L/R) anatomical handedness in C. elegans was originally found to be essentially invariant in the laboratory N2 strain, we have observed that the L/R arrangement of the gonad and gut is frequently reversed under some environmental conditions in N2 and in many of the ~100 wild isolates examined. In some
strains, up to ~11% of males show complete reversal in L/R gut/gonad arrangement, while in others, reversals were never seen. GWAS analysis revealed at least two regions of the genome responsible for these reversals in males. We also found that hermaphrodites of some strains show partial L/R reversals (“heterotaxy”) of only the anterior or only the posterior gonad arm, with a small fraction of these animals showing complete reversals involving both arms. Variation in this phenotype across the wild isolates ranged from 0% to ~10% total reversals of all three types in hermaphrodites. Surprisingly, we found that the propensity for reversals in males and hermaphrodites is not significantly correlated across the isolates, indicating that the genetic basis for L/R reversals is independent in the two sexes. Analysis of 99 RILs generated from two strains at the extreme ends of the distribution of the hermaphrodite phenotypes (N2, 0% reversals, MY16, 10% reversals) revealed that the phenotype is multigenic and provided evidence of transgressive segregation, with one RIL showing ~20% reversals. QTL analysis is being performed to assess the genetic complexity and causal basis for this variation in handeness. Preliminary evidence suggests that the reversals in hermaphrodites may in part be traced back to an embryonic event, during which the developing gut undergoes a L/R asymmetric twist in comma-stage embryos (*Hermann et al., 2000), consistent with our finding that lin-12 mutants, which do not undergo normal gut twist, show frequent L/R gut/gonad reversals. Thus, variations in the anatomical handedness among wild isolates may reflect varying fidelity in the mechanism controlling the rotation of gut cells during mid-embryogenesis.


Most diseases and traits are influenced by multiple genetic factors. However, identifying variants underlying phenotypic differences between individuals is hard and laborious even in model organisms. For instance, Genome-Wide Association Studies (GWAS) require hundreds of C. elegans wild isolates to be individually phenotyped, a process prone to measurement noise, particularly for traits influenced by environmental factors. Alternatively, in Quantitative Trait Loci (QTL) mapping, large recombinant inbred line (RIL) panels need to be generated, maintained and phenotyped, which limits the number of parental genotypes which can be interrogated. Thus, despite the previous success of these strategies in identifying causal loci, a long road still lies ahead for understanding the complex relationship between genotype and phenotype. To gain insights into the genetic basis of complex traits, we developed a novel Bulk Segregant Analysis (BSA) strategy in C. elegans as a versatile and complementary approach to GWAS and panels of recombinant inbred lines (RILs). Our method is based on mating obligate outcrossing strains carrying the fog-2(q71) allele for multiple generations, allowing the accumulation of recombination events, and leading to increased mapping resolution. The resulting population is composed of thousands of genetically unique individuals, which can be used to map traits that vary between the parental strains. Furthermore, the population can be directly interrogated at multiple generations for variants affecting fitness, allowing us to study the dynamics of selection. To guide the choice of parameters for our experiments, we developed a computational framework in R, bulkPop, for simulating the BSA pipeline. This framework takes into account the known genetic map and realistic patterns of linkage disequilibrium, and allows us to optimize the number of individuals and number of generations. We implemented our method to study a cross between the reference strain N2 and the wild isolate CB4856 from Hawaii, and found highly reproducible fitness peaks that were closely matched by our simulations. As a proof of principle, we mapped the known zeel-1/peel-1 genetic incompatibility on Chr. I. and discovered previously unknown loci influencing fitness/growth. In addition, BSA can also be used to map variants affecting gene expression. We used BSA followed by fluorescence sorting of worms (COPAS Biosorter) to map a de novo genetic variant in sti-1 leading to up-regulation of the chaperone daf-21 (HSP90). We will further discuss current limitations of the approach and future directions to improve the mapping resolution.

85 C. elegans microbiomes. W. Yang1, J. Zimmermann2, C. Petersen13, N. Obeng1, J. Aidley3, K. Kissoyan1, B. Pees3, L. Cassidy2, M. Leippe3, A. Tholey3, K. Dierking1, C. Kaleta2, H. Schuleenburg1. 1) Evolutionary Ecology and Genetics, University of Kiel, Kiel, DE; 2) Institute of Experimental Medicine, University of Kiel, Kiel, DE; 3) Physiology, University of Kiel, DE.

The evolution of all higher organisms took place in the presence of microbes. Microbes may serve as food, act as competitors, commensals, or even interact with a host in a mutualistic form. Therefore, the naturally associated microbial interactors are key determinants of the biology of any organism. This also applies to C. elegans, even though the associated microbiome has been neglected in the numerous studies with this nematode. In fact, information on the worm’s native microbiome was only published last year. Based on this knowledge, we here present a model for C. elegans and its interaction with naturally associated microbiome members of the genus Ochrobactrum. These bacteria are notable because of their ability to enter and persist in the nematode gut, even under stressful conditions. We explored the characteristics of this interaction at both phenotypic and molecular level, for the latter using a combination of different omics approaches and metabolic network analysis. Our results revealed an influence of the microbiome members on developmental processes, including development of the nervous system and sex-related traits, on reproduction, and also on ageing. These effects appear to be mediated by different transcription factors, including E-Box, and SP transcription factors. In sum, our consideration of naturally associated microbiome members may help to develop a more realistic understanding of C. elegans life history and gene function.

86 Ecology of bacterial community assembly in a C. elegans host model. Nicole Vega1, Anthony Ortiz-Lopez1,2, Jeff Gore1. 1) Physics, MIT, Cambridge; 2) National Autonomous University of Mexico, Mexico City, Mexico.

Despite decades of theoretical and observational investigation, the factors directing community assembly in real ecosystems are still poorly understood. The problem is particularly acute in host-associated systems such as the intestinal microbiota. Furthermore, while these communities are known to vary between hosts, the causes and consequences of this heterogeneity are not well understood.
Here we use *C. elegans* as a simple host model to determine general rules for community assembly in a biotic environment. Gut-associated communities are assembled *de novo* from a set of bacterial species selected to cover a broad taxonomic range and to include different host interactions, including both probiotics and pathogens of the worm. Worm mutants with and without responsive immune function are used to describe the effects of immunity on bacterial community establishment.

We describe colonization as an interaction between bacterial competition in a spatially structured environment and host effects mediated in part by innate immunity. Migration rate into the intestine and birth/death rates in this environment are evaluated for their contributions to the outcomes of bacterial competition, and the resulting heterogeneity between individual communities is described.

87 Intron loss and gain in the genus *Caenorhabditis*. L. Stevens, M.L. Blaxter  Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, GB.

The evolutionary origin of introns and the mechanisms by which they evolve remain poorly understood. In *Caenorhabditis*, studies of intron evolution have been restricted to a handful of taxa or single genes, and thus cannot reveal global patterns in the genus. We now have genomes for many *Caenorhabditis* species, generated as part of the *Caenorhabditis* Genomes Project (CGP) and other efforts, and this question can be revisited definitively. We developed custom software that defines orthologous introns, and applied it to genes predicted from the genomes of over 20 species of *Caenorhabditis* and outgroups. Placing the patterns of intron presence and absence within a phylogenetic context enabled the inference of intron gain and loss events.

We show that the *Caenorhabditis* ancestor had substantially more introns than many present-day species, including *C. elegans*. Intron loss is thus a major pattern throughout *Caenorhabditis* evolution. We compared the characteristics of lost introns to those of retained introns to identify mechanisms responsible for this pattern. We also analyze intron gain events, which have in previous studies proved difficult to define, to identify the origins of new introns. Our findings highlight the importance of the data being produced by the CGP, allowing us to place *C. elegans* and the vast body of associated research within a rich evolutionary context.

88 Diverse types of regulatory mutations drive the evolution of sex in nematode germ cells. Y. Shen, S.-Y. Lin, R. E. Ellis  Molecular Biology, Rowan University SOM, Stratford, NJ.

In self-fertile nematodes, XX animals have been modified so that larvae make sperm. Since the XX soma remains female, these changes must be restricted to the germ line. Furthermore, they have to be precisely regulated so the animals can switch to oogenesis as adults. To identify the regulatory changes that underlie this trait, we are comparing the nematodes *C. elegans* and *C. briggsae*, and have started work with *C. tropicalis*. Each species evolved self-fertility independently.

These nematodes share a conserved sex-determination pathway that acts through the transcription factor TRA-1, a homolog of the human Gli proteins. Males make HER-1, which binds to and inactivates the TRA-2 receptor on each cell, allowing the FEM proteins to target TRA-1 for degradation. Females and hermaphrodites lack HER-1, allowing TRA-2 to inhibit the FEM complex. As a result, TRA-1 is cleaved, producing a repressor that turns off male genes.

By studying evolutionary change in this pathway we show that: (1) each species has adopted independent and unique solutions for self-fertility, and (2) these regulatory changes can affect almost every step of the pathway upstream of TRA-1. Most of our data come from the direct comparison of similar mutations in different species, made through gene editing.

First, the functions of chromatin regulatory complexes have been altered. The Tip60 HAT complex is essential for spermatogenesis in *C. briggsae*, but plays only a minor role in *C. elegans*. In addition, the NURF complex is needed for spermatogenesis only in *C. tropicalis*. Finally, mutations of the WDR-5 proteins affect oogenesis in *C. briggsae*, but spermatogenesis in *C. elegans*.

Second, translational regulation has changed dramatically. We analyzed translational control of fem-3, and found that 3′-UTR mutations that completely block oogenesis in *C. elegans* result in normal hermaphrodite development in *C. briggsae*. The Schedl lab showed that in *C. elegans*, the unique FOG-2 protein works with GLD-1 to block translation of tra-2 messages, allowing XX spermatogenesis. Finally, the Haag lab has shown that PUF proteins play unique roles in *C. briggsae*.

Third, regulation through protein-protein interactions has been altered. An intracellular fragment of TRA-2 can bind TRA-1 in both *C. elegans* and *C. briggsae*. Mutations that prevent this interaction cause oogenesis in *C. elegans*, but orthologous mutations cause either constitutive spermatogenesis or hermaphrodite development in *C. briggsae*. Furthermore, *C. briggsae* relies on the novel SHE-1 protein for self-fertility, and SHE-1 works in part through its binding partner, PQN-94.

Thus, selection can alter the sex-determination pathway at almost any point to promote self-fertility. We propose that pathways organized around a binary switch are particularly flexible during evolution, since any change to one of several competing factors should change the final decision.

89 Transcriptome analysis reveals a predominant role of cis-elements in regulating allele-specific expression in F1 hybrids between *C. briggsae* and *C. nigoni*. Runsheng Li, Xiaoliang Ren, Yu Bi, Qiutao Ding, Zhongying Zhao  Department of Biology, Hong Kong Baptist University, Hong Kong, HK.

Differential expression of orthologous genes could be a product of divergence in cis-regulatory elements and/or trans-factors. Analysis of transcriptomes in F1 hybrids between closely related species provides an opportunity for systematically dissecting the roles of cis-elements and the trans-factors in controlling allele-specific expression. This analysis could also provide insight into the mechanism controlling genome stability, for example, through transposon silencing. The F1 hybrid transcriptomes have been analyzed in most model organisms, but never been examined in nematode species. Here we perform transcriptome analyses in the F1 female hybrids from reciprocal crosses between *C. briggsae* and *C. nigoni* with their parental
has no typical neural crest either. We therefore propose a common origin of the gene network for lateral borders of neuroectoderm across bilateria. Infective larvae actively locate a suitable host for infection may represent a previously unexplored target for therapeutic development. Skin-penetrating parasitic nematodes infect approximately one billion people worldwide, and are a major source of neglected tropical diseases. These parasitic worms are infective exclusively during a soil-dwelling larval stage. The mechanisms by which infective larvae actively locate a suitable host for infection may represent a previously unexplored target for therapeutic intervention. Previous work has shown that the infective larvae of parasitic nematodes respond to several host-emitted sensory cues, including heat. However, our understanding of how strongly infective larvae rely on thermosensation to drive parasitic reproduction play in shaping the genome. Changes in how an organism sexually reproduces can profoundly alter both its genome and reproductive traits that its genome encodes. In Caenorhabditis nematodes, species with self-fertilizing hermaphrodites have smaller genomes and reduced mating success than male-female, obligate outcrossing species. The connections, if any, between sexual traits and genome size, and the speed with which genome size changes, remain unclear. We compared chromosome-scale genome assemblies for the two most closely related Caenorhabditis nematodes with alternative sexual modes, the outcrossing C. nigoni and the selfing C. briggsae. The C. nigoni genome is 19% larger and encodes 31% more protein-coding genes, resembling other male-female Caenorhabditis and indicating that the C. briggsae genome shrank rapidly. C. nigoni-specific genes were enriched for those encoding small proteins with male-biased expression. We identified the male secreted short (mss) gene family, which encodes sperm surface factors conserved in outcrossing species but recently lost in C. briggsae. In the outcrossing C. remanei, mss-null males had normal fertility, but their sperm failed to compete with wild-type males. Conversely, restoration of mss to C. briggsae males rendered their sperm more competitive than those of wild-type males, further enhanced the precedence of male sperm over the self-sperm of hermaphrodites, and made males more common in mixed-sex populations. These results directly link the reduced mating efficacy of selfing species to the loss of reproductive genes, and highlight the ongoing role that modes of sexual reproduction play in shaping the genome.

90 **Self-fertility triggers rapid genome shrinkage and loss of sperm competition proteins.** E.M. Schwarz1, D. Yin1, C.G. Thomas2,3, R.L. Feldes, I. Korfs, A.D. Cutters, E.M. Schar ters3, E.J. Railtons, B.J. Meyers, E.S. Haags 1) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY, USA; 2) Department of Biology and Biological Sciences Graduate Program, University of Maryland, College Park, MD, USA; 3) Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada; 4) Department of Molecular and Cellular Biology and Genome Center, University of California, Davis, CA, USA; 5) Howard Hughes Medical Institute and Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA.

Changes in how an organism sexually reproduces can profoundly alter both its genome and reproductive traits that its genome encodes. In Caenorhabditis nematodes, species with self-fertilizing hermaphrodites have smaller genomes and reduced mating success than male-female, obligate outcrossing species. The connections, if any, between sexual traits and genome size, and the speed with which genome size changes, remain unclear. We compared chromosome-scale genome assemblies for the two most closely related Caenorhabditis nematodes with alternative sexual modes, the outcrossing C. nigoni and the selfing C. briggsae. The C. nigoni genome is 19% larger and encodes 31% more protein-coding genes, resembling other male-female Caenorhabditis and indicating that the C. briggsae genome shrank rapidly. C. nigoni-specific genes were enriched for those encoding small proteins with male-biased expression. We identified the male secreted short (mss) gene family, which encodes sperm surface factors conserved in outcrossing species but recently lost in C. briggsae. In the outcrossing C. remanei, mss-null males had normal fertility, but their sperm failed to compete with wild-type males. Conversely, restoration of mss to C. briggsae males rendered their sperm more competitive than those of wild-type males, further enhanced the precedence of male sperm over the self-sperm of hermaphrodites, and made males more common in mixed-sex populations. These results directly link the reduced mating efficacy of selfing species to the loss of reproductive genes, and highlight the ongoing role that modes of sexual reproduction play in shaping the genome.

91 **A conserved regulatory network for lateral neural borders from worm to vertebrate.** Yongbin Li1, Di Zhao1, Takeo Horie2,3, Geng Chen1, Hongcun Bao1, Ryoko Horie1, Tao Liang1, Qinghua Tao1, Xiao Liu1 1) School of Life Sciences, Tsinghua Univ, Beijing, Beijing, CN; 2) Shimoda Marine Research Center, University of Tsukuba, Shimoda, Shizuoka, 415-0025, Japan; 3) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, New Jersey 08544, USA; 4) College of Life Sciences, Zhejiang University, Hangzhou 310058, CN.

The lateral neural plate border (NPB) of vertebrates is the precursor of substantial evolutionary innovation, including the neural crest. It has been proposed that NPB may exist in protostomes, such as annelids. In addition, lateral neuroblasts in Caenorhabditis elegans have key features of vertebrate neural crest, such as Epithelial to Mesenchymal Transition, migration and multipotency. Here we present evidence that several components of the NPB gene network are essential for the development of lateral neuroblasts in Caenorhabditis elegans. Using single cell gene expression profiling and genetic analysis, we systematically examined the gene network underlying the development of worm lateral neuroblasts and compared it with vertebrate Neural Crest Gene Regulatory Network (NC-GRN), which is hierarchically composed of NPB specification module, neural crest specification module and differentiation gene batteries. The worm regulator set is significantly enriched in orthologues of vertebrate NPB specification module (Msx/vab-15, Pax3/7/pax-3 and Zic/ref-1). We also revealed a novel NPB specifier Nzl/tll-1 that functions synergistically with Msx/vab-15 both in C. elegans lateral neuroblasts and Xenopus NPB. In the process of worm lateral neuroblasts Q and V5 giving rise to mechanosensory neurons, NPB specifier Msx/vab-15 also deploys a differentiation gene battery related to vertebrate Dorsal Root Ganglion (DRG). Furthermore, we showed that regulation of the differentiation gene battery by Msx/vab-15 is essential for specifying a NPB lineage in proto-chordate Ciona intestinalis, which has no typical neural crest either. We therefore propose a common origin of the gene network for lateral borders of neuroectoderm across bilateria.

92 **Some like it hot: temperature-driven host seeking in human-parasitic nematodes.** A.S. Bryant, E.A. Hallem M IMG, UCLA, Los Angeles, CA.

Skin-penetrating parasitic nematodes infect approximately one billion people worldwide, and are a major source of neglected tropical disease. These parasitic worms are infective exclusively during a soil-dwelling larval stage. The mechanisms by which infective larvae actively locate a suitable host for infection may represent a previously unexplored target for therapeutic intervention. Previous work has shown that the infective larvae of parasitic nematodes respond to several host-emitted sensory cues, including heat. However, our understanding of how strongly infective larvae rely on thermosensation to drive parasitic
behavior is incomplete. We investigate the role of heat in host-seeking behaviors of multiple parasitic nematode species, including the human threadworm, Strongyloides stercoralis. Using a custom-built behavioral setup, we quantified the movement of infective larvae within precisely controlled temperature gradients. We found that S. stercoralis is strongly attracted to heat, with a preferred temperature at least 5°C warmer than human body temperature. In addition, we found that the skin-penetrating rat parasite Strongyloides ratti and the passively ingested mouse parasite Heligmosomoides polygyrus are also attracted to heat, although less robustly than S. stercoralis. The elevated temperature preference of S. stercoralis infective larvae likely generates temperature-driven movements that will not asymptote as worms approach a host, a specialization that may be critical for tracking ephemeral thermal targets such as mobile humans. We also tested whether infective larvae synergize multiple sensory modalities during host seeking. S. stercoralis attraction to host odors is highly dependent on the local thermal environment, implying a hierarchical coding of sensory information during host seeking. Our findings suggest that parasitic nematodes use temperature as a potent sensory cue to locate hosts for infection. We are currently testing the temperature preferences of other parasitic nematode species, including a human hookworm, Ancylostoma ceylanicum. In addition, we are investigating the cellular and circuit adaptations underlying this behavioral specialization.

Friday, June 23   8:30 AM – 11:30 AM
Grand Horizon Ballroom
Aging and Longevity

Co-chairs: Javier Apfeld, Northeastern University, and Collin Ewald, ETH Zurich

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Progressive impairment of protein homeostasis is a universal hallmark of aging, stress, and disease. One potential site for this cellular dysfunction is the lysosome, which is responsible for macromolecular breakdown and recycling. We previously showed in C. elegans that granulin peptides, the cleavage products of the neurodegenerative disease protein progranulin, selectively enhance TAR DNA-binding protein 43 (TDP-43) toxicity and impair its degradation (Salazar J. Neurosci 2015). Now, we provide genetic, biochemical, computational modeling and behavioral evidence supporting a role for C. elegans granulin 3 as a stress and age-responsive inhibitor of the lysosomal aspartyl protease, ASP-3/CTSD.

C. elegans progranulin contains three granulins. We show that granulin production increases with age and stress. Transgenic expression of individual granulins impairs stress response and learning and memory. Granulins localize to the lysosome where they stimulate movement of the autophagy-promoting transcription factor HLH-30/TFEB from the cytosol to the nucleus. Co-immunoprecipitation experiments show that C. elegans granulin 3 interacts with the lysosomal aspartyl protease, ASP-3, which has previously been implicated in necrotic cell death. Protein docking models predict that granulin 3 binds to and occludes the protease active site. Supporting this, ASP-3 activity is significantly reduced in granulin expressing animals. Furthermore, the expression of granulin 3 in a deg-1(d) background delays the cell death of mechanosensory neurons and prolongs response to gentle touch. In addition, human patients carrying progranulin mutations produce more granulin fragments in degenerative brain regions than age-matched controls, and this is accompanied by a significant reduction in CTSD activity.

Our results identify granulins as a novel class of endogenous peptide aspartyl protease inhibitor, which in analogy to serpins and cystatins we call aspartins. Given that age and stress can increase granulin production, we propose that the regulated production of granulin peptides can impair protein homeostasis and contribute to neurodegenerative disease pathogenesis. These results also suggest that dysregulation of lysosomal protease activity may more generally contribute to aging and age-related diseases.

94 Expression patterns and functions of the daf-2 isoforms. Wen-Hong Zhang, Yan-Ping Zhang, Yue Sun, Han-Qing Zhao, Chao Zhai, Cheng Zhan, Meng-Qiu Dong
National Institute of Biological Sciences, Beijing, Beijing, China.

In C. elegans, the sole insulin/IGF-1 receptor DAF-2 has multiple prominent functions in development, metabolism, reproduction and lifespan regulation. Its functional importance has attracted intense research interest since 1992. However, after 25 years and hundreds of research articles, the expression pattern of daf-2 remains unknown, let alone the expression patterns and functions of individual isoforms. The only exception is the daf-2c isoform, which was characterized and found to be a neuronal isoform that regulates learning (Ohno H. Science. 2014; Tomiako M, Nat Commun. 2016).

Taking advantage of the recently developed CRISPR/Cas9 gene editing technology, we sought to dissect the functions and expression patterns of daf-2 in an isoform-specific manner. To visualize the expression of daf-2 mRNA, we knocked in a tandem GFP::mNeonGreen tag with two nuclear localization sequences and a SL2-type ICR (inter-cistronic region) sequence in front of the coding sequence of each predicted isoform of daf-2, except the c isoform. To visualize the expression of DAF-2 proteins, we fused a tandem GFP::mNeonGreen fluorescent protein tag to the DAF-2 C-terminus, which is shared by all six protein-coding isoforms, and then introduced frame-shifts to knock out specific isoforms individually or in combination. The
function of DAF-2 appears intact with the C-terminal GFP::mNeonGreen tag, for this knock-in strain is phenotypically indistinguishable from wild type in development and lifespan.

From the strains described above, we detected the DAF-2 protein on the surface of early embryonic cells starting from the two-cell stage and on the surface of adult neurons, germ cells, oocytes, and vulval cells. The daf-2 mRNA was detected in two additional tissues—intestine and hypodermis.

With respect to the isoforms, we found that the DAF-2a protein was expressed exclusively in the germ line, although expression of daf-2a mRNA was detected in the intestinal and hypodermal cells. Deletion of daf-2a reduced brood size and greatly extended lifespan to a level that is more than half way between wild type and the daf-2(e1370) mutant.

The DAF-2f protein was expressed exclusively in a subset of neurons. This isoform was critically important for dauer formation, and its contribution to lifespan regulation will be analyzed after we overcome a technical issue.

Expression of the d and e isoforms were not detected. Expression of the g isoform, which encodes a truncated DAF-2 protein lacking the extracellular and trans-membrane regions, was found in vulval cells only. These isoforms appeared to make no or little contribution to dauer formation, reproduction, and lifespan regulation.

95 DAF-16 requires Protein Phosphatase 4 to initiate the transcription of stress resistance and longevity promoting genes.

I. Sen1,2, N. Puerta Cavanzo2, M. Liu2, X. Lin1,2, S. Brandenburg2, C. Riedel1,2 1) ICMC, Department of Medicine, Karolinska Institute, Stockholm, Sweden; 2) European Research Institute for the Biology of Ageing, University of Groningen, Groningen, The Netherlands.

The conserved transcription factor DAF-16 is one of the most important aging regulators known to date. It resides downstream of the insulin/IGF signaling (IIS) pathway and relays low insulin/IGF signals into the expression of stress resistance and longevity promoting genes. However, DAF-16 is not exerting its lifespan regulatory role just by itself. Instead it relies on regulators and cofactors, several of which have been described in previous work. One of the first discovered and most potent positive regulators of DAF-16 is SMK-1. It is essential for DAF-16 to promote stress resistance and longevity under low IIS. However, despite this crucial role, little is known about the mechanism by which SMK-1 functions.

By large scale purification of SMK-1 from C. elegans and mass spectrometry-based identification of co-purifying proteins, we found that SMK-1 is part of a specific Protein Phosphatase 4 (PP4) complex. Genetic analyses showed that SMK-1 fulfills its aging-regulatory role as part of this complex. Loss of PP4/SMK-1 under low IIS leads to a mildly delayed nuclear entry of DAF-16 and mildly reduced binding of DAF-16 to its target promoters. However, it suppresses many of the gene expression changes mediated by DAF-16. So, where does this strong effect on DAF-16 target gene expression come from? To address this, we studied the behavior of RNA polymerase II (Pol II) by ChIP-Seq under low IIS and found a PP4/SMK-1-dependent change in the phosphorylation status of DAF-16 under low IIS. Eventually, we used mass spectrometry to identify the full SMK-1-dependent phosphoproteome, followed up the emerging substrate candidates by genetic screening, and intend to complete substrate validation in the near future — to fill the last gap in this mechanistic picture in which PP4/SMK-1 influences PolII transcriptional initiation at DAF-16-activated genes and thereby the promotion of stress resistance and longevity under conditions of low IIS.

96 Using the CRISPR/Cas9 technology to dissect the spatial-temporal expression patterns and the functions of DAF-16 isoforms.

Yan-Ping Zhang, Wen-Hong Zhang, Chao Zhai, Meng-Qiu Dong  National Institute of Biological Sciences, Beijing, Beijing, China.

DAF-16 is a pivotal transcription factor that transmits most, if not all, of the signaling from DAF-2 (the worm insulin/IGF-1 receptor) to regulate development, stress resistance, and aging in C. elegans. Previous studies analyzing the functions of different daf-16 isoforms produced contradictory results (Kwon ES, Nature 2010; Chen AT, Genetics 2015). In this study, we dissected the functions and expression patterns of individual daf-16 isoforms using the CRISPR/Cas9 technology, which became available in recent years. We knocked in a GFP coding sequence before the stop codon of all daf-16 isoforms at the endogenous locus and then introduced frameshifts to delete individual isoforms. We found that isoforms a, b, and d/f are the major isoforms translated into proteins, because deleting all three (d and f are treated as one) completely abolished the DAF-16::GFP signal. The expression patterns are:

- DAF-16a::GFP: neurons, intestine, hypodermis, muscles; from 3-fold embryos to adults
- DAF-16b::GFP: neurons; from bean-stage embryos to adults
- DAF-16d/f::GFP: germ cells, oocytes, occasionally in one or a few intestinal cells; from L4 to adults

The differences in the expression were accompanied by differences in functions. Knocking out daf-16a but not daf-16b or daf-16d/f fully repressed the constitutive dauer formation phenotype induced by daf-2(e1370), indicating that the widely expressed somatic isoform daf-16a is required for dauer formation, whereas the neuronal daf-16b and germline-limited daf-16d/f are not. For thermo tolerance conferred by daf-2(e1370), at least two daf-16 isoforms were required, although a seemed to be more important than b and d/f. In contrast, the three daf-16 isoforms must all be expressed to fully support the longevity phenotype of daf-2(e1370). Ranked by how well each isoform supports daf-2(e1370) longevity, we had d/f > a > b, which was determined by leaving only one daf-16 isoform to be expressed and deleting the other two. Intriguingly, when ranked by how much the daf-2 longevity is suppressed by deleting only one daf-16 isoform, we had a > d/f > b. We also assessed the importance of the three daf-16 isoforms in lifespan extension caused by daf-2a knock-out and obtained the same results (the
protein product of *daf-2a* was detected only in the germline, see abstract by Wen-Hong Zhang *et al.*).

The above results revealed new insights: first and most unexpected, DAF-2/DAF-16 signaling is present in the germline and is important for lifespan regulation; besides, there are complex genetic interactions between *daf-16* isoforms and cross-tissue communications between parallel channels of insulin signaling.

97  **Identification of new pathways involved in the regulation of the UPR^mt reveals a crosstalk between mitochondrial stress response and insulin signaling.** *B. Hernando-Rodriguez* 1, V. Miliar 1, A. Jarit-Cabanillas 1, H. Manchada 2, L. Kaderali 2, M. Artal-Sanz 3 1) Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide, Sevilla, Sevilla, ES; 2) Institut für Bioinformatik-Universitätsmedizin Greifswald, Greifswald, Germany.

Depletion of the mitochondrial prohibitin complex (PHB) shows an opposite effect on aging: it shortens lifespan in wild-type worms while it dramatically extends the longevity of the already long-lived insulin/IGF-1 signaling (IIS) mutants. Moreover, PHB depletion induces a strong mitochondrial unfolded protein response (UPR^mt) in wild-type animals while this response is remarkably reduced in IIS mutants. Interestingly, some of the described UPR^mt components are not required for the activation of the response upon PHB depletion.

We aimed at identifying new pathways involved in the regulation of the PHB-mediated mitochondrial stress response, as well as mechanisms responsible for the opposite longevity outcomes of PHB depletion. Towards this aim, we developed a semi-automated method based on double RNAi and automated image analysis to carry out RNAi screens, in PHB-depleted wild type and IIS mutants.

In addition to a big number of genes involved in protein homeostasis, we describe evolutionarily conserved developmental signal transduction pathways as essential modulators of the mitochondrial stress response. Furthermore, we report pathways regulating the UPR^mt in an insulin-dependent manner. Our results suggest a difference in metabolism between PHB-depleted worms and PHB-depleted;IIS mutants and place carbohydrate and lipid metabolism as possible mechanisms contributing to the opposite effect of PHB depletion in the aging phenotype of wild type and metabolically compromised animals.

98  **Glutamine 5’-tRNA-driven small RNAs promote longevity via increasing mitochondrial activity through AMPK.** *W. Hwang* 1,8, G.W. Shin 2,8, Y. Lee 1, Y.E. Kim 1, M. Seo 1, C.M. Ha 3, G.O. Ahn 3, G.Y. Jung 2,4,9, H.G. Nam 1,7,9, S.J.V. Lee 1,2,9 1) Department of Life Sciences; 2) School of Interdisciplinary Bioscience and Bioengineering; 3) Division of Integrative Biosciences and Biotechnology; 4) Department of Chemical Engineering, Pohang University of Science and Technology, Pohang, Gyeongbuk, South Korea; 5) Research Division, Korea Brain Research Institute, Daegu, Korea; 6) Center for Plant Aging Research, Institute for Basic Science, Daegu, South Korea; 7) Department of New Biology, DGIST, Daegu, South Korea; 8) These authors contributed equally; 9) Co-corresponding authors.

Transfer RNA-derived small RNAs (tsRNAs) are abundant small non-coding RNAs discovered in various organisms, including *C. elegans*. Studies have begun to unravel the biological functions of tsRNAs, but their roles in aging processes remain elusive. Here we demonstrate the functional importance of specific tsRNAs for *C. elegans* longevity. We first determined age-dependent changes in tsRNA levels by analyzing available small RNA sequencing data (1) and confirmed the results by using Northern blot analysis. We found that the level of various tsRNAs gradually increased during aging. We overexpressed one of such tsRNAs, glutamine (Gln) 5’-tsRNA, by using an RNA polymerase III-responsive U6 (K09B11.12) promoter. Interestingly, we found that overexpression of the Gln 5’-tsRNA significantly extended lifespan. Our results suggest that the level of life-extending Gln 5’-tsRNA is increased during normal aging as a compensatory response. We then asked which longevity factors were required for the life extension. We showed that mutations in *aak-2* the catalytic alpha subunit of AMP-dependent protein kinase (AMPK) fully suppressed the Gln 5’-tsRNA-mediated longevity. Through performing RNA seq. analysis, surprisingly, we found that the expression of all 12 mitochondrial DNA-encoded genes was highly increased by Gln 5’-tsRNA overexpression. We further showed that Gln 5’-tsRNA overexpression increased mitochondrial DNA contents in an AMPK-dependent manner and enhanced muscle-specific mitochondrial RFP intensity. Together, our data raised the possibility that age-dependent increases in Gln 5’-tsRNA levels delay aging and promote longevity by enhancing mitochondrial functions through AMPK. Currently we are testing whether our findings regarding the role of tsRNAs in mitochondrial function in *C. elegans* are conserved in mammals by using mice. Our study provides key information regarding anti-aging and mitochondria-enhancing functions of tsRNAs, which are universal small non-coding RNAs. (1) Kato *et al.*, 2011, RNA

99  **Mating and male pheromone kill Caenorhabditis males through distinct mechanisms.** *C. Shi*, A. Runnels, C. Murphy  Department of Molecular Biology and LSI Genomics, Princeton University, Princeton, NJ, United States.

The difference in longevity between sexes is a mysterious yet general phenomenon across great evolutionary distances. *C. elegans* males live significantly shorter when maintained in groups, whereas the longevity of hermaphrodites is not influenced by population density. Previous investigations into the factors that affect the lifespan of *C. elegans* have primarily focused on hermaphrodites, thus how *C. elegans* male lifespan is regulated remains poorly understood. We find that male-specific population density-dependent death in *C. elegans* is due to the perception of male pheromone as a toxin. Neurons and the germline are required for male pheromone-dependent male death. Hermaphrodites with a masculinized nervous system secrete male pheromone and are susceptible to male pheromone killing. Male pheromone-mediated killing is unique to androdioecious *Caenorhabditis*, and may reduce the number of males in hermaphroditic populations; neither males nor females of gonochoristic species are susceptible to male pheromone killing. The toxicity of male pheromone may explain the contradictory results from previous publications in which grouped males were used as the control in testing whether mating affects the lifespan of *Caenorhabditis* males.

We previously showed that mating causes shrinking and shortens the lifespan of *Caenorhabditis* hermaphrodites and females.
Using single worm lifespan assays, we discovered that *Caenorhabditis* males also experience post-mating changes and earlier death. Mating-induced death is characterized by germline-dependent shrinking, glycogen loss, and ectopic vitellogenin expression, utilizes distinct molecular pathways from pheromone-induced death, and is shared between the sexes and across species. The study of sex- and species-specific regulation of aging reveals deeply conserved mechanisms of longevity and population structure regulation.

100 Sexual interactions promote early death in *C. elegans*. L.N. Booth, K. Hebestreit, T.J. Maures, A. Brunet Genetics Department, Stanford University School of Medicine, Stanford, CA.

In the wild, animal interactions can dramatically affect an individual’s health and behavior in many species, but how social interactions impact aging is still unclear. *C. elegans*, with its short lifespan and defined neuronal circuits, is uniquely fit to identify new social signals and mechanisms that influence health and aging. Males induce the rapid aging and premature death of the opposite sex (hermaphrodites) in *C. elegans* through mating-dependent and -independent mechanisms. To better understand the impact of males on hermaphrodites, we generated a new RNA-seq dataset on young and older hermaphrodites exposed to normal males or males deficient in pheromone production. Our analysis reveals that males induce gene expression changes that are hallmarks of aging including metabolic, mitochondrial, and stress response genes. Interestingly, we find that defects in mitochondria protect hermaphrodites from the presence of males. Exposure to pheromone-producing males also induces the expression of neuronal related genes. Knockdown of some of these neuronal genes protects hermaphrodites from the presence of males, consistent with the role of chemosensation of male pheromones in the premature death of hermaphrodites. Interestingly, the majority of male-induced gene expression changes occurred in older hermaphrodites, which were exposed to males for a long period of time. We tested the idea that older hermaphrodites could be more sensitive to the presence of males. Indeed, older hermaphrodites died early following brief male exposure, whereas young hermaphrodites were resistant to males. This work provides new insights into the mechanisms by which interactions with the opposite sex impact health and aging.

101 Sex-specific regulation of lifespan in *C. elegans*. S. Gordon1, D. McCulloch2, A. Antebi3, D. Gems2, D. Portman4, S.S. Lee1, V. Rottiers1 1) Molecular Biology and Genetics, Cornell University, Ithaca, NY; 2) Institute of Healthy Aging, University College London, UK; 3) Max Planck Institute for Biology of Ageing, Køln, Germany; 4) Center for Neural Development and Disease, University of Rochester, Rochester, New York, USA.

Sex specific differences in longevity occur throughout the animal kingdom, including in humans. Those differences are thought to be caused by hormonal, metabolic and behavioral differences but the exact mechanisms are not clear. *C. elegans* is an excellent model system to study aging, and many genes and interventions have been shown to affect hermaphrodite aging dramatically. However, the lifespan of males has been largely neglected, since males leave the agar dishes in search of mates rendering classic aging experiments technically difficult. Using a liquid 96-well aging assay that prevents male leaving, we find a striking sex specific difference for at least one aging intervention: loss of the germline. In contrast to hermaphrodites, male lifespan does not change significantly upon germline loss (either by ablation or *glp-1* mutation). We also show that *glp-1* hermaphrodites are thermo-tolerant (a trait often found in long-lived animals) while *glp-1* males are not. We are profiling other traits of long-lived *glp-1* ablated hermaphrodites such as DAF-16, SKN-1 and HLH-30 nuclear localization (using *gfp* reporters), expression of genes upregulated in *glp-1* animals (QRT-PCR) and metabolic changes (fat storage, hormone levels). Interestingly, two transcription factors important for hermaphrodite *glp-1* lifespan extension, SKN-1 and DAF-16 are activated in both *glp-1* hermaphrodites and males suggesting that they are not limiting the *glp-1* male lifespan. We find that *glp-1* mutation, in contrast to hermaphrodites, does not dramatically increase fat storage in males suggesting this metabolic shift to increased fat storage is required. To determine the tissues important for the sex-specific differences in response to the loss of the germline we are assessing the lifespan and thermo-tolerance of strains with sex reversal in specific tissues. Such sex-reversal is achieved through tissue specific expression of either *tra-2* (feminization) or *fem-3* (masculinization). We find that intestinal masculinization abrogates the thermo-tolerance of *glp-1* hermaphrodites indicating that a female intestine is required for hermaphrodites for increased thermo-tolerance. However, *glp-1* males with feminized intestines are not thermo-tolerant indicating that intestinal feminization is not sufficient to confer increased thermo-tolerance. Our research provides insight into how changes in the germline or different responses to signals from the germline affect the lifespan of *C. elegans* in a sex specific manner.

102 Longitudinal imaging of *C. elegans* with the WorMotel reveals variation in behavioral decline during aging. M. Churgin1, S.K. Jung1, C.C. Yu1, X. Chen1, D. Raizen2, C. Fang-Yen1,3 1) Department of Bioengineering, University of Pennsylvania, Philadelphia, PA; 2) Department of Neurology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 3) Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania, Philadelphia PA.

The roundworm *C. elegans* is a mainstay of aging research due to its short lifespan and easily manipulable genetics. Standard methods for lifespan measurements in *C. elegans* are tedious and dependent on manual observation. In addition, they are largely performed as population assays and do not have access to potentially rich information contained in individual aging trajectories. To address these limitations, we developed the WorMotel, a microfabricated device for long-term cultivation and automated longitudinal imaging of large numbers of *C. elegans* confined to individual wells. Each WorMotel, which is fabricated by PDMS molded from a 3D printed master, consists of an array of 240 wells, each 3 mm in diameter, with a shape optimized for worm cultivation and imaging. Wells contain agar, bacteria, and a single worm. Residents of the WorMotel exhibit behaviors and lifespan very similar to those on standard plates. We use custom machine vision analysis software to automatically quantify aging behavior and lifespan for each animal simultaneously. An aversive blue light stimulus enables measurements of stimulated behaviors in addition to spontaneous behaviors.
We apply the WorMotel to characterize differences in behavioral decline across individuals and strains with differences in lifespan. Previous work has shown that population lifespan curves scale uniformly under different genetic and environmental conditions, suggesting that the shape of behavioral decline might also scale. We find that short-lived and long-lived mutant strains exhibit patterns of behavioral decline that do not temporally scale between individuals or populations, but instead resemble the shortest and longest lived individuals in a wild type population, respectively. We show that the shape of behavioral decline differs significantly between individuals with different lifespans, and that the shape of decline follows a universal dependence on lifespan across multiple genetic backgrounds.

We also apply our method to quantify aging in worms experiencing external oxidative stress. We find that behavioral trajectories of worms subject to oxidative stress, which reduces mean survival by about twenty-fold, resemble trajectories observed during aging. Therefore, our results suggest a complex relationship between genes, environment, and the shape of the aging process. Current work aims to identify molecular mechanisms of inter-individual variability in aging.

103 A Caenorhabditis elegans Model to Study Age-related Complications Associated with Diabetes and Parkinson’s Disease. Neelanjan Bose1,2, Jyotiska Chaudhuri1, Sanjib K. Guha1, Jianke Gong3,4, Sana Khateeb1, David Hall1, Alexander Rifkind1, Jianfeng Liu3, X. Z. Shawn Xu4, Pankaj Kapahi1,2 1) Buck Institute for Research on Aging, Novato, CA; 2) University of California, San Francisco, San Francisco, CA; 3) Huazhong University of Science and Technology, Wuhan, Hubei, China; 4) University of Michigan, Ann Arbor, MI.

Aging and chronic hyperglycemia leads to the accumulation of reactive a-dicarbonyls (a-DCs), like methylglyoxal. a-DCs react rapidly with biological macromolecules, such as proteins, lipid, and DNA to generate Advanced Glycation End-products (AGEs). AGEs can enhance protein aggregation, cause cellular stress and inflammation that have been associated with aging and a number of age-related pathologies, including various forms of diabetic complications and neurodegenerative diseases. Evolutionarily conserved glyoxalases are responsible for a-DC detoxification to limit AGE stress; however, their core biochemical regulation have remained unclear. We have established a Caenorhabditis elegans model, based on an impaired glyoxalase (gld-4/GLO1), to broadly study a-DC-related stress. We show that gld-4 animals rapidly exhibit several diabetes-like phenotypes including hyperesthesia, neuronal damage, and early mortality. We further demonstrate that the ion channel TRPA-1 acts as a novel sensor for a-DCs and AGEs, conserved between worms and mammals. Moreover, TRPA-1 activates SKN-1/Nrf2 via calcium-modulated kinase signaling, ultimately regulating the glutathione-dependent (gld-4/GLO1) and-independent (djr-1.1/DJ1 and djr-1.2/DJ1) glyoxalases to detoxify a-DCs and AGEs. We show that TRPA1-Nrf2 pathway is not only relevant for diabetic complications, but also provide protection against dopaminergic neuronal damage in C. elegans, as observed in Parkinson’s disease (PD). We also show that synthetic AGE supplementation is sufficient to recapitulate signifying diabetic and PD pathologies both in worms and mammalian systems. Finally, a phenotypic drug-screen using C. elegans identified podocarpic acid as a novel activator of TRPA-1 that rescues both diabetes and PD-related pathologies in C. elegans and mammalian cells. We propose that the upstream amelioration of a-DC stress represents a viable option to address related pathologies in diabetes and associated neurodegenerative conditions such as PD.

104 The homeodomain-interacting protein kinase HPK-1 preserves protein homeostasis and longevity through the HSF-1 chaperone network and TORC1-restricted autophagy. R. Das1,2, M. Thondamal3,4, B. Crick1, J.A. Melo1, J. Kim1,6, E. Swartz1,2, P.M. Douglas3, A.V. Samuelson1 1) Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, New York 14642, USA; 2) Department of Biology, University of Rochester, New York 14627, USA; 3) Department of Molecular Biology, Hamon Center for Regenerative Science and Medicine, UT Southwestern Medical Center, Dallas, TX 75390-9041, USA; 4) Department of Cell Biology, Skirball Institute of Biomolecular Medicine, NYU School of Medicine, New York, NY 10016, USA; 5) Dr. Reddy’s Institute of Life Sciences,?University of Hyderabad Campus?Gachibowli, Hyderabad 500046, India; 6) Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY 10029, USA; 7) Interdepartmental Program in Neuroscience, University of California, Los Angeles, CA 90095-1761, USA.

An extensive proteostatic network comprised of molecular chaperones and protein clearance mechanisms functions collectively to preserve the integrity and resiliency of the proteome. The efficacy of this network deteriorates during aging, coinciding with many clinical manifestations, including protein aggregation diseases of the nervous system. A decline in proteostasis can be delayed through the activation of cytoprotective transcriptional responses, which are sensitive to environmental stress and the internal availability of nutrients. The homeodomain-interacting protein kinase (hipk) family members are transcriptional co-factors that have been implicated in both genotoxic and metabolic stress responses. We demonstrate that overexpression of the sole Caenorhabditis elegans Hipk homolog, hipk-1, is sufficient to delay aging, maintain proteostasis, and promote stress resistance. We find that HPK-1 promotes proteostasis through induction of two separate but complimentary genetic pathways defined by the heat shock transcription factor (HSF-1) and the target of rapamycin complex 1 (TORC1). Consistently, HPK-1 defers aging-associated decline by potentiating the transcriptional activation of molecular chaperones after thermal stress and the expression of autophagy genes under nutrient stress. HPK-1 is induced by post-transcriptional mechanisms in response to thermal stress and transcriptionally by nutritional stress. Collectively our results position HPK-1 at a major regulatory node upstream of the protein homeostatic machinery, promoting protein folding via chaperone expression and protein turnover via expression of autophagy genes. HPK-1 therefore provides a potentially powerful intervention point for pharmacological agents targeting the protein homeostasis system as a means of preserving robust longevity.
Spatiotemporal regulation of autophagy in C. elegans aging. J. Chang, C. Kumsta, Y. Yang, A. Hellman, M. Hansen Sanford Burnham Prebys Med Discovery Institute, La Jolla, CA.

Autophagy is a cellular degradation process important for lifespan extension in several conserved longevity paradigms. While multiple links exist between autophagy and aging, it remains unclear how autophagy changes during normal organismal aging, and how long-lived mutants regulate autophagy to promote longevity. We used the nematode C. elegans to characterize age- and tissue-specific changes in autophagy in wild-type (WT) and in long-lived daf-2/insulin receptor and glp-1/germline-less animals. Using a GFP-tagged and a new tandem-tagged LGG-1/Atg8 reporter, we quantified autophagic vesicles as well as autophagic activity by Bafilomycin A ‘flux’ assays throughout adulthood in the C. elegans intestine, muscle, pharynx, and neurons. Our data are consistent with a decline in autophagic activity in these major tissues of wild-type animals. In contrast, daf-2 and glp-1 mutants showed unique age- and tissue-specific differences consistent with select tissues displaying elevated or reduced autophagic activity compared with wild-type animals. Although autophagy appeared active in the intestine of both long-lived mutants, inhibition of intestinal autophagy significantly abrogated lifespan extension only in glp-1 mutants. Collectively, our data suggest that autophagic activity normally decreases with age in C. elegans, but daf-2 and glp-1 long-lived mutants regulate autophagy in distinct spatiotemporal-specific manners to extend lifespan.

Friday, June 23  8:30 AM – 11:30 AM
De Neve Auditorium
Behavior

Co-chairs: Christopher Fang-Yen, University of Pennsylvania, and Jin Il Lee, Yonsei University

C. elegans detect the color of pigmented food sources to guide foraging decisions. D. Dipon Ghosh, Xin Jin, Michael N. Nitabach Cellular and Molecular Physiology, Yale University, New Haven.

Here we establish that contrary to expectations, Caenorhabditis elegans nematode worms possess a color discrimination system despite lacking any opsins or other photoreceptor genes. We found that simulated daylight guides C. elegans foraging decisions with respect to harmful bacteria that secrete a blue pigment toxin. By absorbing yellow-orange light, this blue pigment toxin alters the color of light sensed by the worm, and thereby triggers an increase in avoidance of harmful bacteria. These studies thus establish the existence of a color detection system that is distinct from those of other animals. In addition, these studies reveal an unexpected contribution of microbial color display to visual ecology.

The chaotic worm: Capturing the continuous complexity of natural behavior in the movement of C. elegans. Tosif Ahamed, Greg Stephens OIST Graduate University, Onna-son, Okinawa, JP.

Animal behavior is often thought to be composed of discrete stereotyped motifs and stochastic transitions between them. This view has been strengthened by recent studies that utilize advances in Machine Learning to map the behavioral motifs for several different model animals, including C. elegans. However, this discrete description is only an approximation, and doesn’t take into account the variability within each motif. Here, we propose an alternative description of C. elegans behavior based on the fact that it is fundamentally a continuous dynamical system. The equations of any dynamical system define a flow in a phase space and the behavior of the system can be completely described by the properties of this flow. We leverage the low dimensional space of C. elegans postures to reconstruct the dynamical phase space of C. elegans locomotion and study the resulting flow. We show that the dynamics lie in a 6 dimensional phase space, which is globally composed of three sets of cyclic trajectories that form the animal’s most stereotyped behaviors: forward, backward and turning locomotion. In other words, these 6 numbers tell us almost everything about worm locomotion: whether it’s going forward, backward or making a turn; the speed at which it’s moving, its turning rate, its wave frequency and amplitude etc. In this sense, the 6D phase space provides an almost complete description of C. elegans behavior.

In contrast to the global stereotypy, we also observe substantial local variability, i.e. every body wave the animal makes is different in the detail. Surprisingly, our tests reveal that simple noise models cannot account for the observed behavioral variability. Instead, we find that the variability is due to the chaotic nature of the flow, arising from exponential divergence of neighboring trajectories in the phase space. Further analysis of the phase space flow reveals even more surprises. We find that C. elegans dynamics, although chaotic, are highly constrained. In fact, they turn out to be closely associated with Hamiltonian systems, a well understood class of dynamical systems that conserve energy. We propose that the connection with Hamiltonian dynamics might underlie the flexibility and efficiency of C. elegans behavior.

In summary, we have come up with a mathematically precise description of C. elegans behavior that is not only complete but different in the detail. Surprisingly, our tests reveal that simple noise models cannot account for the observed behavioral variability. Instead, we find that the variability is due to the chaotic nature of the flow, arising from exponential divergence of neighboring trajectories in the phase space. Further analysis of the phase space flow reveals even more surprises. We find that C. elegans dynamics, although chaotic, are highly constrained. In fact, they turn out to be closely associated with Hamiltonian systems, a well understood class of dynamical systems that conserve energy. We propose that the connection with Hamiltonian dynamics might underlie the flexibility and efficiency of C. elegans behavior.

Neuropeptide signalling is required to regulate arousal in C. elegans. Y.L. Chew1, B Zhao1, Y Tanizawa1, L Grundy1, I Beets2,3, W Schaefer 1) Neurobiology Division, MRC Laboratory of Molecular Biology, Cambridge, Cambridgeshire, UK; 2) Cell Biology Division, MRC Laboratory of Molecular Biology, Cambridge, Cambridgeshire, UK; 3) Functional Genomics and Proteomics Group, Division of Animal Physiology and Neurobiology, KU Leuven, Belgium.
One of the central functions of the nervous system is to enable survival. Arousal is the sustained state of hyper-vigilance that produces increased locomotor activity and sensory responsiveness. Research in invertebrates has demonstrated the ubiquity of arousal and revealed important conceptual insights. Despite these advances, the mechanistic processes underlying arousal remain unclear. We are interested in how neuromodulators control arousal in *C. elegans*. We found that the neuropeptide FLP-24 is required for locomotor arousal – the sustained increase in speed following a noxious stimulus – as *flp-20* mutants show a defective arousal response when subjected to a mechanical “tap” stimulus. This defect can be rescued by re-expression of *flp-20* specifically in the gentle touch neurons, indicating that *flp-20*-encoded peptides are released from the touch neurons to facilitate arousal. Through a candidate screen, we found that FLP-20 binds the G-protein coupled receptor FRPR-3 in an *in vitro* binding assay. Interestingly, *frpr-3* mutants are also defective in arousal. We are currently identifying FRPR-3-positive neurons to determine which responding cells are required to bind FLP-20 and drive locomotor arousal after a tap stimulus.

We also found that the corticotrophin releasing factor (CRF) signalling pathway in *C. elegans* acts to modulate arousal. We recently identified the ligand for the CRF receptor orthologue, SEB-3, to be encoded by a peptide precursor gene we have named *crf-1*. A gain-of-function mutation in *seb-3, eg696*, leads to increased arousal, and this is suppressed in mutants containing a nonsense allele for *crf-1*. Conversely, CRF-1 over-expression leads to an enhanced tap arousal phenotype that is lost in the absence of the SEB-3 receptor. CRF-1 is expressed in a small number of head neurons, including the AVK interneuron and SMB motorneurons. We found that transgenic over-expression of *crf-1* solely from AVK is sufficient to drive enhanced arousal, but this is not the case for the nearby SMBs. The SEB-3 receptor is widely expressed throughout the nervous system, including in chemosensory and mechanosensory neurons, command interneurons, and cholinergic motor neurons in the head and ventral cord. We are currently investigating which of these SEB-3-expressing cells are required to respond to high levels of CRF-1 and promote an aroused behavioural state. Interestingly, CRF in humans, which is released from the hypothalamus in response to stress, acts to promote arousal and anxiety. Therefore, the SEB-3/CRF-1 system in *C. elegans* could represent a conserved stress pathway, and presents an attractive model to investigate CRF-mediated stress responses in a compact nervous system.

A fundamental question in neuroscience is how global brain states like sleep and wakefulness are reversibly generated and maintained. Specifically, how do populations of individual neurons contribute to the emergent properties of global brain states? During developmental stages termed lethargus *C. elegans* exhibits prolonged phases of behavioural quiescence, which share fundamental properties with sleep in other animals. Using behavioural genetics, we have identified a chemosensory arousal circuit involving the neuropeptide receptor NPR-1 that can trigger rapid and robust switching between behavioural quiescence and wake: in lethargic *npr-1* animals atmospheric oxygen levels induce sustained arousal, while preferred intermediate oxygen levels permit quiescence. We have exploited this paradigm to image brain-wide neuronal activity during these state transitions. Prelethargic larval stage 4 (L4) animals display continuous dynamical neuronal network activity, which has been previously shown by our lab to represent motor command states (e.g. Forward and Reverse). On the contrary, during the L4 lethargus brain activity is predisposed to enter periods of global Quiescence characterised by systemic down-regulation of neuronal activity. While motor command states are represented by transient neuronal population dynamics in the wake worm brain, the sleep-like brain state arises by converging from the Forward command state towards a fixed-point attractor described by maintained tonic activity of the GABAergic head neuron classes RME and RIS. Interestingly, these neurons are active already during the Forward command state, indicating that while signalling from these neurons may promote Quiescence they are not Quiescence specific. Spontaneous exits from Quiescence can occur through either Forward or Reverse command states, however, sensory-evoked wake only occurs through a Reversal command state, and has a significantly different neuronal activation pattern compared to spontaneous Reversals from Quiescence. Based on our data we propose that during sleep prone periods like lethargus neuronal networks are drawn to a baseline attractor state, and that this attractor mechanism could be and effective means for reactivating network dynamics by signalling from arousing circuits.

Regulation of locomotion quiescence during stress-induced sleep by FMRFamide-like peptide FLP-24. H. Wang1,2, P. Liu3, S Gharib1,2, Z.W. Wang3, P.W. Sternberg1,2 1) Biology and Biological Engineering, California Institute of Technology, Pasadena, CA; 2) Howard Hughes Medical Institute, California Institute of Technology, Pasadena, CA; 3) Department of Neuroscience, University of Connecticut Health Center, Farmington, CT.

The ALA neuron is the master control center for stress-induced sleep in *C. elegans*. The molecular and cellular mechanisms underlying ALA-mediated sleep are not clear. Our previous work suggests that multiple neuropeptides from the ALA neuron work collectively to regulate sleep, each of which primarily regulates particular aspects of sleep that include pumping quiescence, locomotion quiescence, and sensory depression. Here, we focus on the FMRFamide-like peptide FLP-24. We found that *flp-24* is expressed in the ALA neuron and a few other neurons. Constitutive overexpression (OE) of *flp-24* specifically led to a lethargic phenotype, characterized by the lack of movement. Preliminary electrophysiological data showed that *flp-24* worms have normal evoked neurotransmitter release at the neuromuscular junction and normal muscle sensitivity to exogenous levamisole, nicotine and GABA, but may have defects in muscle excitability. From a forward genetic screen, we identified multiple strong suppressors, all of which define the same gene, encoding a G protein-coupled receptor, temporarily called FLIPPER. Transcriptional reporter analysis of *flipper* revealed that it is strongly expressed in body wall muscles and a handful of neurons. Tissue-specific rescue experiments showed that *flipper* is likely to function in body wall muscle. We also found that *flp-24* is sufficient to inhibit sensory function when its putative receptor *flipper* is ectopically expressed in sensory
neurons. Based on these results, we propose that FLP-24 controls locomotion quiescence during stress-induced sleep in *C. elegans* primarily by reducing the excitability of muscles.


The ability to form associative memories is one of the most important functions of an organism’s nervous system. Identifying molecules involved in these processes is important not only to gain an understanding of normal brain function, but can also lead to an understanding of disease states such as Alzheimer’s disease and age-related cognitive decline. Associative memories are generally divided into three categories: short-term (STAM), intermediate-term (ITAM), and long-term associatory memory (LTAM), based on duration and molecular requirements of each type of memory. We have developed two positive olfactory association assays that pair a conditioned stimulus (CS) with an unconditioned stimulus (US) to measure S/ITAM and LTAM (Kaufmann et al., 2010; Stein and Murphy, 2014). S/ITAM is induced following a single CS-US pairing, while 16 hour LTAM requires seven CS-US pairings. These behaviors require processes that are conserved in higher organisms: STAM is both transcription- and translation-independent, ITAM is translation-dependent, and LTAM requires translation and CREB-mediated transcription. Here, we have identified a constitutively active mutant in the Gαq signaling pathway that exhibits a >10-fold extension of memory following S/ITAM training. This extended memory requires CREB and the AIM interneuron pair, which we previously found to be the site of CREB activity during LTAM formation (Lakhina et al., 2015). Increased CREB-dependent transcription appears to “prime” the mutants for memory consolidation. Surprisingly, altering Gαq signaling in a single chemosensory neuron is sufficient to cause a CREB-dependent memory extension following a single CS-US pairing, indicating a cell non-autonomous role for this pathway in enhancing memory circuit function. Activation of this pathway also appears to ameliorate age-related deficits in associative learning and memory; animals maintain their ability to learn and remember at an age when wild-type animals no longer exhibit long-term memory and associative learning is reduced by ~80%. These findings suggest that activation of Gαq in the AWG sensory neurons non-autonomously induces consolidation after a single CS-US pairing, bypassing the spaced training normally required for LTAM formation, and enables the maintenance of cognitive function with age. Understanding mechanisms by which S/ITAM can be converted into LTAM will enhance treatments to prevent deleterious memory consolidation, such as in PTSD, and to maintain and repair memory function lost with age-related cognitive decline and neurodegenerative disease.

**112 Temporal structure of mechanosensory signal and current behavioral state determine locomotory response.** M. Liu¹, J. Shaevitz¹, Y. Suehiro¹, Y. lino², S. Mitani¹ ¹) Lewis-Sigler Institute for Integrative Genomics, Princeton, NJ; 2) Department of Physics, Princeton University, Princeton, NJ; 3) Princeton Neuroscience Institute, Princeton University, Princeton, NJ.

We systematically measured sensorimotor transformations from the soft touch sensory neurons to locomotory behaviors in *Caenorhabditis elegans* to investigate how the nervous system interprets time-varying mechanosensory signals. Our results show that the animal’s sensorimotor response depends greatly on the temporal details of the mechanosensory signal, and is modulated by the animal’s current behavioral state.

We delivered white-noise-like optogenetic stimuli to the soft touch mechanosensory neurons of populations of animals while simultaneously recording their behavior, using a novel high throughput optogenetic assay. We then used machine learning to identify transitions between behaviors (Berman et al. 2014), and a reverse-correlation approach (Gepner et al. 2015) to mine our recordings to reveal how mechanosensory stimuli influence behavioral transitions. We measured more than 2,000 worm-hours of optogenetic stimulation containing millions of behavioral transitions that provided us with unprecedented statistical power.

We identified many discreet behaviors (e.g. fast reverse locomotion) that are enriched when the worm is experiencing mechanosensory stimulation and we measured the precise temporal properties of the stimuli that bias the animal to adopt one behavior over another. Our measurements posited a set of models of sensorimotor transformations that predict how the worm would respond to novel stimuli. By delivering these novel stimuli in separate experiments, we demonstrated that we can evoke specific behaviors just as predicted. For example, we anticipated and then observed that an increasing temporal ramp of stimulus intensity drives animals that are currently moving forward to slow down; conversely, a decreasing temporal ramp of stimulus intensity causes them to speed up. We also found that the optimal stimuli for inducing behavioral transitions depend on the animal’s current behavioral state, which suggests that the worms exhibit task-based modulation of sensory processing. For example, we observed that a specific time varying stimulus will bias the animal to transition into fast reverse locomotion when the animal is in a slow forward or slow reverse locomotion state. However, when the worms are in an omega turn state, transition into fast reverse locomotion appeared entirely uncorrelated with stimuli. Furthermore, we found that neuronal system integrates mechanosensory signals on a time scale of ~10 seconds.

**113 OFF-responding interneurons controls stimulus strength-dependent sensory processing.** S. Hori¹, S. Oda², Y. Suehiro¹, Y. Iino², S. Mitani¹ ¹) Tokyo Women’s Medical University School of Medicine, Tokyo; 2) The University of Tokyo, Tokyo; 3) MRC Laboratory of Molecular Biology, Cambrid.

Sensory processing is a neurological function to integrate multi or monosensory information depending on the context by the central nervous system, and each sensory stimulus leads to proper behavior for survival in a complex environment. Particularly, monomodal sensory integration is the important neural function to gauge stimulus strength for proper behavior. *C. elegans* exhibits three aversive behaviors, defined as short reversal, long reversal, and omega turn. Omega turn generates the largest change in direction with a deep ventral head bend. Probabilities of each type of avoidance behavior vary on the contexts, for
example, by the type of stimulus, on or off food, and timing. However, it falls behind multi-modal integration in identification of the neural basis. Here we report monomodal sensory integration in *C. elegans* using both optogenetics and osmolality to control stimulus strength. Behavioral screening of 279 neural transcription factors identified an *atonal* homolog *lin-32*, which is required for stimulus strength-dependent behavioral adjustment by selecting from reversal and turn. *lin-32* regulates AIB differentiation through expression of electrical and chemical synapses, *inx-1* and *glt-1*. Removal of strong stimulation induced OFF-response depolarized of AIB to turn, suggesting that AIB is a key neurons to gauge stimulation intensity. *inx-1* mutants showed a similar defect to that of *lin-32* mutants and it was completely rescued by INX-1 expression in AIB cell lineage, indicating that AIB gap junction is required as a main synapse. Finally, AIB sends aversive information to RIS via INX-1 and INX-6 heterotypic gap junction to promote powerful contraction of neck muscle leading to turn. We have also obtained 18 candidates involved in aversive behaviors by RNAi screening. Some belongs to the same genetic pathways, suggesting that they act in cascade. Analyses on other candidate genes will clarify the whole gene network for sensory integration in the future.

114 Neural pulses code chemical gradients dictating chemotaxis strategy. R. Ruach, E. Itskovits, A. Zaslaver Department of Genetics, The Silberman Life Science Institute, Edmond J. Safra Campus, Hebrew University, Jerusalem, Israel.

Animals navigate through chemical gradients in a process known as chemotaxis. While extensively studied, little is known about how sensory neurons encode intricate spatio-temporal gradients. We developed a microfluidic-based device that allows delivering virtually any desired temporal gradient to the worm while simultaneously measuring its neural activity. Surprisingly, smooth monotonic gradients are encoded cell-autonomously by pulsatile activity in the AWA neuron, where the steeper the gradient, the higher is the amplitude and the frequency of the response. Moreover, we find a similar pulsatile activity in freely-chromotaxing animals: increasing neural activity locks the animal to move forward, and the subsequent decreasing activity is followed by a reorientation; Interestingly, the downstream interneuron AY pulses in-sync with AWA, indicating that ‘run and tumble’ behavioral outputs are encoded already at the sensory level.

Furthermore, we uncovered a novel intriguing principle: while commonly accepted that animals sense only the first derivative of a chemical gradient, we find that neurons adapt to the magnitude of the first derivative, effectively sensing higher derivatives of the gradient. This strategy becomes advantageous for animals located remotely from their target where gradients are typically shallow and up-gradient trajectories are difficult to infer; indeed, simulations show that such a strategy outperforms the classical biased random walk strategy.

Increasing turn probabilities when adapting to the first derivative of the gradient bears a cost of possible erroneous reorientations despite continuously climbing up the gradient. To this end, the AWC sensory neuron responds persistently in a non-pulsatile manner whenever the animal senses decreasing gradients, leading to a rapid reorientation that may reduce costs incurred following off-course turns. Together, these rich orchestrated activities expose novel principles in coding intricate chemical gradients which underlie efficient and robust chemotactic performance.

115 The *C. elegans* MAST Kinase Acts through Stomatin and Diacylglycerol Kinase to Regulate Thermotaxis Behavior. S. Nakano1, M. Ikeda1, A. Giles1, T. Suzuki2, A. Sano1, R. Kondo1, T. Higashiyama2, I. Morit1 1) Grad. Sch. of Science, Nagoya Univ; 2) ERATO, JST; 3) IITbM, Nagoya Univ.

*C. elegans* navigate themselves toward the past cultivation temperature when placed on a thermal gradient. Previous studies showed that temperature sensation and its memory in AFD thermosensory neuron is essential to achieve this thermotaxis behavior. However, how AFD exploits the temperature information to generate a proper neuronal output remains elusive. Through newly conducting a genetic screen for thermotaxis-defective mutants, we revealed here that two genes, *kin-4* and *mec-2*, play important roles in the AFD function.

A null mutation in the gene *kin-4*, which encodes the *C. elegans* homolog of MAST (Microtubule Associated Serine Threonine) kinase, caused a cryophilic phenotype, while a gain-of-function mutation in *mec-2*, which encodes a stomatin-like membrane-associated protein, caused a thermophilic phenotype. We also observed that this *mec-2(gf)* mutation suppressed the *kin-4* null phenotype, suggesting that *mec-2* might act downstream of *kin-4*. Both *kin-4* and *mec-2* are expressed and function in AFD.

Temperature-evoked calcium responses in *kin-4* and *mec-2(gf)* mutants were grossly normal, suggesting that these genes act downstream of the calcium influx in AFD.

To identify the molecular mechanism by which *kin-4* and *mec-2* regulate thermotaxis, we screened for mutants that can suppress the thermophilic phenotype of *mec-2(gf)* and isolated loss-of-function mutations in *dgk-1*, which encodes a *C. elegans* homolog of diacylglycerol (DAG) kinase. We found that like *kin-4* and *mec-2*, *dgk-1* acts in AFD to regulate thermotaxis.

Our results suggest a novel signal transduction pathway in which *kin-4/MAST kinase and MEC-2/Stomatin might regulate DGK-1. Since DGK-1 has been shown to be important for synaptic transmission by controlling the level of DAG, *kin-4* and *MEC-2* may also regulate the DAG level in AFD to coordinate the synaptic transmission. We are currently examining the dynamics of DAG in AFD of wild-type and mutant animals, and are assessing whether *kin-4* and *MEC-2* affect the subcellular localization or the activity of DGK-1. Further study will reveal a fundamental role of DAG in integrating the sensory information to neuronal output and uncover a new mechanism of the regulation of DAG, an important lipid second messenger that functions in a diverse biological processes, including synaptic vesicles cycling.

116 Sexually dimorphic control of gene expression in sensory neurons regulates decision-making behavior in *C. elegans*. Z.A. Hilbert, D.H. Kim Department of Biology, MIT, Cambridge, MA.

Animal behavior is directed by the integration of sensory information from internal states and the environment. Neuroendocrine
regulation of diverse behaviors of *Caenorhabditis elegans* is under the control of the DAF-7/TGF-β ligand that is secreted from sensory neurons. We have recently shown that *C. elegans* males exhibit an altered, male-specific expression pattern of *daf-7* in the ASJ sensory neuron pair with the onset of reproductive maturity, which functions to promote male-specific mate-searching behavior (Hilbert and Kim, eLife, 2017). We demonstrated that the switch-like expression of *daf-7* expression in the ASJ neuron pair is regulated by a hierarchy of inputs—sex, age, nutritional status, and microbial environment— which function in the modulation of behavior. Our recent work has begun to reveal critical regulators upstream of *daf-7* expression, including a conserved neuromodulatory signaling pathway previously implicated in the control of food related behaviors. We are currently characterizing these upstream regulators and their effects on *daf-7* gene expression and decision making behavior to further elucidate the mechanism through which multi-sensory experience can be integrated at the level of gene expression in a single pair of sensory neurons.

117 Insulin-like signaling regulates the prioritization of feeding behavior by regulating the chemoreceptor ODR-10 in *C. elegans*. Emily Wexler, Douglas Portman University of Rochester, Rochester, NY.

The feeding state of an animal can have deep effects on its behavioral decisions. Although many internal signals of nutritional state and hunger are known, the mechanisms by which these modulate neural circuits and behavior are less well understood. The *C. elegans* male exhibits an interesting example of such plasticity. Well-fed males prioritize mate-searching over feeding, but food-deprived males prioritize feeding until their nutritional needs are met. This behavioral modulation results in part from regulation of *odr-10*, the chemoreceptor for the food-associated odorant diacetyl. In adult males, expression of *odr-10* in the AWA chemosensory neurons is low, promoting exploratory behavior. Transient starvation activates *odr-10* expression increasing animals' attraction to diacetyl and food. By screening candidate mechanisms, we found that this regulation is mediated by the insulin-like signaling pathway. Loss of the insulin receptor *daf-2* results in increased *odr-10* expression and diacetyl attraction in males. Upregulation of *odr-10* requires the FoxO transcription factor *daf-16*, suggesting that changes in nutritional state regulate *odr-10* expression through the insulin signaling pathway. Consistent with this hypothesis, *daf-16* mutant males do not upregulate *odr-10* upon starvation. In addition, a candidate DAF-16 binding site lies upstream of the *odr-10* promoter, and disruption of this element eliminates the starvation-mediated upregulation of *odr-10* in males. Preliminary results also show that rescue of *daf-16* function in AWA restores the ability of *daf-16* males to upregulate *odr-10* in response to starvation, suggesting that insulin signaling acts in AWA to directly regulate chemoreceptor expression and food detection. While many targets of insulin signaling in the nervous system are known, the direct regulation of *odr-10* by *daf-16* provides new insight into the state-dependent modulation of sensory behavior by this pathway. Interestingly, the *odr-10* promoter also integrates other aspects of internal state. A separate downstream element necessary for the regulation of *odr-10* by genetic sex seems to recruit an unknown hermaphrodite-specific activator, generating high levels of *odr-10* expression in well-fed hermaphrodites. Because of this high baseline expression compared to males, the regulatory effects of insulin signaling are less apparent in hermaphrodites. This modular architecture of the promoter allows integration of distinct internal states to regulate chemoreceptor expression at the level of transcription, enabling behavioral flexibility in response to multiple internal cues.

118 Nitric oxide sensation and avoidance in *C. elegans*. Y. Hao1,2, W. Yang3, J. Ren3, H. Liu3, Y. Zhang3, J. Kaplan1,2 1) Department of Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Department of Neurobiology, Harvard Medical School, Boston, MA; 3) Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.

As a signaling molecule, nitric oxide (NO) regulates key physiological events in both prokaryotes and eukaryotes. NO is also a commonly encountered environmental cue that is generated by lightning or released into the air by organisms that produce NO with nitric oxide synthases. However, whether terrestrial animals respond to NO as a sensory cue has not been determined. We find that while *C. elegans* avoids a pathogenic bacteria (P. aeruginosa PA14), the avoidance of a PA14 mutant that is defective for NO production is much reduced, revealing the role of NO in eliciting avoidance behavior in the nematode. Using a NO donor solution, we show that *C. elegans* senses and avoids NO as an external sensory cue. NO sensation and avoidance depend on the function of the cyclic nucleotide gated ion channel subunit TAX-4 in the ciliated sensory neuron ASJ. Using intracellular calcium imaging, we also demonstrate that ASJ responds to the onset and removal of NO in a cell-autonomous manner. NO sensation by ASJ is mediated by a transmembrane guanylyl cyclase DAF-11 and a de-nitrosylating enzyme TRX-1. We are currently investigating our hypothesis that DAF-11 acts as an NO sensor to elicit the avoidance behavior.

Friday, June 23 8:30 AM – 11:30 AM
Carnesale Palisades Ballroom
Cell Biology

Co-chairs: Ann Wehman, University of Würzburg, and Francois Robin, Institut de Biologie Paris-Seine

119 Reconstitution of the PAR polarity networks in a heterologous cell system. Z. Han1, F. Motegi1,2,3 1) Temasek Lifesciences Lab, Singapore; 2) Mechanobiology Institute, Singapore; 3) Department of Biological Science, National University of Singapore, Singapore.

Cell polarity is essential for the structure and function in many cell types. A hallmark of polarized animal cells is the segregation of well-conserved PAR proteins into two distinct compartments at the cortex. Spatial patterning of PAR proteins
relies on antagonistic interactions between two complexes, one consists of Cdc42/PAR-6/PKC-3/PAR-3 and the other contains PAR-1/PAR-2. The design principle that governs local molecular interactions into global cellular patterning remains elusive. Here, we reconstitute the Caenorhabditis elegans PAR polarity networks in a heterologous system, budding yeast. The introduced complexes of PAR proteins yielded spatial segregation into a bipolar pattern in yeast cells. Active form of Cdc42p recruits PAR-6, which then accumulates PKC-3 and PAR-3 at the cortex. PAR-3 forms cortical clusters, which is interfered by PKC-3 alone but not by the Cdc42p/PAR-6/PKC-3 complex. PAR-2 helps PAR-1 localize at the cortex complementary to the pattern of cortical PAR complexes. Our approach will also permit synthetic control of spatial behaviors of cell polarity proteins in living cells.

120  Spatiotemporal control of gene knock-out and protein-protein heterodimerization to study mitotic spindle positioning.  Lars-Eric Fielmich, Ruben Schmidt, Anna Akhmanova, Sander Van den Heuvel  1) Developmental Biology, Utrecht University, Utrecht, NL; 2) Cell Biology, Utrecht University, NL.

Tissue development and homeostasis depend on the correct axis and plane of cell division, which are determined by the position of the mitotic spindle. Studies of the C. elegans one-cell embryo have provided much insight in the regulation of spindle positioning during asymmetric cell division. Pulling forces that act from the cell cortex on astral microtubules were found to position the spindle. These pulling forces depend on a force-generating complex (FG) that anchors the dynein motor to the cortex. The FG is a conserved complex consisting of a membrane-bound Ga protein that, in the GDP-bound form, binds the GPR-1/2 linker protein, which interacts with the LIN-5 coiled-coil protein. The discovery of a non-canonical role of Ga as a membrane-anchor for the FG was surprising at the time, and still is only partly understood.

The Guanine-nucleotide Exchange Factor (GEF) RIC-8 and GTPase-Activating Protein (GAP) RGS-7 regulate the GDP/GTP cycle of Ga and are also required for proper force generation. It remains unclear how this corresponds to Ga functioning as a membrane anchor. Hampering analysis, complete inactivation of ric-8 and rgs-7 appears difficult to achieve by RNAi and null mutants are not viable. We therefore developed an inducible, tissue-specific gene knock-out system based on Cre-Lox recombination. We introduced Lox sites in the endogenous ric-8 and rgs-7 loci to enable KO of the genes. To assure spatiotemporal control of the KO, we use a two-component recombinase system. Thus, gene KO can be induced in the tissue of choice, for example the germline, while maintaining a healthy strain.

A complementary approach focuses on the individual functions of FG components. Here, we use the light-inducible heterodimerization of LOV and ePDZ to reconstitute cortical FG complexes that lack one or several components. Using a PH::LOV as membrane anchor and an ePDZ::dynein knock-in, we were able to recruit endogenous dynein directly to the membrane and bypass the FG. This was insufficient for pulling force generation and suggests that the FG is more than a dynein anchor. To test the role of Ga signaling, we generated and are currently analyzing an ePDZ::GPR-1 knock-in to reconstitute a cortical FG that lacks the Ga component. We will present our latest results at the meeting.

121  The RNA-binding protein ATX-2 regulates cytokinesis through PAR-5 and ZEN-4.  Megan M. Gnazzo, Eva-Marie E. Uhlemann, Alex R. Villarreal, Masaki Shirayama, Eddie G. Dominguez, Ahna R. Skop  1) Laboratory of Genetics, UW-Madison; 2) University of Massachusetts Medical School; 3) Department of Medicine, UW-Madison.

The spindle midzone harbors both microtubules and proteins necessary for furrow formation and the completion of cytokinesis. However, the mechanisms that mediate the temporal and spatial recruitment of cell division factors to the spindle midzone and midbody remain unclear. Here, we describe a mechanism governed by the conserved RNA Binding Protein (RBP), ATX-2/Ataxin-2, that targets and maintains ZEN-4 at the spindle midzone. ATX-2 does this by regulating the amount of PAR-5 at the spindle midzone. Preventing ATX-2 function leads to elevated levels of PAR-5, enhanced chromatin and centrosome localization of PAR-5-GFP, and ultimately a reduction of ZEN-4-GFP at the spindle midzone. Co-depletion of ATX-2 and PAR-5 rescued the localization of ZEN-4 at the spindle midzone, indicating that ATX-2 mediates the localization of ZEN-4 upstream of PAR-5. Together, we provide the first direct evidence that ATX-2 is necessary for cytokinesis and suggest a model in which ATX-2 facilitates the targeting of ZEN-4 to the spindle midzone by mediating the posttranscriptional regulation of PAR-5.

122  Mitochondrial redox signaling induces polarization in the C. elegans embryo.  S. De Henau, T.B. Dansen  Molecular Cancer Research, University Medical Center Utrecht, Utrecht, NL.

Mitochondrial redox signaling plays a critical role in many biological processes and relies on the well-regulated production of H₂O₂ as signaling molecule. However, the regulatory mechanisms that lead to specificity in mitochondrial H₂O₂-signaling remain elusive, mainly because good model systems to address this question are not available. Cell polarization requires the dynamic regulation of signaling cascades in both time and space, making it an attractive model to study localized, subcellular signal transduction. We take advantage of this and use the C. elegans early embryo, one of the most-studied systems for cell polarization, to analyze the spatiotemporal regulation of redox signaling.

We find that, coinciding with polarization, a subgroup of mitochondria relocates to the cell membrane at the site of symmetry breaking. After this, mitochondria become highly motile and localize closely to the posterior cortex of the embryo. An ultrasensitive H₂O₂-specific sensor shows that mitochondrial relocation to the cell membrane is accompanied by a striking increase in cortical H₂O₂-levels. Furthermore, mitochondrial H₂O₂ directly influences polarization, since compounds that alter mitochondrial H₂O₂-production affect symmetry breaking and maximal polarization.

Key in polarization is the asymmetric rearrangement of cortical actomyosin-rich cables away from the newly formed posterior
pole. We find that mitochondrial H$_2$O$_2$ inhibits cortical NMY-2 dynamics as well as activity of RHO-1, an actomyosin regulator. RHO-1 displays highly conserved and well described redox sensitivity, and we are currently mutating its redox-sensitive cysteines to determine if this protein is a direct target of mitochondrial H$_2$O$_2$ during polarization.

In conclusion, we have developed a pioneering model based on cell polarization to study spatiotemporal redox signaling. Based on our results, we propose that 1) mitochondria induce symmetry-breaking by increasing H$_2$O$_2$-levels at the cell cortex and so inhibit actomyosin contractions, and 2) by moving anteriorly along the cortex during polarization, mitochondria generate a "H$_2$O$_2$ wave" that signals to collapse the actomyosin network in the anterior direction.

123 A potential role for midbodies in developing tissues of *C. elegans*. Xiaofei Bai$^1$, Bi-Chang Chen$^2$, Ryan Simmons$^1$, Chris Turpin$^1$, Lindsey Klebanow$^1$, Diana Mitchell$^1$, Eric Betzig$^2$, Joshua Bembenek$^1$ 1) Biochemistry and Cellular and Molecular Biology Department, University of Tennessee, Knoxville, Knoxville, TN; 2) Janelia Research Campus, HHMI, Ashburn, VA.

The midbody forms at the end of cytokinesis and facilitates abscission, the final separation of the daughter cells. Recently, the midbody has been implicated in several developmental processes, including cell fate specification, dorsoventral axis formation, neurite growth, cilium formation, and apical polarity during epithelial lumen formation. To investigate developmental roles of the midbody, we examined midbody fate in the invariant lineage of the *C. elegans* embryo. Cytokinesis occurs in stereotypical patterns that are unique and tissue specific with a defined pattern of midbody inheritance. In the first mitosis, a relatively symmetric furrow results in a centrally positioned midbody that is always internalized by the P1 daughter cell. In the next AB cell division, a highly asymmetric furrow positions the midbody next to EMS, which internalizes it instead of either AB daughter cell. A dramatic shift in midbody behavior is observed in several tissues at around the 300-cell stage when the embryo undergoes global morphogenetic changes. In two lumen-forming tissues, the intestine and the pharynx, midbodies form after symmetric furrowing and migrate across the cell to the future apical midline. This coordinated midbody migration event coincides with polarization events in intestinal epithelia. At the apical midline, the midbody ring is internalized and disappears. However, Aurora B kinase (AIR-2) remains on the apical surface for over an hour after cytokinesis and polarization. A similar apical localization pattern is observed for AIR-2 in the pharyngeal primordium. Finally, in cells that form the inner labial sensilla (ILS), we observe symmetrical cytokinesis followed by a midbody migration event that leads to a focal aggregation of AIR-2. AIR-2 persists along the leading edge of developing dendrites (which also may be apical) as they migrate towards the anterior end of the embryo, anchor at the tip of the embryo and elongate. Other midbody markers are either internalized or maintained with AIR-2 in a tissue-specific manner. Inactivating several fast-inactivating temperature sensitive cytokinesis mutants late in embryogenesis causes severe defects in positioning, continuity and shaping of the intestinal and pharyngeal lumen. Many animals fail to hatch, but among those that do, a high percentage show defects in neuronal DiI staining. These data suggest that the proper execution of cytokinesis, which shows surprising flexibility during development, and specific cytokinesis regulators such as AIR-2, may regulate different aspects of development including the final interphase architecture of a terminally dividing cell.

124 Cilia length and Intraflagellar transport regulation by kinases PKG-1 and GCK-2 in *C. elegans* sensory neurons. Prerana Bhan, Muniesh Shanmugam, Hsin-Yi Huang, Ron Hsieh, Victor Lee, Helly Punjabi  Department of Life Science, National Tsing Hua University, Hsinchu, TW.

Statement of Purpose: A plethora of human diseases are based on cilia dysfunction including polycystic kidney disease, Bardet-Biedl and Meckel-Gruber syndrome. Fortunately, basic mechanisms underlying cilia development and intraflagellar transport (IFT) became more understandable in recent years. Though at the same time a considerably complex ciliary machinery was unravelled leading to new questions, specifically, how IFT cargo assembles at the cilia base, how it localises to cilium, and how “IFT trains” are regulated. One intriguing recent finding describes the ciliary regulating function of two *C. elegans* kinases DYF-5 and DYF-18, and we hypothesised that even more kinases and phosphatases may be uncovered. We, therefore, employed data mining tools to identify kinases and phosphatases specifically expressing in *C. elegans* ciliated sensory neurons.

Methods: We then used a broad range of methods like dye-filling, Chemotaxis, IFT component expression pattern etc to investigate the effects of selected kinases and phosphatases on ciliogenesis and IFT, and have identified PKG-1 as well as GCK-2 as potential candidates that significantly affect cilia development and cargo transport.

Results: In pkg-1 mutants, severe accumulation of homodimeric kinesin-2 OSM-3 at the cilia tip was observed in conjunction with an overall reduction in retrograde speeds of ciliary dynein XBX-1, leading to abnormal cilia morphology, likely as a function of reduced tubulin acetylation. While in gck-2 mutants OSM-3 and IFT-particle A (CHE-11) motility was significantly elevated in conjunction with increased tubulin acetylation, further KAP-1 motility was decreased, confirming a recent model in which the slow KAP-1 motor restricts the motility of the fast OSM-3 motor. Crucially, all observed effects in mutant animals can be rescued by overexpressing the respective protein PKG-1 or GCK-2 (under the cilia specific Posm-5 promoter). Both, PKG-1 and GCK-2 follow similar expression pattern in cilia, localising distally of the middle segment as well as near the tip of the distal segment. Because PKG-1 is related to the cGMP pathways, we knocked down the upstream effectors DAF-11 and ODR-1, respectively, used rapamycin to inhibit this pathway leading to similar effects as seen in gck-2 mutants. In summary, we identified and by overexpressing the respective protein PKG-1 or GCK-2 (under the cilia specific Posm-5 promoter). Both, PKG-1 and GCK-2 conjunction with increased tubulin acetylation, further KAP-1 motility was decreased, confirming a recent model in which the

125 An unexpected link between fusogen activity and intracellular lumen elongation. F. Soulavie$^1$, D. Hall$^2$, M. Sundaram$^3$ 1) Department of Genetics, University of Pennsylvania, Philadelphia, PA 19104; 2) Department of Neuroscience, Center for C. elegans Anatomy, Albert Einstein College of Medicine, Bronx, NY 10461.

Unicellular tubes with intracellular lumens are found in the mammalian microvasculature and in some invertebrate organs, including the *C. elegans* excretory (renal-like) system. Many unicellular tubes are seamless—they lack autocellular junctions...
along their length – and they adopt elongated or branched shapes. How cells become seamless tubes with such complex shapes is poorly understood. One proposed mechanism for seamless tube formation involves endocytosis of basal membrane followed by vesicle merging or exocytosis to form a lumen. An alternative mechanism involves cell wrapping to form a lumen lined by an autacellular junction, followed by membrane auto-fusion to remove that junction and become a seamless toroid. We provide evidence that the plasma membrane fusogen AFF-1 is involved in both mechanisms of intracellular lumen growth. The C. elegans excretory duct tube forms by wrapping and auto-fusion, and subsequently elongates more than five-fold and adopts an unusual asymmetric shape. Both auto-fusion and subsequent tube elongation depend on the EGF-Ras-ERK signaling cascade and its downstream target, aff-1. In the absence of aff-1, the duct retains an autacellular junction and has a dramatically shortened morphology, with the lumen only ~a third of its normal length. Super-resolution and electron microscopy showed that aff-1 mutants accumulate excess vesicles with apical cargoes adjacent to the main lumen, and large inclusions with highly convoluted membranes adjacent to and continuous with the basal membrane. Accumulation of the membrane-binding dye FM4-64 in aff-1 mutant duct cells suggests that the basal inclusions may correspond to a blocked endocytic intermediate.

AFF-1 is an exoplasmic fusogen, and therefore could be suitably positioned to mediate dynamin-independent vesicle scission during endocytosis. Based on our observations, we propose that EGF signaling stimulates basal endocytosis in the growing duct tube, and that AFF-1 could be needed for scission of the internalized tubulovesicles, which ultimately contribute apical membrane to drive lumen growth. To further test this model, we are currently using the ZIF-1-dependent proteolysis system to remove AFF-1 from the duct cell after auto-fusion is complete, and following the subsequent progress of membrane trafficking. This work was supported by NIH grant GM58540 to M.S.

126 The fusogen EFF-1 promotes a novel cell process-specific engulfment program. Piya Ghose1, Meera Trivedi1, Peter Insley1, Anupriya Singhal1, Yun Lu1, Pavan Shah2, Zhirong Bao2, Shai Shaham1 1) The Rockefeller University, New York, NY; 2) Memorial Sloan Kettering, New York, NY.

Programmed cell death and cell process pruning are common in metazoan development and homeostasis. Dismantling of morphologically complex cells, sporting long processes, poses a particularly interesting problem, as different regions of a cell are often in different microenvironments, and contact different cells. We have used the C. elegans tall-spine cell (TSC) to understand the death and clearance of a morphologically complex cell. The TSC extends a microtubule-laden process towards the tail tip during embryonic morphogenesis and then dies. TSC ablation reveals that this cell promotes tall morphogenesis. Tracking of TSC demise using still images and long-term light-sheet microscopy reveals that this morphologically complex cell undergoes three distinct degeneration/clearance events. The proximal TSC process is dismantled first, and undergoes beading reminiscent of Wallerian degeneration. The cell soma then dies and is cleared in a manner resembling other apoptotic cells. The distal process retracts and accumulates in a distinct varicosity. All three mechanisms depend independently on CED-3/caspase. Importantly, a similar sequence accompanies the demise and clearance of embryonic CEM neurons in hermaphrodites. Thus, the TSC death program may represent a general mechanism for dismantling morphologically complex cells, including neurons.

Clearance of the proximal and distal TSC fragment is independent of known apoptotic clearance proteins, except for SAND-1, which promotes phagosome maturation, suggesting that novel mechanisms are at play. From a genetic screen for mutants disrupting TSC clearance, we identified two mutants with lesions in eff-1, encoding a previously-described C. elegans fusogen. Mutant animals, and animals carrying the canonical eff-1(hy21) allele, are defective in TSC distal process varicosity clearance, and do not display cell body or proximal process clearance defects. EFF-1 is expressed in and functions in hyp10 cells, which surround and engulf TSC process remnants. While hyp10 appears to recognize the distal process varicosity, the phagosome that is formed seems not to be closed, suggesting that EFF-1 may mediate the fusion event required for membrane scission and phagosome sealing. Supporting this idea, the undegraded open phagosome is labeled with PLCd-PH::mKate2, a reporter for undegraded open phagosome. Furthermore, a fusion-dead version of EFF-1 fails to rescue distal process clearance. EFF-1 also localizes to puncta adjacent to the TSC remnant, perhaps corresponding to the site of coalescing phagosome arms.

Direct mediators of membrane scission that promote the formation of endosomes, phagosomes, and other plasma membrane-derived organelles are not known. Our data reveal a novel paradigm for complex cell dismantling that may be broadly conserved, and suggest a novel role for EFF-1 in the scission event promoting phagosome sealing.


NIMA-related kinases (NEKs) are highly conserved among eukaryotes and have been reported to regulate cell cycle processes along with ciliogenesis and DNA repair. Nevertheless, relatively little is known about NEK kinase targets or NEK pathway components. Whereas the human genome encodes for 11 NEK kinases, C. elegans encodes for only 4, including NEKL-2/NEK8 and NEKL-3/NEK6/7. We have reported a requirement for NEKL-2 and NEKL-3 in C. elegans molting and have shown that these kinases influence intracellular trafficking in epidermal cells. Furthermore, NEKL-2 and NEKL-3 appear to function in complementary pathways and within distinct molecular complexes containing several conserved ankyrin-repeat proteins (MLT-2/ANKS6, MLT-3/ANKS3, MLT-4/Inversin). To gain insight into the molecular and cellular functions of NEKL–MLT network, we carried out genetic screens to identify suppressors of the synthetic lethal molting-defective phenotype of nekl-2; nekl-3 double mutants. For these screens, we used weak aphenotypic alleles generated by CRISPR (nekl-2) and the million-mutation project (nekl-3). Our screens were therefore designed to simultaneously identify both nekl-2 and nekl-3 pathway suppressors, thus increasing efficiency. Moreover, by using very weak nekl alleles, our screens should have high sensitivity and thus capture a wide range of suppressor mutations. We also incorporated a novel counter-selection method, using the peel-1 toxin, to reduce the amount of labor. Finally, a unique subtraction method was carried out in conjunction with whole genome
sequencing to help narrow down candidate causal mutations within the suppressor strains. Among the identified suppressors were strong LOF alleles of fcho-1, and apm-2/dpy-23. fcho-1 encodes the C. elegans ortholog of FCHO1, an F-bar protein that regulates the conformation and activity of AP2, a multimeric adapter complex involved in clathrin-mediated endocytosis. Strikingly, apm-2 encodes the mu2 subunit of AP2. Further studies have shown that RNAi of the alpha (apa-2) or sigma2 (apa-2) subunits of AP2 also suppress nekl-2; nekl-3 double-mutant lethality and that fcho-1(RNAi) suppresses molting defects in other members of the NEK-L–MLT network. Our data suggest that the NEK-L–MLT network may regulate the activity of the AP2 complex by balancing the actions of FCHO-1. Consistent with this, clathrin puncta, which are mislocalized in nekl-2; nekl-3 mutants, are restored to a more wild-type morphology in nekl-2; fcho-1; nekl-3 triple mutants. Interestingly, proteome studies identified several AP2 subunits in complex with human NEK7, suggesting that NEK family members may regulate clathrin-mediated endocytosis and AP2 in a wide range of species. These results, together with other data, strongly suggest that the regulation of trafficking and endocytosis is a conserved but previously overlooked function of NEK kinases.

128  Retromer-associated proteins and PAD-1 control TAT-5 flipase activity to inhibit extracellular vesicle budding.  Katharina Beer1, Gholamreza Fazeli1, Jeremy Nance2, Christian Stigler1, Ann Wehman1 1) Rudolf Virchow Center, University of Würzburg, Würzburg, Bayern, DE; 2) Skirball Institute, NYU School of Medicine, New York, NY; 3) Biocenter, University of Würzburg, Würzburg, Bayern, DE.

Cells release extracellular vesicles (EV) that can mediate intercellular communication to influence development and disease (Beer & Wehman, Cell Adh Migr 2017). Despite their pleiotropic functions, the molecular details of EV release are poorly understood, especially for plasma membrane budding (ectocytosis). Previously, we showed that TAT-5 phospholipid flipase activity inhibits ectocytosis and maintains the asymmetric localization of the lipid phosphatidylethanolamine (PE) in the inner leaflet of the plasma membrane (Wehman et al., Curr Biol 2011). In a screen for additional proteins that inhibit BV budding, we identified new TAT-5 regulators related to the retromer recycling pathway (PI3Kinase VPS-34, Beclin1 homolog BEC-1, and RME-8) together with the Dopey domain protein PAD-1. PI3K, RME-8, and sorting nexins are required for the localization of TAT-5 to the plasma membrane, which is important to maintain PE asymmetry. PAD-1 also localizes to the plasma membrane, but is not required for TAT-5 localization. Rather, PAD-1 is required for the lipid flipping activity of TAT-5, further supporting the model that PE asymmetry regulates plasma membrane budding. Our study identifies new proteins that regulate extracellular vesicle release and pinpoints TAT-5 and phosphatidylethanolamine as key regulators of plasma membrane budding. Understanding the mechanisms of EV release will enable us to determine the in vivo roles of EVs during development and homeostasis.

129  The lysosomal endoribonuclease RNST-2 degrades ribosomal RNA to support embryonic and larval development in C. elegans.  Y. Liu1,2, W. Zou1, X. Wang1,2  1) National Institute of Biological Sciences, Beijing, China; 2) Institute of Biophysics, Chinese Academy of Sciences, Beijing, China.

Lysosomes degrade cargoes derived from endocytosis, autophagy and phagocytosis and the resulting catabolites are transported out of lysosomes and re-utilized in cellular metabolism. Thus, lysosomes play important roles in cell homeostasis. We isolated a recessive mutant qx245 from an EMS screen, which contains enlarged lysosomes in hypodermal and sheath cells. By SNP mapping and whole genome sequencing, we found that qx245 affects mst-2, which encodes a RNase T2 family protein. RNST-2 is widely expressed and localizes to lysosomes. Loss of RNST-2 function leads to accumulation of rRNA, ribosomal proteins and autophagic cargoes in enlarged lysosomes and these phenotypes were partially suppressed by autophagy-defective mutations. This indicates that tRNA and ribosomal proteins accumulated in mst-2 lysosomes are delivered through autophagy. mst-2(qx245) mutants are defective in embryonic and larval development. Importantly, loss of both mst-2 and de novo synthesis of pyrimidine leads to almost 100% embryonic lethality, which can be rescued by addition of uridine and cytidine. These data suggest that lysosomal degradation of rRNA by RNST-2 provides pyrimidine required for embryogenesis. Our data reveal the essential role of autophagy- and lysosome-dependent degradation of ribosomal RNA in animal development.

130  Interdependent accumulation of Cadherin-Catenin complexes and WAVE/SCAR branched actin regulators at the apical junction promotes epithelial morphogenesis.  S. Sasidharan, S Borinskaya, E Larsen, M Soto  Department of Pathology and Laboratory Medicine, Robert Wood Johnson Medical School, Rutgers University, 675 Hoes Lane, Piscataway, NJ 08854.

Our lab studies embryonic morphogenesis in C. elegans. Our earlier work showed that branched actin, powered by the WAVE/SCAR and Arp2/3 complexes, promotes polarized events during epithelial tissue development. These events include epidermal cell migration during ventral enclosure, and the polarized formation of the embryonic intestine. Actin is an integral component of the epithelial apical junction, yet the interactions of branched actin regulators with apical junction components are still not clear. We used live imaging of C. elegans embryos and newly available CRISPR-tagged strains (from our lab and the Goldstein lab) to define the developmental time course of accumulation of apical junction components relative to the branched actin regulators WVE-1 and ARP-2/ARX-2 as the embryonic intestinal epithelium becomes polarized. Live imaging of apical F-actin accumulation in the developing intestine showed that the two main apical junction complexes, namely Cadherin-Catenin Complex (CCC) and DLG-1 AJM-1 complex (DAC), branched actin regulators, and non-muscle myosin were essential for apical actin enrichment. Biochemical data have shown that alpha catenin (a CCC component) inhibits Arp2/3-dependent branched actin, which suggests branched actin is only needed at the earliest stages of apical junction development. We found that loss of WVE-1 led to increased levels of CCC components after junction formation. The CCC components, in turn, regulated the levels of WAVE. Intriguingly, loss of two components of the CCC, E-Cdh/HMR-1 and b-catenin/HMP-2, reduced levels of WVE-1 at apical junctions whereas loss of a-catenin/HMP-1 led to elevated apical WVE-1. Due to the interdependency in the localization
of CCC and WVE-1 at the apical junction (AJ) and the temporal analysis of CCC and WAVE complex accumulation, we conclude that the CCC accumulates at AJ first and aids in recruitment of WAVE complex. Later CCC and DAC help in the maintenance of WAVE complex levels at AJ. WVE-1 in turn regulates HMR-1 turnover and shows inversely complementary levels with respect to a-catenin at the AJ. Therefore branched actin regulators are continually recruited to junctions to set up dynamic junction formation and maintenance of the AJ. Our on-going studies are investigating the mechanism for the interdependency of WAVE and CCC components at the apical junction.

131 Linker cell migration uses neuronal signaling through the muscarinic acetylcholine receptor GAR-3. M. Kato, P. Sternberg Biology, California Inst Technology, Pasadena, CA.

The male gonadal leader cell, the linker cell (LC), migrates along the ventral nerve cord during the L4 stage. Transcriptional profiling previously revealed that the LC expresses neuronal receptors, including a muscarinic acetylcholine receptor gar-3. We found that gar-3 mutants have a gonad migratory path defect in 22% of males. gar-3::GAR-3::YFP is expressed in the LC in both the L3 and L4 stages, but the localization of the receptor changes with stage. In the L3 stage when the LC mostly migrates along the dorsal bodywall, GAR-3::YFP has a uniform membrane expression pattern, while in the L4 stage when the LC migrates closely along the ventral nerve cord, GAR-3::YFP localizes asymmetrically in the membrane towards the ventral side. We found that increasing acetylcholine signaling using aldicarb, an acetylcholine esterase inhibitor, caused the LC to change its orientation from posterior-facing to anterior-facing in the L4 but not L3 stage. This response to aldicarb does not occur in specific rescue of gar-3 mutants while GAR-3::YFP overexpression increases the percentage of reversed LCs. We showed through performing tissue-specific rescue of gar-3 in mutants that this change in LC orientation requires the expression of gar-3 in the LC rather than in cholinergic neurons, where gar-3 is also expressed. This aldicarb induced, gar-3-dependent response to LC orientation uses the egl-30/Gaq, unc-73/TRIO, and egl-8/PLCβ downstream signaling pathways. In summary, we have identified signaling between the nervous system and a nonneuronal migratory cell, and specifically have found a stage-dependent role for acetylcholine signaling through the GAR-3 receptor in changing LC orientation.

Friday, June 23 3:00 PM – 4:30 PM
Royce Hall
Plenary Session 3

Chair: Susan Mango, Harvard University

132 Primordial germ cell survival depends on contact with the somatic gonad in the C. elegans embryo. Daniel McIntyre, Jeremy Nance NYU Medical Center, New York, NY.

We are studying the formation of the germline stem cell niche in the C. elegans gonad primordium, and investigating how niche cell contact promotes stem cell survival. Niche-derived cues can control stem cell quiescence, proliferation and differentiation. As such, establishment of niche-stem cell interactions is a critical stage in development. In C. elegans embryos, two primordial germ cells (PGCs, stem cell precursors), and two somatic gonadal precursor cells (SGPs, niche cells) come together to form the gonad primordium. This happens through a fascinating morphogenetic process in which the SGPs migrate to the PGCs and extend their cell membranes, wrapping around these cells. This process is critical for PGC survival. Ablation of the SGPs at this time causes death of the PGCs (Kimble and White, 1981) through an unknown mechanism. We repeated this experiment using fluorescent reporters to follow the fate of the PGCs after SGP ablation. Surprisingly, we observed that unprotected PGCs were often engulfed by neighboring endodermal cells. In a normal embryo, PGCs produce large lobes that are pinched off and digested by endodermal cells, suggesting that endodermal cells may inappropriately cannibalize the PGC cell body when SGPs are absent. Loss of endodermal cells in embryos lacking SGPs prevented PGC death, indicating that this is the case. Endodermal cells remove PGC lobes by assembling a ced-10 and lst-4 dependent scission complex composed of actin and dynamin around PGC lobe necks. We show that this pathway is also needed for PGC death following SGP ablation. We hypothesize SGP wrapping protects the PGC cell body, ensuring only the lobe is eaten by the endoderm. We are testing this hypothesis by specifically disrupting SGP wrapping of PGCs. While signaling between niche and stem cell is important in many systems, our work suggests that close physical contact with the niche also plays an unexpected role in protection of the primordial germ cells from cannibalism by neighboring cells.

133 X chromosome topology driven by condensin binding to eight high-affinity sites. Erika Anderson, Qian Bian, Caitlin Schartner, Barbara J. Meyer HHMI and UC Berkeley, Berkeley, CA.

Interphase chromosome structure is regulated at multiple length scales, and each level of organization controls nuclear functions such as transcription, replication, and recombination. To learn how these structures are formed, we dissected the mechanism by which a condensin complex imposes a distinct, higher-order structure across X chromosomes of C. elegans hermaphrodites. Here our deletion analysis of the endogenous X revealed that architectural proteins can remodel chromosome-wide topology by binding to a small number of sites. The dosage compensation complex (DCC), a specialized condensin complex, is recruited to dozens of specific sites (rex sites) on both hermaphrodite X chromosomes and represses X-linked gene
expression by half. The DCC establishes a higher-order structure composed of megabase-scale topologically associating domains (TADs) that is distinct from the structure of autosomes and male X chromosomes. The DCC-dependent TAD boundaries all contain a strong rex site, and the DCC promotes long-range interactions both within TADs and between rex sites within TADs. To discern the contributions of these different rex-site interactions to TAD boundary formation, we deleted the eight rex sites at DCC-dependent TAD boundaries and examined X structure using an updated in-nucleus Hi-C protocol that detects distant interactions efficiently. In the rex-delete strain, the specific loops between adjacent DCC-dependent TAD boundaries were eliminated. All eight DCC-dependent boundaries were lost or significantly weakened, producing a structure that recapitulates the X structure of a DCC mutant. Therefore, though the DCC binds dozens of sites on X, the TAD boundaries are established by binding to these eight high-affinity sites. Disruption of TAD structure by deleting a series of cis elements uniquely allows us to assess the effects of TAD boundaries on gene expression. The rex-deleted worms lack strong dosage compensation phenotypes, indicating that TAD boundaries alone are insufficient to enact full repression of X gene expression. Ongoing sensitive gene expression assays are determining whether TAD boundary disruption causes local or subtle transcriptional changes. Intra-TAD interactions present in wild-type and rex-delete animals but absent in DCC mutants likely underlie mechanisms that control X gene expression.

Furthermore, Hi-C results showed that in C. briggsae, which also uses a condensin DCC, X chromosomes have a unique TAD structure compared to that of autosomes, even though the rex sites are in different locations relative to C. elegans’ sites. Hi-C also revealed errors in C. briggsae’s genome assembly, which are now being corrected based on Hi-C data. This improved genome assembly facilitates studies of how condensin’s role in shaping higher-order chromosome structure is maintained as its binding sites evolve.

134 Nutrient-mediated environmental sex determination: fatty acids regulate germline sex-determination and germ cell fate through ACS-4-dependent myristoylation. Hongyun TANG, Min Han MCDB, HHMI AND CU BOULDER, Boulder, CO.

Fat metabolism has been linked to fertility and reproductive adaptation in animals and humans, but cellular and molecular mechanisms involved in the response of reproductive development to fat levels, including a potential role of lipids in environmental sex-determination, remain to be explored. Hypothesizing that a regulatory mechanism perceives and interprets lipid cues to regulate reproductive development, we investigated whether and how FA metabolism impacts the specification of germ cell fate. We first observed that somatic fatty acid metabolism is critically involved in the impact of food availability on germ cell fate. Subsequent screen and analysis identified ACS-4, an acyl-CoA synthetase, and its FA-CoA product, as key germline factors that mediate the role of FA in promoting oocyte fate. We next demonstrated that ACS-4-dependent protein myristoylation in the germ line can regulate germ cell fate by sensing the change of FA levels. Further genetic and molecular analyses indicate that this ACS-4-dependent protein myristoylation regulates the sex-determination pathway, and MPK-1/MAPK plays a critical role in mediating this modulation. Finally, by showing alternation in germ cell fate in C. remanei female with acs-4 knocked down, we obtained evidence that this FA impact on sex-destination and germ cell fate specification is conserved in male/female species. Our findings uncover a likely conserved mechanism by which FA, an environmental factor, regulates sex determination. This mechanism translates nutrient availability into instructive cue for reproductive development.

135 The C. elegans sperm epigenome and the fate of sperm epigenetic marking in early embryos. T Tabuchi, A Rechtsteiner, T Egelhofer, S Strome University of California, Santa Cruz, Santa Cruz, CA.

If and how fathers’ lifestyles impact their children’s development through non-genetic (epigenetic) information is an area of intense interest. Paternal contributions via chromatin marking is especially mysterious. This stems in part from the repackaging of sperm DNA in some organisms with protamines and reduced levels of histones. Our lab showed that in C. elegans H3K36me3 and H3K27me3, histone modifications associated with gene expression and gene repression, respectively, are transmitted to embryos from sperm and persist on paternal chromosomes for at least a few cell divisions. These findings raised the possibility that C. elegans sperm transmit epigenetic information to offspring in the form of modified histones. We have now defined the epigenetic landscape of C. elegans sperm and the fate of sperm marking in early embryos using ChIP-seq. We found that sperm retain histones genome-wide, and that sperm and embryos generally display similar antagonistic marking of the genome by H3K36me3 and H3K27me3. A surprising exception is a set of genes expressed exclusively in male germlines (spermatogenesis genes), which are marked by both active marks (H3K36me3 and H3K4me3) and a repressive mark (H3K27me3) in sperm. Interestingly, this “trivalent” marking of spermatogenesis genes resolves in embryos; the active marks disappear and the repressive mark persists. Another surprise was that many genes thought to be transcribed exclusively in early embryos (H3K27me3). They became sterile adults in a sensitized genetic background. These findings demonstrate that epigenetic information provided by sperm is necessary and sufficient to guide proper germ cell development. Our studies identify C. elegans as an excellent model for elucidating paternal contributions to epigenetic inheritance.
Heterochromatin factors collaborate with small RNA pathways to combat repetitive elements and germline genotoxic stress. Tessa Gaarenstroom, Alicia McMurchy, Przemyslaw Stempor, Brian Wysolmerski, Yan Dong, Darya Aussianikava, Alex Appert, Ni Huang, Paulina Kolasinska-Zwierz, Alexandra Sapetschnig, Eric Miska, Julie Ahringer. Department of Genetics, The Gurdon Institute, University of Cambridge, Cambridge.

Repetitive sequences derived from transposons make up a large fraction of the genome and must be silenced to protect genome integrity. Silencing of these elements is especially important in the germ line, where the piRNA pathway has been shown to be involved. Repeats are often found in heterochromatin, which in C. elegans are regions dispersed over the chromosome arms, however the roles and interactions of heterochromatin proteins are poorly understood. We have shown that a diverse set of heterochromatin factors act together with the piRNA and nuclear RNAi pathways to silence repetitive elements and prevent genotoxic stress in the germ line. HPL-2/HPL1, LIN-13, LIN-61, LET-418/Mi-2 and the H2K9me2 histone methyltransferase MET-2/SETDB1 show genome-wide co-binding and enrichment at repetitive elements, and mutants show a derepression of a subset of transposons. Furthermore, heterochromatin mutants are characterized by functionally redundant and temperature sensitive sterility, and display increased germline apoptosis and activation of DNA damage signalling. Remarkably, fertility of heterochromatin mutants could be partially restored by inhibiting expression of MIRAGE1 DNA transposons or endogenous meiotic double strand breaks. Loss of CEP-1/p53 also ameliorates both their fertility and somatic defects, suggesting that DNA damage signalling contributes to the phenotypes observed. Through genetics and transcriptional profiling, we uncovered complex interactions between heterochromatin factors and the piRNA and nuclear RNAi pathways, including functional redundancy in repetitive element repression between let-418 and ndre-2. This redundancy underlies the importance of safeguarding the genome through multiple means. It is also becoming evident that heterochromatic silencing occurs through different mechanisms at different genomic elements. We are currently dissecting interactions between the various factors, as well as H3K9me2/3, to uncover their mechanisms of action at heterochromatic regions, as well as investigating their roles in development and adult homeostasis.

MORC-1 integrates nuclear RNAi and transgenerational chromatin architecture to promote germline immortality. N.E. Weiser3,4, D.X. Yang3,4, S. Feng3,4, N. Kalinava4, K.C. Brown4, J. Khanika1a, M.A. Freeberg1, M.J. Snyder9, G. Csankovszki2, R.C. Chan4, S.G. Gu6, T.A. Montgomery4, S.E. Jacobsen5,10, J.K. Kim1 1) Department of Biology, Johns Hopkins University, Baltimore, MD; 2) Program in Cellular and Molecular Biology, University of Michigan, Ann Arbor, MI; 3) Life Sciences Institute, University of Michigan, Ann Arbor, MI; 4) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 5) Department of Molecular, Cell and Developmental Biology, University of California-Los Angeles, Los Angeles, CA; 6) Eli and Edythe Broad Center of Regenerative Medicine and StemCell Research, University of California-Los Angeles, Los Angeles CA; 7) Department of Molecular Biology and Biochemistry, Rutgers the State University of New Jersey, Piscataway, NJ; 8) Department of Biology, Colorado State University, Fort Collins, CO; 9) Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI; 10) Howard Hughes Medical Institute, Los Angeles, CA.

Germline-expressed endogenous siRNAs (endo-siRNAs) transmit multigenerational epigenetic information to ensure fertility in subsequent generations. In C. elegans, nuclear RNAi ensures robust inheritance of endo-siRNAs and deposition of H3K9me3 marks at target loci. The progressive loss of heterochromatin at endo-siRNA targets in nuclear RNAi mutants is thought to contribute to their mortal germline (Mrt) phenotype. However, the specific mechanisms by which nuclear RNAi mediates heterochromatin deposition and germline maintenance remain to be clarified. We have identified MORC-1, a highly-conserved zinc-finger protein, as a novel factor which links the nuclear RNAi pathway to heterochromatin formation and germline mortality. MORC-1 is essential for transgenerational fertility and acts as an effector of endo-siRNAs. Unexpectedly, MORC-1 is dispensable for siRNA inheritance but required for target silencing and maintenance of siRNA-dependent chromatin organization. To further elucidate the mechanism underlying the Mrt phenotype of morc-1(-) and nuclear RNAi mutants, we performed a forward genetic screen to identify mutations that suppress morc-1(-) Mrt. We identified four mutations in the gene encoding the H3K36 histone methyltransferase MET-1 as potent suppressors of morc-1(-) Mrt. Our finding that morc-1(-) and nuclear RNAi mutants exhibit progressive, MET-1-mediated gain of H3K36 methylation suggests that MORC-1 and nuclear RNAi play an important role in protecting their genomic targets from encroachment of H3K36me3.


Maternal effects of appropriate environmental conditions produce intergenerational phenotypic plasticity. Adaptive value of these effects depends on appropriate anticipation of environmental conditions in the next generation, and mismatch between conditions may contribute to disease. However, regulation of intergenerational plasticity is poorly understood. Dietary restriction (DR) delays aging but maternal effects have not been investigated. We demonstrate maternal effects of DR in the roundworm C. elegans. Worms cultured in DR produce fewer but larger progeny. Nutrient availability is assessed in late larvae and young adults, rather than affecting a set point in young larvae, and maternal age independently affects progeny size. Reduced signaling through the insulin-like receptor daf-2/InsR in the maternal soma causes constitutively large progeny, and its effector daf-16/FoxO is required for this effect. nhr-49/Hubf4, pca-4/FoxA, and skn-1/Nrf also regulate progeny-size plasticity. Genetic analysis suggests that insulin-like signaling controls progeny size in part through regulation of nhr-49/Hubf4, and that pca-4/FoxA and skn-1/Nrf function in parallel to insulin-like signaling and nhr-49/Hubf4. Furthermore, progeny of DR worms are buffered from adverse consequences of early-larval starvation, growing faster and producing more offspring than progeny of worms fed ad libitum. These results suggest a fitness advantage when mothers and their progeny experience nutrient stress, compared to an environmental mismatch where only progeny are stressed. This work reveals maternal provisioning as an organisinal response.
to DR, demonstrates potentially adaptive intergenerational phenotypic plasticity, and identifies conserved pathways mediating these effects.

Friday, June 23  5:00 PM – 6:00 PM
Royce Hall
Keynote Address
Judith Kimble, University of Wisconsin, Madison

Chair: Susan Mango, Harvard University

139 Of niches and naïveté.  J. Kimble  University of Wisconsin, Madison.
no abstract submitted

Saturday, June 24  8:30 AM – 11:30 AM
Grand Horizon Ballroom
Germline: Small RNAs and Chromatin

Co-chairs: Alyson Ashe, University of Sydney, Australia, and Germano Cecere, Institut Pasteur

140 Global profiling reveals new sub-classes of microRNAs and identifies germline microRNAome.  A.L. Gervaise1, S. Arur1,2  1) Program in Developmental Biology, Baylor College of Medicine, Houston, TX; 2) Genetics, UT MD Anderson Cancer Center, Houston, TX.

MicroRNAs (miRNAs) are small noncoding RNAs that regulate gene expression via post-transcriptional repression of target mRNAs. Since their discovery in C. elegans in 1993, miRNAs have been found to play a critical role in varied biological processes across many systems. Germ cells in particular rely on post-transcriptional and translational control, in part because oocyte development is largely transcriptionally silent. Despite a key role for post-transcriptional and translational mechanisms in controlling oogenesis, a role for miRNAs is highly debated in this process. To investigate whether miRNAs regulate oocyte development, and female germ cell specification, I systematically profiled all 399 mature C. elegans miRNAs during oocyte development using two different genomic assays. A microarray analysis on whole adult C. elegans (wild type and germline-deficient) revealed that 28 of the 399 miRNAs are germline-expressed. Strikingly, a Fireplex™ analysis on dissected germlines revealed that ~95% of the miRNAs are not expressed in the oogenic germline, suggesting a global repression of miRNA production. Surprisingly, of the 28 germline-expressed miRNAs, 19 are Drosha-independent. Thus, a majority of miRNAs generated in the germline seem to be generated through a non-canonical miRNA biogenesis pathway. To determine the spatial and temporal expression pattern of each of the 28-germline miRNAs, a locked nucleic acid based in situ hybridization was performed. In situ analysis thus far reveals that a large proportion of the germline miRNAs are expressed in arrested oocytes, and maybe maternally contributed to the developing embryo. This suggests that miRNAs may not mediate meiotic progression, but rather regulate oocyte meiotic maturation and early embryo/larval development. Together these analyses reveal a novel class of Drosha-independent germline-expressed miRNAs, which reveals that those expressed during oogenesis largely regulate oocyte maturation and/or early embryogenesis.

141 GTSF-1 is required for the formation of small RNA-producing complexes.  M. Vasconcelos Almeida1, S. Dietz2, S. Redl1, E. Karaulanov3, F. Butter2, R. Ketting1  1) Biology of Non-Coding RNA Group, Institute of Molecular Biology, Ackermannweg 4, 55128 Mainz, Germany; 2) Quantitative Proteomics Group, Institute of Molecular Biology, Ackermannweg 4, 55128 Mainz, Germany; 3) Bioinformatics Core Facility, Institute of Molecular Biology, Ackermannweg 4, 55128 Mainz, Germany.

In every domain of life, Argonaute proteins and their associated small RNAs were found to regulate gene expression. In the eumetazoan germline, Piwi clade Argonautes associate with piRNAs to control transposable elements. Given the potentially detrimental role of transposon expression, Piwi activity has a pivotal role in maintaining fertility. The conserved CHHC zinc-finger protein gametocyte-specific factor 1 (Gtsf1), as well as its paralogs Gtsf11 and Gtsf2, interact with Piwi proteins in flies and mice. In Drosophila, Gtsf1 interacts with Piwi to elicit transcriptional gene silencing. However, mechanistic insights into Gtsf1 function are lacking. In Caenorhabditis elegans, Piwi is required for transgenerational fertility while Argonautes and the RNA-dependent RNA Polymerase (RdRP) RRF-3 of the 26G-RNA pathway are required for fertility at elevated temperatures. We identified T06A10.3 as the C. elegans Gtsf1 ortholog and named it gtsf-1. Given its importance in Piwi-mediated transcriptional silencing in D. melanogaster, we addressed the role of gtsf-1 in the context of the small RNA pathways of C. elegans. To this purpose, we used CRISPR-Cas9 technology to produce mutant alleles of gtsf-1. Surprisingly, using reporter strains, we found that gtsf-1 is not involved in the piRNA pathway and transposon silencing in C. elegans. Instead, gtsf-1 mutant animals recapitulate the mutant phenotypes of factors operating in the 26G-RNA pathway, such as those of RRF-3. These mutant phenotypes include,
notably, temperature-sensitive sterility at 25°C. Accordingly, small RNA sequencing showed that 26G-RNAs are severely depleted in gtsf-1 mutants. Strikingly, by immunoprecipitation of GTSF-1 followed by quantitative proteomics, we identified RRF-3 as a strong interactor of GTSF-1. Furthermore, GTSF-1 interacts with RRF-3 via its two CHHC zinc-fingers. In embryos, where a functional RRF-3 associated small RNA-producing complex is present, GTSF-1 is required for complex assembly. We propose that GTSF-1 homologs in other species may similarly act to drive the assembly of larger complexes that subsequently act in small RNA production or in imposing small RNA-mediated silencing activities.

142 The highly conserved factor ZNFX-1, regulates gene silencing and crosstalk between germline small RNA pathways in C. elegans. T. Ishidate1,2, R. Sharma1, D. Durning1, M. Seth1, M. Shirayama1,2, C. Mello1,2 1) RNA Therapeutics Institute, UMass Med School, Worcester, MA; 2) Howard Hughes Medical Institute.

In the C. elegans germline Argonaute pathways propagate epigenetic "memories" of parental gene expression to offspring. The CSR-1 Argonaute engages small RNAs (sRNAs) antisense to most expressed mRNAs, while WAGO Argonautes engage sRNAs that target transposons and other silent genes. These Argonautes and their sRNA co-factors are loaded abundantly in oocytes and sperm and are thought to reinforce and propagate the expression state of their targets by recruiting RNA-dependent RNA polymerases (RdRPs) to template de-novo sRNA production in each generation. A third Argonaute pathway involving the conserved Piwi Argonaute PRG-1 and tens of thousands of genomically encoded piRNAs is thought to interface with the CSR-1 and WAGO pathways. PRG-1 initiates de novo WAGO pathway recruitment to mRNAs (such as transgene mRNAs), whose sequences are not protected by CSR-1 targeting.

To identify genes required for this intricate epigenetic mechanism we mutagenized animals carrying both a temperature-sensitive lethal allele of cdk-1(ne2257) along with a silenced rescuing transgene, cdk-1::gfp. Mutations that activate the cdk-1::gfp were identified at 25°C as viable strains with CDK-1::GFP positive germlines. As expected this screen yielded new alleles of many previously identified silencing factors as well as approximately 20 mutations that define new complementation groups. Among these we found two alleles of a previously uncharacterized but highly conserved gene, znfx-1(zk1067.2). These two alleles as well as CRISPR induced null mutations in znfx-1 are viable and healthy but render BOTH the CSR-1 and WAGO epigenetic pathways unstable. As expected, znfx-1 mutants exhibit a gradual de-silencing of germline transgenes over a period of a few generations. However, surprisingly these same mutants can also cause a progressive silencing of an otherwise stably expressed CSR-1 targeted transgene. The ZNFX-1 protein contains a conserved cysteine rich motif, and a upf1-type helicase domain also found in the human protein ZNFX1. The helicase domain is similar to the HRR1 helicase implicated as an RdRP co-factor in fission yeast. Perhaps consistent with this finding, ZNFX-1 localizes to P-granules and interacts with the prg-1 3 as a strong interactor of GTSF-1. Furthermore, GTSF-1 interacts with RRF-3 via its two CHHC zinc-fingers. In embryos, where a functional RRF-3 associated small RNA-producing complex is present, GTSF-1 is required for complex assembly. We propose that GTSF-1 homologs in other species may similarly act to drive the assembly of larger complexes that subsequently act in small RNA production or in imposing small RNA-mediated silencing activities.

143 Dissecting 21U RNA biogenesis and activity in the germline and the early embryo. M. Placentino1, R. Rodrigues1, A. Domingues1, S. Dietz2, F. Butter3, R. Ketting1 1) Biology of non-coding RNA, IMB, Mainz, DE; 2) Quantitative Proteomics, IMB, Mainz, DE.

Many different small RNA pathways exist in C. elegans. Some of these affect the expression of ‘regular’ genes, while others are more geared towards the silencing of repetitive elements in the genome. In general, they have a large impact on fertility. Most of these pathways, with the notable exception of miRNAs, act in a two-step manner. First, so-called primary Argonaute proteins, such as PRG-1 that binds to 21U RNAs, recognize a target transcript based on homology between small RNA and target RNA. This is followed by a second step in which an RNA-dependent RNA polymerase (RdRP) enzyme is recruited to the target, resulting in 22G RNA production. As the target-RNA is used as template by the RdRP, 22G RNAs are anti-sense with respect to the target of the primary small RNA. 22G RNAs are bound by secondary Argonaute proteins that induce silencing. In case of 21U RNAs, the secondary phase can become self-sustainable and independent of PRG-1, in which case the target is said to be under control of RNAe (RNA induced epigenetic silencing). The molecular differences between a PRG-1 target that is under control of RNAe and one that is not are presently completely unclear. We are further dissecting both the biogenesis of 21U RNAs as well as how and when 21U RNAs induce RNAe.

Based on IP-mass spec experiments on PID-1, a factor we previously showed to be essential for 21U RNA production, we found evidence for a complex that drives the production of 21U RNA. The identity of the PID-1 interactors has implications for 21U RNA precursor recognition and the stabilization and/or transfer of processing intermediates. We established that 21U RNA-mediated silencing most likely takes place during early embryogenesis, since maternally provided 21U RNAs are both required and sufficient to induce an RNAe-like state. We then identified a novel protein, PID-2, that is required for PRG-1-mediated silencing activity, but is dispensable for RNAe. In pid-2 mutants, PRG-1 is normally loaded with 21U RNAs. Therefore, PID-2 acts downstream of PRG-1 loading, but upstream of the secondary RNAe pathway that is PRG-1 independent. Consistent with PRG-1 silencing activity in the early embryo, zygotic PID-2 is sufficient to induce silencing. We are currently further dissecting PID-2 activity, but it is already clear from our analyses that PID-2 defines an as yet poorly understood step in between target recognition by PRG-1 and establishment of a stably inherited RNAe-state.

144 Rules for PIWI targeting and a practical approach to prevent transgene silencing by avoiding piRNA recognition. Donglei Zhang1, Shikui Tu1, Zhiping Weng2, Heng-Chi Lee1 1) Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL; 2) Program in Bioinformatics and Integrative Biology, University of Mass. Medical School, Worcester, MA.

In diverse animals, PIWI Argonaute and its associated piRNAs play an essential role in genome defense against foreign
nucleic acids, such as transposons. Intriguingly, the vast majority of the 15,000 sequence-distinct piRNAs encoded by C. elegans genome do not target transposons, suggesting additional function for piRNAs. It has been known for decades that transgenes carrying foreign nucleic acids, such as GFP or mCherry, are frequently silenced in the germline of C. elegans. Recent studies have shown that the PIWI protein PRG-1 plays a critical role in triggering silencing of these transgenes. Transgenes have thus served as an invaluable model for foreign nucleic acids to study the mechanism of the piRNA pathway. While piRNA targeting can tolerate mismatches, none of the worm piRNAs can pair with less than three mismatches to several silenced transgenes. If and how piRNAs can directly recognize these foreign sequences remain unknown. By identifying the targets of individual piRNAs and systematically manipulating piRNA reporters, we have revealed the piRNA-targeting signature. This finding has allowed us to predict piRNA binding sites. Importantly, by introducing silent mutations that disrupt these predicted piRNA targeting sites in several silencing-prone transgenes, we demonstrate that these transgenes can be stably expressed in the germline. Surprisingly, many endogenously expressed genes also contain predicted piRNA targeting sites but exhibit strong resistance to piRNA-mediated gene silencing. Together, our study reveals insights to the piRNA targeting mechanism and suggests a cellular mechanism that protects self genes from piRNA silencing.

145 Evolutionary analysis of piRNA genomic organisation reveals two fundamental modes of piRNA biogenesis in nematodes. A. Beltran1,2, L. Stevens3, H. Schwartz2, H. Fradin5, F. Piano5, P. Sternberg4, M. Blaxter1, P. Sarkies1,2 1) MRC London Institute of Medical Sciences, London, GB; 2) Imperial College London, GB; 3) Institute of Evolutionary Biology, The University of Edinburgh, GB; 4) Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, USA; 5) Department of Biology and Center for Genomics and Systems Biology, New York University, New York, USA.

Piwi-interacting RNAs (piRNAs) are crucial for germ line development across metazoans. Puzzlingly however, despite their wide conservation, piRNA pathways evolve extremely rapidly and have been lost independently in nematode clades I, II, III and IV. To better understand piRNA pathway evolution, we undertook an extensive study of piRNAs across nematodes. We find that the key Ruby motif required for piRNA production in C. elegans is widely conserved in clade V nematodes, and is also present in Plectus murrayi and Plectus sambesi, dating back to at least 350mya. However, despite motif conservation, we identify two fundamentally different modes of genomic organisation of piRNAs. Within the Caenorhabditis genus up to Oscheius tipulae, piRNAs are are densely packed into a small number of intergenic piRNA clusters. Conversely, in more ancestral nematodes, typified by Pristionchus pacificus, piRNAs are not clustered but instead are found within the introns of highly expressed genes. Consistently, piRNA clusters are enriched for H3K27me3 chromatin whereas piRNAs in non-clustered species are associated with H3K36me3. Analysis of SNP data from C. elegans and P. pacificus confirms that both these types of piRNA organisation are under selection. Although distinct in organisation, we show that both types of piRNAs rely on a common mechanism for their production: through comparison of common features across nematodes we identify a hitherto unknown RNA polymerase II (Pol II) pausing signal downstream of the piRNA locus, which is required for precursor production and robust piRNA expression. Taken together our data uses comparative genomics to uncover two novel features unifying nematode piRNA biogenesis. First, piRNA expression requires a high density of Pol II. This can be achieved either through a high local piRNA density or through localisation of piRNAs within introns. Second, Pol II must be regulated to produce a short piRNA precursor, necessitating the use of a strong pausing signal downstream of the mature piRNA sequence.

146 A potential role for RSD-3-coated extracellular vesicles in trafficking RNA from the soma to the germline. D.J. Pagano, S. Kennedy Harvard Medical School, Boston, MA.

Animals have evolved sophisticated systems that allow their many cells to communicate with each other. These systems enable cells and tissues to act in coordination during development and in response to changing environmental conditions. One such system involves extracellular vesicles (EVs), phospholipid membrane-enclosed organelles found in animal circulatory systems that carry molecular signals between cells. Some EVs contain RNA, hinting that RNA may be an important mediator of cell-cell communication in animals.

We use RNA interference (RNAi) in C. elegans as a model to study RNA-based cell-cell communication. RNAi is systemic in C. elegans: dsRNAs expressed in one somatic cell can move to other somatic cells. Interestingly, RNAi is also heritable in C. elegans: RNAs can be trafficked from somatic cells to germ cells (termed RNAi inheritance). RNAi inheritance requires RSD-3, a conserved lipid-binding protein that associates with intracellular vesicles and is thought to contribute directly to vesicle formation in mammals. We found that in C. elegans, RSD-3 is expressed in somatic cells that surround germ cells and that RSD-3 acts in these somatic cells to allow silencing RNAs to accumulate in germ cells. Additionally, we found that RSD-3 localizes to vesicles that are trafficked out of somatic cells and deposited into the germline. We hypothesize that during the normal course of reproduction RSD-3-coated vesicles are the vehicles for transmitting RNA-based information from the soma to the germline. This type of communication system may have evolved to enable the soma to send gene regulatory information to the germline which could allow for inheritance of acquired traits, transmission of RNA-based immunity, and protection of the germline genome from parasitic DNA.

147 Transcription alterations associated with nuclear Argonautes pathways are accompanied by chromatin reorganization. E. Gushchanskaia, A. Grishok Boston University, Boston, MA.

The subfamily of C. elegans Argonaute proteins localized to the nucleus is known to participate in transcription regulation. Thus, WAGO Argonautes bind endogenous siRNAs (endo-siRNAs) corresponding to transposable and repetitive elements and maintain the heterochromatic status of these regions [Gu W. et al., 2009]. In contrast to WAGO, nuclear Argonaute CSR-1 [Claycomb J. et al., 2009] was shown to promote sense-oriented RNA polymerase II transcription in active chromatin regions.
In the previous study, Global Run-On sequencing (GRO-seq) analysis demonstrated both a decrease in active genes transcription and an increase in antisense transcription and expression of silent genes genome-wide. This effect was similar in both csr-1 hypomorphic strain that has reduced expression of CSR-1 and in a loss-of-function mutant of Dicer-related helicase, drh-3 [Cecere G. et al., 2014]. Dicer-related helicase (DRH-3) represents an essential component of all C. elegans RNA-dependent RNA polymerase (RdRP) complexes involved in the production of secondary siRNAs, that also exist endogenously. The loss of DRH-3 affects both CSR-1 bound endo-siRNAs and WAGO interacting ones [Gu W. et al., 2009]. Keeping in mind the transcription alterations demonstrated by GRO-Seq, we are focusing on CSR-1 target genes characteristics, their positioning in the nucleus and chromatin organization changes that accompany the transcription alterations. Silent, heterochromatic regions of the genome are localized close to the nuclear periphery [Akhtar A. et al., 2007, Sexton T. et al., 2007] and are anchored there via nuclear membrane proteins [Ikegami K. et al., 2010]. Here, we show that the global changes in transcriptome profile in drh-3 and csr-1 loss-of-function mutants occur together with global alterations in chromatin organization. Repeat-rich autosomes arm loses their contact with the nuclear envelope. On the contrary, the central gene-rich parts of the chromosomes are coming closer to LEM-2 transmembrane protein as transcription of active genes is decreasing. This effect is more pronounced in drh-3 mutant, which is depleted of endo-siRNAs belonging to both “silencing” and “activating” pathways. Interestingly, drh-3 mutant also demonstrates a dramatic decrease in LEM-2 proximity of the chromosome regions associated with the WAGO pathway activity. Therefore, in the absence of WAGO siRNAs, these regions become less heterochromatic and tend to detach from the nuclear membrane.

We are further investigating the connection between transcription changes, chromatin organization and histone modifications in csr-1 and drh-3 mutants.


Proper regulation of the germline transcriptome is essential for the maintenance of fertility and survival of a species. In C. elegans, germline transcriptome homeostasis hinges on a complex repertoire of small RNA pathways that act in both activating and silencing capacities. Our understanding of how fundamental RNA processing steps, such as splicing, capping, nuclear export, and polyadenylation intersect with these small RNA machineries in the germline remains relatively limited. Here, we link the conserved intron binding protein and splicing factor, EM-4/AQR/IBP160 to two key 22G-RNA pathways in the C. elegans germline. EM-4 associates with the Argonautes CSR-1 and HRDE-1, and is enriched at the genomic loci of CSR-1 and HRDE-1 target genes. Loss of em-4 leads to distinct alterations in CSR-1 vs. HRDE-1 small RNA and mRNA transcriptomes. Our transcriptome-wide analysis shows that EM-4 is enriched along pre-mRNAs of nearly 10,000 transcripts. For a subset of these genes, including mostly CSR-1 pathway targets, EM-4 enriches for intronic, but not exonic, sequences. Notably, loss of em-4 or the Argonautes with which it associates leads to similar defects in germline chromatin resetting in the primordial germ cells of embryos. Together these data point to EM-4 as a factor that may help to enable the biogenesis of small RNAs and distinguish the targets of these two germline nuclear small RNA pathways in the worm. In turn, these activities are important for proper germline development and contribute to fertility over multiple generations.

149 A P-granule-localized Argonaute protein transmits siRNA from germline to zygote to promote transgenerational inheritance of RNAi. Fei Xu, Xuezhu Feng, Shouhong Guang School of Life Sciences, University of Science and Technology of China, Hefei, Anhui, China, Anhui, CN.

RNAi-elicited gene silencing is heritable and can persist for multiple generations after its initial induction in C. elegans. However, it remains mysterious how parental acquired trait-specific information of RNAi are transmitted into the progenies. Here, we identified a cytoplasmic Argonaute protein WAGO-4 necessary for the inheritance of RNAi. WAGO-4 accumulates at the perinuclear P-granule foci in the germline and is required for exogenous RNAi targeting germline expressed genes. WAGO-4 binds to 22G-RNA and the mRNA targets. Interestingly, WAGO-4-associated endogenous 22G-RNA targets the same cohort of 22G-RNA and the mRNA targets. Interestingly, WAGO-4-associated endogenous 22G-RNA targets the same cohort of germline genes as CSR-1 and similarly contains untemplated addition of uracil at 3' ends. The poly(U) polymerase CDE-1 is required for the untemplated polyuridylation and transgenerational inheritance of RNAi as well. WAGO-4 exhibits an asymmetrical translocation to the germline along generations and deposits siRNA to the zygotes. Therefore, we identified a mechanism that cytoplasmic Argonaute protein can transport siRNAs to the progeny to promote the inheritance of RNAi.

150 Sperm-inherited chromatin states shape gene expression in offspring. K.R. Kaneshiro, S. Strome MCD Biology, UCSC, Santa Cruz, CA.

Proper development requires that gene expression information be transmitted across generations and though cell division. Part of this information is carried in epigenetic packaging of the genome into repressed and expressed domains. Our studies in C. elegans have shown that 1) the repressive mark H3K27me3 generated by PRC2 is transmitted to the embryo on both sperm and oocyte chromosomes, 2) in the absence of PRC2, inherited H3K27me3 is transmitted through several cell divisions and 3) in the presence of PRC2, inherited patterns of H3K27me3 are transmitted through many divisions (Gaydos et al. Science 345:1515, 2014). I demonstrated that in embryos that inherit oocyte chromosomes with H3K27me3 and sperm chromosomes without H3K27me3, PRC2 activity maintains H3K27me3 exclusively on oocyte-derived chromosomes in daughter cells. This analysis made use of a mutant that fails in prnuclear fusion and keeps oocyte-inherited chromosomes and sperm-inherited chromosomes in separate nuclei for many divisions. The ability of PRC2 to maintain gamete-inherited patterns of H3K27me3 depends on the antagonistic
activity of MES-4 and the histone modification (H3K36me3) it generates. These findings demonstrate that H3K27me3 transmits a memory of repression from parent to offspring and that PRC2 and MES-4 cooperate to preserve that memory through cell divisions. Does the memory inherited on sperm chromosomes matter? It does, as inheritance of sperm chromosomes lacking H3K27me3 leads to changes in germline gene expression and compromised fertility in subsequent generations. I am currently analyzing the patterns of misexpression that result from this altered inheritance by identifying transcripts that emanate from sperm- vs oocyte-inherited genomes. My preliminary findings indicate that inheriting sperm chromatin lacking H3K27me3 results in altered transcription from both sperm- and oocyte-inherited genomes. My findings demonstrate that sperm-inherited chromatin states transmit gene expression information and that altering those states results in changes to offspring gene expression and fertility. The specific patterns of misexpression indicate that inherited chromatin states influence transcription at several levels and suggest that, for at least a subset of genes, transcription is directly controlled by local chromatin states.

151 Temperature increases cause transposon-associated DNA damage specifically during spermatogenesis. N.A. Codd, J.M. Helm, K Schoellhorn, D.E. Libuda Department of Biology, Institute of Molecular Biology, University of Oregon, Eugene, OR.

During meiosis the faithful inheritance of the genome is necessary for successful gamete formation. While many tissues are affected by extreme temperature changes, developing sperm in the testes are particularly sensitive to small fluctuations in temperature, with spermatogenesis requiring a narrow isotherm of 2-7°C below core body temperature. Testes exposed to high temperature display reduced fertility. Studies in mammals have linked elevated temperatures with an increase in DNA damage in spermatocytes, however the underlying mechanisms remain unknown. Here we show that upon a heat shock at 34°C, the spermatocytes (but not oocytes) of Caenorhabditis elegans exhibit an increase in double strand DNA breaks (DSBs) via a SPO-11 independent pathway which is dependent on the presence of transposons. Using a RAD-51 antibody that marks sites of DSBs, we have found that heat shock of both males and L4 hermaphrodites produces a dramatic increase in RAD-51 foci at all phases of meiosis I, and causes a 30-40 fold increase in RAD-51 foci in a spo-11 mutant background where no endogenous meiotic DSBs are formed. Moreover, using a marker for a crossover repair outcomes (COSA-1), we found that heat shock can restore COSA-1-marked crossovers in spo-11 males, indicating that at least a subset of the heat shock induced RAD-51 foci represent DSBs with the capacity to be repaired as crossovers. Further, we observed disorganization of chromosome structures specifically in late pachytene spermatocytes upon heat shock, suggesting that increased temperature alters chromosome structure. Interestingly, we found that the heat-shock induced RAD-51 foci are Tc1 transposon dependent as males carrying a low Tc1 copy number do not display increased RAD-51 foci following heat-shock. Previous studies have demonstrated that certain small RNA molecules such as PIWI-interacting RNAs (piRNAs) regulate transposon activity in the germline and some small RNA pathways promote male fertility at elevated temperatures. To determine the role of small RNA pathways in temperature-induced DNA damage in spermatocytes, our on-going studies include: 1) examining the effects of mutations in small RNA pathways and other associated proteins known to act in spermatocytes and, 2) performing deep sequencing of small RNAs in male and obligate female germ lines at specific time points after heat shock. Our preliminary data suggest that a null mutation in csr-1 leads to variability in the ability of worms to undergo temperature-induced DNA damage in spermatocytes. Taken together, our data suggest that the increased DNA damage from heat-shock observed during spermatogenesis is due to transposon mobilization, and may contribute to impaired fertility in heat-exposed males.

152 Developmental relaxation of nuclear RNAi targeted LTR retrotransposons in C. elegans germ cells. J.Zhou. Ni, Natalia Kalinava, Sam Gu Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ.

In C. elegans, the germline nuclear RNAi pathway serves as a genome surveillance mechanism against foreign genetic material, such as LTR retrotransposons, by small interfering RNA (siRNA)-mediated transcriptional silencing. Despite recent progress, it remains elusive how germ cells recognize LTR retrotransposon as “self” DNA and trigger the siRNA response. LTR retrotransposon siRNAs belong to the 22G siRNA class and require corresponding transcripts as the template for their biogenesis. Therefore, in order to understand the triggering mechanism, one must know when and where LTR retrotransposons are expressed during the reproductive cycle of C. elegans. In this study, we resolve this gap by combining single-molecule RNA FISH (smFISH) and high-throughput RNA-seq. We found that expression of germline nuclear RNAi targeted LTR retrotransposons is tightly linked to germline development, and is regulated by distinct mechanisms. We found that, compared to mRNA from protein-coding genes (“self” DNA), LTR retrotransposon transcripts exhibit numerous aberrant features at cellular and molecular levels. We are currently investigating the potential roles of these features as the triggering mechanisms that allow germ cells to distinguish “self” and “foreign/unwanted” genetic elements and will report our progress in this meeting.

Saturday, June 24  8:30 AM – 11:30 AM
Northwest Auditorium
Interactions with Pathogens and the Environment

Co-chairs: Michalis Barkoulas, Imperial College London, and Patricja van Oosten-Hawle, University of Leeds

153 Identification of an evolutionarily conserved pathway essential for virus infection using Orsay virus infection of C. elegans. Hongbing Jiang, Kevin Chen, Luis Sandoval, Christian Leung, David Wang Molecular Microbiology Department,
The RNA virus Orsay is the only known natural viral pathogen infecting the intestine cells of the nematode C. elegans. The C. elegans intestine cells resemble human intestinal epithelial cells not only in morphological features such as microvilli, but also in molecular mechanisms such as antibacterial innate immune response. As Orsay was discovered only six years ago, the C. elegans antiviral innate immune system remains largely unknown. Only two pathways, RNAi and ubiquitin-mediated protein degradation, have been discovered to be involved in the C. elegans antiviral innate immunity. To explore the genetic landscape of the C. elegans antiviral innate immunity, we conducted a genome-wide RNAi screen for genes whose inactivation sensitizes worms to Orsay infection. 110 genes were identified to be required for C. elegans antiviral innate immunity. 72 of these genes have human orthologs. Tissue-specific RNAi experiments showed that the majority of these genes function in the intestine cells to modulate antiviral innate immunity. In addition to RNAi and ubiquitin-mediated protein degradation, these genes encompass pathways in autophagy, mitochondrial unfolded protein responses, collagens, cytoskeletal organization, RNA processing, and transcription. To identify the gene products that are druggable, we screened 2000 chemicals for drugs that can alleviate Orsay infection symptoms, and discovered four innate immunity enhancing drugs, resorcinol monoacetate (RM), berberine (BBR), bismuth subsalicylate, 3,3'-diiodomethane (DIM). BBR and bismuth subsalicylate are known antidiarrhea drugs, with possible antiviral functions against human gastrointestinal viruses. Chemical-genetic experiments revealed that these drugs have different mechanism of actions: RM strengthens the collagen barrier for viral entry; BBR and DIM enhances the ubiquitin-mediated protein degradation pathway. Together, these data revealed a multifaceted antiviral innate immune system in the C. elegans intestine. Aspects of genetic and chemical modulation of this system may be conserved in human innate immunity against gastrointestinal viruses.

154 Genetic and chemical modulation of C. elegans intestinal antiviral innate immunity. Y Zhou, L Bedian, Y Tao, W Zhong  BioSciences, Rice University, Houston, TX.

The RNA virus Orsay is the only known natural viral pathogen infecting the intestine cells of the nematode C. elegans. The C. elegans intestine cells resemble human intestinal epithelial cells not only in morphological features such as microvilli, but also in molecular mechanisms such as antibacterial innate immune response. As Orsay was discovered only six years ago, the C. elegans antiviral innate immune system remains largely unknown. Only two pathways, RNAi and ubiquitin-mediated protein degradation, have been discovered to be involved in the C. elegans antiviral innate immunity. To explore the genetic landscape of the C. elegans antiviral innate immunity, we conducted a genome-wide RNAi screen for genes whose inactivation sensitizes worms to Orsay infection. 110 genes were identified to be required for C. elegans antiviral innate immunity. 72 of these genes have human orthologs. Tissue-specific RNAi experiments showed that the majority of these genes function in the intestine cells to modulate antiviral innate immunity. In addition to RNAi and ubiquitin-mediated protein degradation, these genes encompass pathways in autophagy, mitochondrial unfolded protein responses, collagens, cytoskeletal organization, RNA processing, and transcription. To identify the gene products that are druggable, we screened 2000 chemicals for drugs that can alleviate Orsay infection symptoms, and discovered four innate immunity enhancing drugs, resorcinol monoacetate (RM), berberine (BBR), bismuth subsalicylate, 3,3'-diiodomethane (DIM). BBR and bismuth subsalicylate are known antidiarrhea drugs, with possible antiviral functions against human gastrointestinal viruses. Chemical-genetic experiments revealed that these drugs have different mechanism of actions: RM strengthens the collagen barrier for viral entry; BBR and DIM enhances the ubiquitin-mediated protein degradation pathway. Together, these data revealed a multifaceted antiviral innate immune system in the C. elegans intestine. Aspects of genetic and chemical modulation of this system may be conserved in human innate immunity against gastrointestinal viruses.

155 Mitochondria as a Hub for Cellular Surveillance. N.V. Kirienko  Department of Biosciences, Rice University, Houston, TX.

Mitochondria are crucial for cellular biochemistry, generating ATP and reactive oxygen species for signaling, regulating metabolic activity, and controlling programmed cell death. Lying at the center of cell physiology, they represent a nearly ideal hub for surveilling the nutritional and metabolic status of the cell. Here we present two stories about mitochondrial surveillance will be presented.

In the process of characterizing a liquid-based C. elegans-P. aeruginosa pathogenesis system, we made the rather startling discovery that a bacterial exoproduct, pyoverdine, is capable of translocating into the cells of C. elegans, hijacking its iron, and exiting the host, only to transfer this important micronutrient to the pathogen. Pyoverdine is a siderophore, a small, water-soluble molecule excreted by P. aeruginosa. Its most important function is to acquire iron from the bacterial environment (whether soil, water, or biological tissue) and return it to the bacterium to support growth and division. Removing iron causes mitochondrial damage to the host, which responds by activating mitochondrial quality control pathways, including mitophagy. Interestingly, our data demonstrate that compromising this process dramatically curtails host survival.

While using transcriptional profiling to study the host defense against pyoverdine-mediated pathogenesis, we identified an 11-nucleotide motif overrepresented in the promoter regions of genes upregulated specifically by pyoverdine intoxication. This motif, known as the ESRE motif, has been independently identified at least six times and each time it has associated with stress resistance in worms, flies, or humans. However, it has not previously been linked to innate immune function. Using reverse genetics, we have identified four bZIP proteins that regulate the expression of ESRE genes. Much like disrupting mitophagy, inhibiting ESRE gene expression by RNAi-mediated knockdown of these transcription factors significantly impairs host survival. Interestingly, we also determined that other, non-pathogenic mitochondrial toxins induce expression of ESRE genes, suggesting that this mitochondrial surveillance pathway has broad importance.

Two large conclusions can be drawn from our findings. First, C. elegans (and likely all metazoans) use several mechanisms to ensure mitochondrial health. Upon disruption of mitochondrial function (by iron removal or by chemical damage), the ESRE network is activated to attempt to restore homeostasis. If this fails and mitochondrial damage remains pervasive, the organelle is...
targeted for degradation and recycling. The defenses are not redundant, however, as disruption of either shortens lifespan in the face of stress. Second, the processes of innate immunity and abiotic stress response are not as separable as we like to think; rather, biological organisms perceive them as a single continuum.

156 Role of host damage and bacterial genomic diversity in the interaction between P. aeruginosa and C. elegans. A. Vasquez-Rifo, I. Veksler-Lublinsky, Z. Cheng, E. Ricci, M. Moore, F. Ausubel, V. Ambros

Program in Molecular Medicine, UMass Medical School, Worcester, MA; 2) Department of Microbiology & Immunology, Dalhousie University, Halifax, Nova Scotia, Canada; 3) Department of Genetics, Harvard Medical School, Boston, MA 02115; 4) CIRI, Centre International de Recherche en Infectiologie, INSERM U1111, Ecole Normale Supérieure de Lyon, France; 5) RTI, University of Massachusetts Medical School, Worcester, MA 01605.

Pseudomonas aeruginosa is a free-living bacterium that participates in opportunistic pathogenic interactions with a broad range of hosts, including nematodes, insects, mammals and plants. Many strains of P. aeruginosa can lethally infect the nematode Caenorhabditis elegans. However, the mechanisms underlying the pathogenicity towards the nematode are incompletely understood. The interaction between C. elegans and P. aeruginosa involves a variety of P. aeruginosa regulatory processes (such as quorum sensing) that the bacterium uses to infect a variety of hosts as well as C. elegans-specific processes.

With the goal of understanding the bacterial and host processes that underpin the pathogenic interaction between P. aeruginosa and C. elegans, we have analyzed: (1) the variability of virulence towards worms among bacterial isolates; (2) the effect of the host-microbe interaction on the nematode's RNAs.

Various isolates of P. aeruginosa display broadly varied virulence against wild type C. elegans. Using a comparative genomic and phylogenetic approach, we analyzed the genetic determinants that drive evolutionary changes in P. aeruginosa virulence towards C. elegans. Based on our results, we present an evolutionary model that accounts for differences in virulence amongst P. aeruginosa strains based on the gain/loss of genes that govern virulence, and on the evolutionary history of CRISPR presence/absence in the different strains. (2) We investigated how P. aeruginosa infection of C. elegans impinges on distinct classes of worm RNAs, and observed that, upon infection of adult hermaphrodites, worm RNA profiles are significant altered. In particular, infected worms exhibit significant damage of their rRNA, with extensive degradation and accumulation of cleaved rRNA species. The induction of rRNA damage is independent of the action of pathogen defense pathways that are activated by the worm during infection, and therefore appears to represent a previously unrecognized consequence of bacterial virulence.

157 The Patched-related protein PTR-15 (BUS-13) is essential for larval viability (in the intestine) and pathogen resistance (in the epidermis). Jonathan Hodgkin, Dave Stroud, Christopher Cammies, Victoria Deaner, Laurence Dean

1) Dept Biochem, Univ Oxford, Oxford UK; 2) School of Biochem, Univ Bristol, Bristol UK.

PTR-15 is one of 24 C. elegans proteins sharing homology to the Hedgehog receptor Patched. PTC and PTR proteins carry both sterol-sensing and RND domains as well as 12 predicted transmembrane domains. Earlier work revealed that several of the PTR proteins are involved in molting (Zugasti et al. 2005). Other work showed that bus-13, which exhibits surface abnormalities and sensitivity to some bacterial pathogens (Gravato-Nobre et al. 2005, Hodgkin et al. 2013), carries a missense mutation in ptr-15. Fluorescent transgenic reporters further showed that ptr-15 is expressed strongly in pharynx and intestinal cells and weakly in the epidermis. Recently, CRISPR/Cas9 was used to generate a ptr-15 deletion allele, which exhibits a larval lethal arrest phenotype. This lethality, as well as pathogen sensitivity, is fully rescued by wildtype ptr-15::mRFP transgenes. The larval lethality is also rescued by intestinal ptr-15 expression driven from the mtl-2 promoter, which surprisingly does not rescue the pathogen sensitivity, suggesting that normal pathogen resistance requires epidermal expression. Further characterization of the ptr-15(null) phenotype is in progress.

The missense mutant (pka bus-13) grows normally on E. coli, but unlike wildtype, bus-13 mutants are completely inviable in the presence of the bacterial pathogen Leucobacter Verde1, owing to lethal surface infection. Large scale selections for suppressor mutations allowing viability of this mutant on Verde1 have yielded two intragenic partial revertants, which have implications for PTR-15 structure and function. Surprisingly, none of the known and expected Verde1-resistance mutants were recovered in these selections, suggesting that the role of ptr-15 in generating surface coat is distinct from that of other bus and srf genes. We are investigating the possibility that both intestinal and epidermal functions of PTR-15 are involved in transport of lipids or other small molecules.

158 Intestinal colonization by bacteria alters chemosensory responses to alcohols. M.P. O'Donnell, P.H. Chao, P. Sengupta

Biology, Brandeis University, Waltham, MA.

C. elegans and bacteria exhibit a complex interdependent relationship in which bacteria can be food sources, pathogens, as well as commensal organisms. In the wild, the C. elegans intestine is commonly colonized by live bacteria, which could in principle exert diverse effects on the physiology and metabolism of their nematode hosts. Moreover, vertical and horizontal transmission of bacteria between nematodes and to different substrates suggests that worm behavior can in turn influence the ecological distribution of these microbes. Although C. elegans avoids strongly pathogenic bacteria through associative learning and chemosensory responses, it is currently unknown whether colonization by commensal or mildly pathogenic bacteria alter C. elegans behavior, and/or whether alteration of worm behavior presents an advantage for the colonizing microbe. We have identified a bacterial strain, Providencia sp (Ps), that reduces chemosensory avoidance of long chain alcohols, hexanol and octanol, by C. elegans. We show that Ps efficiently colonizes the C. elegans intestine with little apparent pathogenic effect, that live bacteria are essential to induce behavioral plasticity, and that increased colonization is correlated with reduced avoidance of octanol. In this context, chemosensory plasticity occurs independently of serotonergic aversive learning and likely occurs
159 Complex dynamic subcellular patterning underlies the innate immune response. C. Taffoni, J. Ewbank, D. Marguet, N. Pujol Aix Marseille Univ, CNRS, INSERM, CIML, Marseille, France.

Sterile wounding or infection by the fungus D. coniospora leads to a rapid increase in the transcription of antimicrobial peptide (AMP) genes in the worm epidermis. Forward and reverse genetic screens identified many players involved in this immune response [1] including the GPCR DCAR-1 that acts upstream of a conserved p38 MAPK pathway. Its activation by HPLA, a tyrosine derivative, leads to the expression of AMPs through the STAT-like transcription factor STA-2. STA-2 physically interacts with vesicle-bound SNF-12, a member of the SLC6 family of bioamine transporters [2].

To characterise further the response of the epidermal cell to damage, we are monitoring in vivo the dynamics of these signaling proteins upon wounding, together with that of membrane and cytoskeletal components. We operate a laser wound under a spinning disc microscope. As previously reported, this produces an immediate calcium wave (in less than 1 sec) and the formation of an actin ring (in 20 min) [3].

In between these 2 events, using a CAAX::GFP reporter, we observe a rapid (1 min) reorganization of membranes, forming a barrier for cytoplasmic diffusion, maintained for up to 20 min. It is followed by the accumulation of both highly motile RAB-11 and RAB-5 vesicles (10 min). In the main epidermis, the microtubules (MT) are found either circumferentially orientated in parallel bundles over the muscle quadrants, or randomly orientated in the main lateral cytoplasm. They are highly dynamic as revealed by the rapid movement of the MT + end binding EBP-2 protein. Upon wounding, EBP-2 is recruited (2 min) in a highly directional way to form a ring closely juxtaposed inside the actin ring. These rings progressively close the wound (20 min). Our observations reveal a rapid stabilization of MT at the wound site and an unexpected association with the actin ring.

The DCAR-1 receptor, which has a dotted pattern at the plasma membrane, accumulates at the wound site within 10-15 min. Remarkably, SNF-12 that localizes in apical vesicles disappears within 30 sec after wounding. This could be either due to vesicle internalization, or release of SNF-12 from these vesicles. The SNF-12 vesicles progressively reappear apically and subsequently are recruited around the wound.

We could show that inactivating genes encoding specific tubulins (TBA-2 and TBA-4), while not affecting the development of the worm, destabilizes the epidermal MTs, impacts SNF-12 vesicle dynamics and prevents the induction of AMP. We thus propose a direct link between the reorganization of the cytoskeleton upon wounding and the activation of the innate immune response.

2. Dierking, K., et al., Cell Host Microbe, 2011.

160 Probing organismal proteostasis through the response to intracellular infection. K. Reddy, T. Dror, J. Panek, E. Troemel Division of Biological Sciences, University of California, San Diego, La Jolla, CA.

Maintenance of protein homeostasis, or proteostasis, is critical for organismal health. Through analysis of the host transcriptional response to pathogen infection, we have identified a new pathway that appears to increase proteostasis capacity. We have previously reported that intracellular infection of C. elegans by either microsporidia or virus upregulates expression of several predicted components of a ubiquitin ligase complex (including the cullin cul-6) and many genes of unknown function (including pals-5) (Bakowski et al, PLoS Pathogens 2014). These genes are also induced by proteasome inhibition as well as by a prolonged heat stress at 30°C. We have named this response the IPR (Intracellular Pathogen Response). Notably, the IPR is distinct from previously described pathways such as the heat shock response.

Using a GFP reporter for the IPR gene pals-5, we isolated mutants that constitutively express IPR genes (including ubiquitin ligase components). These mutants are defective in the gene pals-22 (named for a domain implicated in the disease ALS), which functions as a repressor of the IPR. We found that pals-22 mutants have enhanced resistance to heat shock at 37°C, and this phenotype is dependent on cul-6. We also examined whether pals-22 mutants have improved function in a polyglutamine (polyQ) aggregation assay. We found that pals-22 mutants have a greatly reduced number of polyQ aggregates in the intestine after osmotic shock compared to wild-type, and this phenotype is also dependent on cul-6. pals-22 mutants have no increased resistance to direct proteasome inhibition and no increased expression of proteasome subunits, indicating that the pals-22 phenotypes are not due to upregulation of proteasome function. Together these results suggest that upregulated expression of ubiquitin ligase components may increase the ability to target misfolded or toxic proteins for ubiquitylation and subsequent destruction in this mutant.

The PALS-22 protein appears to be expressed broadly throughout the worm, including the intestine, hypodermis, and neurons. We are currently using tissue-specific rescue to identify the tissues in which pals-22 functions to affect stress resistance. We are also taking a biochemical approach to look for evidence of increased ubiquitylation in pals-22 mutants (see abstract by Panek et al).

161 Characterization of neuronal receptor NPR-8 for its role in regulating C. elegans immunity. D. Sellegounder, Y. Liu, C-H. Yuan, J. Sun Department of Biomedical Sciences, Elson S. Floyd College of Medicine, Washington State University, Spokane, WA.

G protein-coupled receptors (GPCRs) are membrane-bound signaling proteins with diverse functions regulating important pathways from development to immunity. Caenorhabditis elegans has evolved as a powerful model for studying G-protein
signaling in tissues as well as in the entire organism. In *C. elegans*, a single neuron expresses numerous GPCRs that responds to diverse stimuli, including damage signals from non-neuronal cells. With advancement in genetic tools and imaging techniques, the function of several GPCRs has been deciphered recently. However, the mechanisms by which distant non-neuronal cells communicate with the nervous system remain unclear. In the current study, we characterized the role of NPR-8, a neuronal GPCR related to the mammalian neuropeptide Y-like receptor, in immune regulation. Survival assays against bacterial pathogens revealed that functional loss of NPR-8 in *C. elegans* enhances the nematode resistance to the pathogen and increases survival. The observed extension in life span was not due to enhanced pathogen avoidance but facilitated through pathogen clearance. Expression of *npr-8* was localized to AWB, AWC, and ASJ sensory neurons. Through forward and reverse genetic approaches, we identified that NPR-8 functions to 1) suppress abu genes involved in maintaining proteostasis through unfolded protein response pathway at an early stage of infection, and 2) regulate structural integrity in non-neuronal tissues by controlling the expression of collagen genes during late phase against bacterial infection. Blocking or silencing individual neurons expressing *npr-8* did not enhance the nematode survival. Expressing NPR-8 in AWB, AWC, or ASJ neurons in *npr-8* mutant animals partially rescued their mutant phenotype of enhanced survival. This study reveals that alongside the immune activation the structural components play a crucial role in enhancing resistance and longevity against pathogenic insults. Our findings suggest that NPR-8 may regulate *C. elegans* immunity by maintaining the organismal proteostasis and structural integrity.


Neuropeptides are important modulators of physiology and adaptive behaviors, such as feeding, reproductive behaviors, learning and sensorimotor integration. In *C. elegans* over 250 neuropeptides have been isolated and predicted, representing the largest group of neuropeptides. However, knowledge on neuropeptide receptors is limited, hampering our understanding of general principles in peptidergic modulation. Using reverse pharmacology, we have performed a large-scale deorphanization screen to identify neuropeptide ligands of ~150 peptide GPCRs predicted in the *C. elegans* genome. This unbiased screening approach sheds light on the diverse RFamide signaling network in *C. elegans*. From this screen, we also identified a novel neuropeptide pathway that is related to vertebrate thyrotropin-releasing hormone (TRH) signaling. Neuropeptides from the *trh-1* precursor dose-dependently activate the *C. elegans* TRH receptor ortholog, TRHR-1, in mammalian cultured cells and are cognate ligands of this receptor in *vivo*. Using CRISPR/Cas9 gene editing and RNAi reverse genetics, we found that TRH-1 signaling has conserved functions in regulating growth. The *trh-1* precursor is expressed in the pharyngeal motor neurons M4 and M5. TRH-1 neuropeptides from these neurons promote growth by activating their receptor TRHR-1 in pharyngeal muscle cells. We found that TRH-1 neuropeptide signaling is a novel growth-regulating pathway that acts independently of TGF-beta signaling, and regulates body size dependent on the bacterial diet. Our results provide first evidence of a functional TRH neuropeptide/receptor system in invertebrates that may ancestrally have been involved in the control of postembryonic growth.

163 Serotonergic control of the conserved heat shock factor transcription factor (HSF-1). V. Prahlad, Felicia Ooi. Department of Biology, University of Iowa, Iowa City, IA.

One of the recent developments in the study of protein homeostasis is the discovery that in a metazoan such as *Caenorhabditis elegans*, the cellular response to protein misfolding is not autonomously controlled by individual cells, but instead is under the regulation of the animals’ nervous system. However, the functional significance of such regulation remains to be shown. We have previously shown that serotonergic signaling in *C. elegans* can activate HSF-1-dependent *hsp70* expression through cell non-autonomous mechanisms. Serotonin (5-hydroxytryptamine, 5-HT) plays a central role in the neuronal mechanisms of learning and memory that allows organisms to synthesize information from their surroundings to predict danger. We find that olfactory learning in *C. elegans* mediated by the serotonergic system not only enhances avoidance behaviors to potential threats, but also induces neuronal and non-neuronal cells throughout the organism to increase expression of their molecular chaperones if they subsequently encounter this threat. Specifically, neurosensory training potentiates HSF-1 activity in anticipation of the actual encounter with the stressor, while not in itself, resulting in chaperone expression. Molecular chaperones are subsequently expressed if and when animals encounter the actual stressor. Our data show that serotonergic control of HSF-1 equips *C. elegans* to better survive environmental dangers by pre-emptively and specifically enhancing cellular homeostasis using the neuronal circuitry underlying memory and learning. We will discuss the mechanisms by which neuronal 5-HT and learning potentiate HSF-1 to enhance chaperone expression.


Isogenic cells or animals exhibit profound differences in many phenotypes, including drug resistance, penetrance of mutations, lifespan and fertility. These nongenetic differences in physiology occur even in well-controlled, homogeneous environments (Mendenhall et al 2017). Thus interindividual differences in physiology occur independent of genetic differences. This intrinsic, nongenetic physiological variation represents a considerable challenge for understanding the aging process and age-related disease. While some physiological variation in isogenic populations is attributed to epigenetic differences, the actual mechanisms of such variations are largely unknown. Yet, in 2017, we have the tools to identify and quantify sources of nongenetic variation in many quantitative traits, such as variation in the expression level of a particular gene. Here we are focused on quantifying the mechanisms of cell-to-cell variation in gene expression for a small heatshock protein, *hsp-16.2*. We
focused on this gene because prior investigations showed that individual animals express different amounts of hsp-16.2 reporter genes and that these differences predict differences in lifespan and are correlated with differences in the penetrance of mutations. To investigate the source of these physiological differences we developed an approach for in vivo quantitative analysis of gene expression at the single cell level (Mendenhall et al 2015). We measured three types of experimentally tractable noise in gene expression. During the course of our studies, we found strong, cell-specific expression patterns for many reporter genes; that is, for many genes, the ratio of gene A to gene B was fixed in intestine cell type X, and different than intestine cell type Y. We discovered that a fundamental property of cells, gene expression capacity (the ability to express genes into functional protein), accounts for most of the cell-to-cell variation in intestine cell gene expression for the genes we have tested. Our experiments suggest that gene expression capacity affects the ability of animals to produce functional proteins, and thus, may underlie many interindividual differences in physiology. Our discovery highlights the need for careful examination of animal physiology at single cell resolution, as bulk analysis of macromolecules (e.g., RNAsseq, mass-spectrometry) alone may fail to capture the physiological heterogeneity that exists within cells in the same tissue or organ.

165 Electron microscopy based high resolution nuclear atlas of C. elegans dauer. E. Bahry1, L. Breimann1, M. Sun2, Y. Wang3, E. Myers3, S. Preibisch1 1) Max Delbruck Center for Molecular Medicine, Berlin-Buch, DE; 2) Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, USA.

We are creating a high-resolution nuclear atlas of the C. elegans dauer from a nanometer-resolved transmission electron microscopy (TEM) reconstruction of an entire dauer larva. Onto this atlas, we map fixed and live light microscopy data exploiting the fixed lineage of C. elegans. This high-resolution atlas can therefore serve as template for any type of microscopy acquisition containing a nuclear marker. This atlas will contribute to C. elegans dauer research as a resource. It will shed light on the dauer lineage, it will allow to directly compare microscopy acquisitions from different dauer larvae at single cell resolution and thus pave the way for studying the dauer diapause systemically.

We acquired a TEM dataset of an entire Dauer C. elegans (daf-2 e1370 mutant) consisting of 25,000 high-resolution images, covering 561 sections, and totaling a size of approximately one terabyte. Following non-rigid alignment using TrakEM2, we developed a semi-automatic approach based on machine learning and dynamic programming to extract all nuclei including their location, size, shape, and specifics of nuclear architecture. We therefore manually annotated a small subset (~10%) of the TEM sections and used them as a training set for machine learning based classification that was subsequently applied to all non-annotated sections to retrieve the probability for each pixel being inside a nucleus. The final outline of each nucleus in each section was then identified by computing an optimal contour that balances its distance to the most probable outline as defined by the machine-learning classification, its smoothness, and its similarity to neighboring sections. To verify the resulting dauer nuclei atlas from the TEM dataset and to assess variability, we acquired confocal images of fixed dauer larvae expressing LMN-1::GFP and stained with DAPI to highlight nuclei locations and outlines. The nuclei were segmented using a combination of local thresholding and machine-learning approaches, while unique geometric constellations of nuclei facilitated registration of the confocal images to the TEM-based atlas.

We aim for the dauer atlas to be an accessible web-based open source and open access resource for the community that will ultimately allow integration of a wide range of different types of imaging data into a common model.

Saturday, June 24 8:30 AM – 11:30 AM
Carnesale Palisades Ballroom
Neuronal Degeneration, Regeneration, Synapses and Circuits


166 The epidermis protects sensory axons from degeneration. S. Coakley, F. Ritchie, M.A. Hilliard Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane, Australia.

Maintenance of neuronal integrity is essential for the preservation of correct neuronal function. Sensory neurons and their axons are subject to continuous mechanical stress due to their location within the skin, muscles, and moveable joints. Despite the strong forces experienced, these neurons are able to maintain their structure and functional circuitry. The ability to resist strain has been shown to be a combination of intrinsic and extrinsic protection mechanisms, but the precise interplay between cell autonomous and cell-non-autonomous stress resistance is not known. Mutations in C. elegans β-spectrin/unc-70 cause spontaneous axonal breakage due to mechanical strain. Through an unbiased forward genetic screen using unc-70 mutants as a sensitised background, we have identified a novel mutant allele of the conserved gene tbc-10, which results in enhanced axonal damage to the PLM mechanosensory neuron. TBC-10 is a Rab-GTPase-activating protein that we demonstrate localises to the membrane of the hypodermis surrounding the PLM neuron and functions non-cell-autonomously within this tissue to exert an axonal protective effect via inactivation of the conserved small GTP-ase RAB-35. Inactivation of RAB-35 within the hypodermis, by either expression of a GDP-locked RAB-35 or a loss of function mutation, is sufficient to rescue the enhanced axonal breakage phenotype in tbc-10 mutants. We show that in C. elegans the epidermis acts to protect the axons of mechanosensory neurons from spontaneous degeneration induced by disruption of the spectrin network, demonstrating a crucial role for non-neuronal support cells in maintaining an intact and functional nervous system.
167 Deciphering the mechanisms of the ZIG-10 immunoglobulin-domain superfamily protein in maintaining synapse density. Salvatore J. Cherra¹, Yishi Jin¹²³ 1) Section of Neurobiology, University of California, San Diego, La Jolla, CA; 2) Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA; 3) Howard Hughes Medical Institute, University of California, San Diego La Jolla, CA.

Synapse maintenance refers to the process of preserving the number and pattern of synaptic connections, which is balanced by synapse formation and synapse elimination. Too few or too many synapses have been associated with various neurological disorders, such as autism spectrum disorders or schizophrenia. While the mechanisms of synapse formation during development have been widely investigated, how established synaptic connections are maintained for the lifetime of an organism is still under debate. Using the C. elegans locomotor circuit as a model system, we have previously shown that ZIG-10, an immunoglobulin-domain superfamily member, is required to constrain the density of excitatory synapses, but not inhibitory synapses (1). Here, we report two new signaling molecules differentially required for ZIG-10-mediated maintenance of excitatory synapses. We show that a novel guanylate kinase, MAGU-2, is required for ZIG-10 to restrict synapse density. Null mutants in magu-2 display an increase in excitatory synapses, similar to zig-10 null mutants, but double mutants of magu-2 and zig-10 display a similar number of synapses as compared to either single mutant. This suggests that MAGU-2 and ZIG-10 function in the same pathway. Our previous data showed that ZIG-10 localizes to the cell surface of excitatory neurons and epidermal cells. In the epidermis ZIG-10 activates the phagocytosis pathway to eliminate excitatory synapses. MAGU-2 displays a similar expression pattern to ZIG-10, but is only required in either neurons or epidermis. KCA-1 is a MAGU-2 interacting protein (2); however, in contrast to MAGU-2, a deletion mutation in kca-1 reduces the excitatory synapse density in zig-10 mutants. This suggests that in the absence of ZIG-10, KCA-1 drives excessive synapse formation. Therefore, we hypothesize that the ZIG-10 normally opposes KCA-1 to maintain proper synapse density. Overall, our data uncover how optimal neuronal interventions to regrow and restore function are a crucial goal in regenerative medicine.


168 UNC-16 inhibits the function of regeneration promoting isoform of DLK-1. S.S. Kulkarni², S. Sheoran², V. Sabharwal¹, K. Matsumoto³, N. Hisamoto³, S.P. Koushika¹ 1) Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai, India; 2) NCBS, Tata Institute of Fundamental Research, Bangalore, India; 3) Department of Molecular Biology, Graduate School of Science, Nagoya University, Japan.

Neurons in the adult nervous system have a limited ability to regenerate after injury. The extent of neuronal regeneration after injury depends on the intrinsic growth potential of neurons and their extracellular environment, both influenced by several genes. We show that UNC-16 plays an inhibitory role during the early stages of neuronal regeneration after axotomy. UNC-16, a C. elegans JIP3 (JNK Interacting Protein 3) homologue, is a scaffolding protein for MAP kinases and binds to the Kinesin-1 and Dynein motors. JIPs are a family of classical scaffolding molecules and they are known to be able to switch their roles from growth promoting to inhibitory based on their levels and times of activation or deactivation. We also show UNC-16’s inhibitory role is independent of JNK-1, Kinesin-1 and Dynein but is dependent on Dual Leucine Zipper Kinase-1 (DLK-1). DLK-1 is an essential MAPKKK for neuronal regeneration and has been reported to interact with JIP3. DLK-1 has two isomers, long and short, of these, DLK-1 long promotes regeneration while DLK-1 short inhibits regeneration. We show that UNC-16 inhibits the regeneration promoting activity of DLK-1 long but does not influence the activity of DLK-1 short. We find that UNC-16 promotes the DLK-1 long punctate localization in a concentration dependent manner limiting DLK-1 long availability at the cut site. We also show that UNC-16 levels are responsible for negatively regulating microtubule dynamics at the cut site immediately after axotomy which we think leads to the observed faster rate of neuronal regrowth in unc-16 animals. We suggest a model where UNC-16 may hold DLK-1 long in a complex and restrict the availability of active DLK-1 long and thereby inhibit regeneration. The dual inhibitory control by both UNC-16 and DLK-1 short can calibrate the intrinsic growth promoting function of DLK-1 long in vivo. We thus show that JIP3 could play its inhibitory role to allow tight temporal and spatial control of DLK-1 function.

169 O-linked N-beta-acetylglucosamine (O-GlcNAc) post-translational modifications dynamically enhance axon regeneration. D.G. Taub, M.A. Awal, C.V. Gabel Physiology and Biophysics, Boston University, Boston, MA.

Regeneration within the mammalian central nervous system following traumatic damage or disease is limited and therapeutic interventions to regrow and restore function are a crucial goal in regenerative medicine. Caenorhabditis elegans’ simple nervous system and neuroanatomy allow for the determination of intrinsic genetic programs that govern axonal regrowth. Here we demonstrate that alterations in O-linked N-beta-acetylglucosamine (O-GlcNAc) post-translational modifications can considerably increase the regenerative potential of individual neurons following laser axotomy in adult C. elegans. O-GlcNAc signaling is known to act as a critical intrinsic sensor of cellular nutrients in part through modulation of the insulin-signaling pathway. We find that loss of O-GlcNAc via mutation of the O-GlcNAc Transferase (ogt-1 (ok1474)) dramatically enhances regeneration by ~70% in C. elegans. Likewise pharmacological inhibition of OGT increases regeneration to a similar extent. Remarkably, hyper-O-GlcNAcylation via mutation of the O-GlcNAcase (oga-1 (ok1207)) or pharmacological inhibition also enhances regeneration by ~40%. Our results shed light on this apparent
contradiction by demonstrating that ogt-1 and oga-1 mutants differentially modulate the insulin-signaling pathway. ogt-1 mutants act on regeneration through the AGC kinase, AKT-1 to modulate local glycolysis independent of DAF-16. In contrast oga-1 mutants appear to act through DAF-16 to improve the mitochondrial stress response and regeneration. Thus, alterations in O-GlcNAc signaling appear to modulate metabolism to enable a pro-regenerative response. These findings reveal for the first time the importance of O-GlcNAc post-translational modifications in axon regeneration and suggest that altering cellular metabolic status is a viable target for regenerative therapies.

170 Inhibition of axon regeneration by aggregation of the prion-like domain containing protein TIAR-2. M.G. Andrusiak1, P. Sharifnia1,2, Y. Jin1,2 1) Biological Sciences - Neurobiology Section, University of California, San Diego, La Jolla, CA; 2) Howard Hughes Medical Institute, University of California, San Diego, La Jolla, CA.

Acute injury to the nervous system, as observed following spinal cord or traumatic brain injury, triggers a number of cellular stresses. Injury-related cellular stresses lead to impaired nervous system function and loss of neuronal connections. Using a laser axon injury model, we can examine how a neuron responds to acute stress in an intact organism. Here, we report that the conserved stress-associated protein TIAR-2 acts as a negative regulator of axon regeneration. tiar-2 acts cell autonomously and early in the regenerative process with mutants showing increased growth and pro-regenerative structures as early as 3h post-injury. Although TIA proteins are defined primarily by their three RNA recognition motif (RRM) domains, we find that the conserved prion-like domain (PrLD) of TIAR-2, a domain prone to aggregation, is both necessary and sufficient to rescue TIAR-2 loss. Moreover, expression of its human orthologue TIA1 is sufficient to rescue the axon regeneration phenotype. By live imaging, we find that TIAR-2 aggregates increase within 5min following axon injury and return to baseline by 3hrs. TIAR-2 possesses liquid-like properties in vivo, an emerging mechanism utilized by PrLD containing proteins to create membrane-less signaling centers. Following axon injury, the liquid-like dynamics of TIAR-2 are inhibited. TIAR-2 aggregates are negatively regulated by the unfolded protein response (UPR) and autophagy pathways, both of which are key in the regenerative process. Our work has identified acute axonal injury as a cue that modulates liquid-phase transitions and aggregation of the PrLD containing protein TIAR-2. Furthermore, we link UPR and autophagy signaling with the ability of TIAR-2 to aggregate and negatively influence axon regeneration.

171 Fifty years of levamisole: do we really know how it works? Viviane Lainé, Luis Briseno-Roa, Benjamin Bonneau, Maelle Jospin, Jean-Louis Bessereau INMG, Université Lyon 1, CNRS 5310 INSERM U1217, France.

Levamisole was identified in 1966 by Paul Janssen and collaborators as a potent broad spectrum anthelminthic. It activates acetylcholine receptors (AChR) at the neuromuscular junction, which causes worm hypercontraction and irreversible paralysis at high concentrations. Genetic screens conducted over 4 decades for mutants either insensitive to the drug (resistance) or able to recover movement after initial paralysis (adaptative-resistance) have been instrumental to identify subunits and regulators of the levamisole-sensitive AChR (L-AChR). Yet, the precise physiological mechanisms of the levamisole response in the wild type (WT) and in adaptative-resistant mutants remain elusive. Using a combination of electrophysiological, Ca2+ imaging and genetic tools we have identified a complex sequence of events that underlie the behavioral features observed at the whole-organism level.

First, when worms are dropped onto plates containing 1 mM levamisole, their muscles hypercontract within a few minutes. This initial response is similar in WT and adaptive-resistant mutants, and abolished in mutants lacking L-AChRs. We show that this early contraction is not due to the entry of Ca2+ only through L-AChR, but requires the activation of the voltage-gated Ca2+ channels (VGCC) EGL-19. Second, after 10 minutes on levamisole, WT start to relax, whereas adaptive-resistant mutants such as lev-10 remain hypercontracted. This long-lasting contraction is correlated with the maintenance of a high intracellular Ca2+ concentration in muscle cells. The difference of behavior between WT and lev-10 worms is puzzling: in lev-10 mutants L-AChRs are spread over the muscle surface instead of being clustered at post-synaptic sites, but the total number of L-AChRs is unchanged (Gally 2004). How can levamisole have an effect independent on L-AChR subcellular localization? We show that the difference between WT and mutants is explained by VGCC properties. In WT, L-AChRs remain opened on levamisole and the muscle depolarization causes a complete inactivation of VGCC. In lev-10 worms, muscle cells are less depolarized on levamisole and a fraction of VGCC is still open, explaining why lev-10 mutants remain hypercontracted when WT worms relax over time.

Third, after 8 hours of levamisole exposure, WT worms are still paralyzed whereas lev-10 mutants partially recover locomotion. In these mutants, we show that the membrane resting potential is back to normal, suggesting that L-AChRs are not functional anymore.

Our results demonstrate that slightly reducing the magnitude of muscle depolarization during the acute phase of levamisole exposure is the key to explain adaptive-resistance. A new large-scale screen conducted in our laboratory has demonstrated that levamisole-resistance screens are not saturated and could still reveal new molecular players involved in the implementation of L-AChR activation.

172 Motor neuron coupling by the presynaptic gap junction protein INX-1 ensures robustness of calcium oscillation generation during a rhythmic behavior. U. Choi1, H. Wang1, M. Hu1, D. Sieburth1 1) Zilkha Neurogenetic Institute, Los Angeles, CA; 2) Howard Hughes Medical Institute, California Institute of Technology, Pasadena, CA.

Rhythmic behaviors are often controlled by calcium oscillations in underlying circuits, but little is known about how these oscillations are shaped to maintain proper periodicity and to avoid arrhythmia. During the expulsion step of the C. elegans defecation motor program, calcium oscillations are generated every 50 seconds at presynaptic terminals of a pair of GABAergic motor neurons (AVL/DVB) by a peptide signal from the pacemaker (intestine), and they drive the rhythmic release of GABA and the subsequent contraction of the enteric muscles (Exp). Here we show that the gap junction protein inx-1/innexin functions to
suggesting that innexin/pannexin proteins may play a broadly conserved role in neuronal mechanotransduction. The also required for harsh touch in both the TRNs and the PVD nociceptors, but not for thermal responses in PVD, indicating that it indicating that UNC-7 acts as hemichannels to sense gentle touch. An isoform known to make gap junctions does not rescue, that lacks four cysteine residues necessary for gap junction formation, robustly rescues the second mechanosensor, the innexin UNC-7, is also essential for gentle touch. A mutant

Major depression (MD), one of the most common mental disorders, is highly heritable and is commonly treated with selective serotonin reuptake inhibitors (SSRIs), which increase serotonin signaling in the brain. Yet, genome-wide association studies have failed to definitively implicate genetic defects in serotonin signaling as the cause of MD. We are studying the mechanism

Mechanosensation is central to a wide range of functions, including tactile and pain perception, hearing and proprioception, but identifying the mechanotransducer molecules responsible has proved challenging. The response to gentle body touch in C. elegans is mediated by the touch receptor neurons (TRNs). We find that, in addition to the well-established role of MEC-4, a second mechanosensor, the innexin UNC-7, is also essential for gentle touch. A mutant unc-7 transgene, encoding a derivative that lacks four cysteine residues necessary for gap junction formation, robustly rescues the unc-7 mechanosensory defect, indicating that UNC-7 acts as hemichannels to sense gentle touch. An isoform known to make gap junctions does not rescue, suggesting that the functions of UNC-7 in gap junctions and mechanosensory hemichannels are genetically separable. UNC-7 is also required for harsh touch in both the TRNs and the PVD nociceptors, but not for thermal responses in PVD, indicating that it functions specifically in mechanosensation. We present evidence that UNC-7 and MEC-4 function independently. Heterologous expression of unc-7 in touch-insensitive chemo sensory neurons confers ectopic touch sensitivity, demonstrating that UNC-7 hemichannels are sufficient for mechanosensation. The unc-7 touch defect can be rescued by the mouse Panx1 gene, suggesting that innexin/pannexin proteins may play a broadly conserved role in neuronal mechanotransduction.

We propose that the HSN releases both serotonin and NLP-3 neuropeptides, which act cooperatively in a partially redundant

We found that the HSNs release a neuropeptide in addition to serotonin to stimulate egg laying. The nlp-3 neuropeptide gene as well as the enzymes necessary for serotonin biosynthesis and release are all expressed in the HSNs. Knocking out either nlp-3 or serotonin biosynthesis caused mild egg-laying defects, but knocking out both caused a strong egg-laying defect that phenocopies that of animals lacking HSNs. Further, we optogenetically stimulated the HSNs and observed that the resulting egg-laying behavior is profoundly dependent on nlp-3, and is completely silenced in animals lacking both NLP-3 neuropeptides and serotonin.
fashion to stimulate egg laying, explaining the absence of strong egg-laying defects in mutants lacking serotonin. Serotonin receptors are found only on the muscles within this circuit, and sensitize them to contract in response to acetylcholine. We are currently analyzing the Ca2+ activity in the circuit to determine roles for NLP-3 neuropeptides in circuit activity. Co-release of serotonin and neuropeptides also occurs in the mammalian brain, and by analogy with the worm egg-laying circuit may be an important feature in explaining the absence of a strong genetic link between MD and serotonin.


176 A compressed C. elegans locomotory circuit utilizes excitatory motor neurons as local central pattern generators for reversal locomotion. S. Gao1,2, S. Guan3, A. Fouad4, J. Meng2,3, Y. Huang2, Y. Li1, S. Alcaire2, W. Hung2, T. Kawano2,6, Y. Lu2,3, B. Qi7, Y. Jin7, M. Alkema7, C. Fang-Yen7, M. Zhen2,3 1) Key Laboratory of Molecular Biophysics of the Ministry of Education, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, China; 2) Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Ontario M5G 1X5, Canada; 3) Department of Molecular Genetics, Department of Physiology, University of Toronto, Ontario M5S 1A8, Canada; 4) Department of Bioengineering, Department of Neuroscience, University of Pennsylvania, Philadelphia, PA 19104, USA; 5) Department of Neurobiology, University of Massachusetts Medical School, Worcester, MA 01605, USA; 6) Present address: Graduate School of Science, Kobe University, Rokkodaiho, Nadaku, Kobe 657-8501, Japan; 7) Neurobiology Section, Division of Biological Sciences, University of California, San Diego, La Jolla, CA 92039, USA.

Central pattern generators (CPGs), neurons or neural circuits that exhibit and sustain oscillatory activities, drive motor rhythmicity. Through cell ablation, electrophysiology, and calcium imaging, we reveal the CPGs that underlie C. elegans reversal locomotion. We show the following: first, the cholinergic and excitatory A class motor neurons exhibit intrinsic oscillatory activity; second, the intrinsic activity from multiple A class motor neurons suffices for reversal locomotion; third, their oscillatory activity requires intrinsic P/Q/N voltage-gated calcium currents; fourth, attenuation and potentiation of their oscillatory activity by the descending premotor interneuron, via gap junctions and chemical synapses, respectively, determines the initiation and duration of reversal locomotion. Hence, the A class motor neuron themselves serve as local oscillators; regulation of their CPG activities determines the forward versus reversal motor state. These findings exemplify functional compression at the small C. elegans motor circuit: excitatory motor neurons assume the role for rhythm and pattern generation.

177 Repetitive behavior mediated by glutamate is controlled by astrocyte-like glia in C. elegans. Menachem Katz1, Francis Corson2, Anupriya Singhal1, Wolfgang Keil3, Andrea Bae4, Yun Lu1, Yupu Liang4, Shai Shaham1 1) Laboratory of Developmental Genetics, The Rockefeller University, New York, NY; 2) Laboratoire de Physique Statistique, Ecole Normale Supérieure, Paris, France; 3) Center for Studies in Physics and Biology, The Rockefeller University, New York, NY; 4) Research Bioinformatics, The Rockefeller University, New York, NY.

Neural circuits control animal behavior, and their dysfunction promotes neurological diseases manifesting aberrant behaviors. Neural circuits are tightly associated with astroglial processes, yet glial roles in animal behavior are not well understood. C. elegans has a compact nervous system driving a limited and well-characterized behavioral repertoire. C. elegans glia are not essential for neuron viability, making this animal a useful setting for investigating glial influences on circuits and behavior. We have characterized roles of the brain-associated C. elegans CEPsh glia in animal locomotion. We demonstrate that, like astrocytes, CEPsh glia infiltrate the brain neuropil and abut glutamatergic synapses. Postembryonic ablation of CEPsh glia results in foraging defects, including an increased reversal rate. CEPsh glia transcriptome analysis reveals similarities to astrocytes, including expression of the glial glutamate transporter, glt-1. Behavior studies reveal that glt-1 mutants, unlike wild-type animals, have increased reversal frequency, as well as repetitive reversal bouts. AVA neurons control reversal initiation, and our in vivo measurements of synaptic glutamate adjacent to AVA reveal roles for glial GLT-1 in rapid glutamate clearance. Inappropriate clearance leads to high-frequencies oscillations in synaptic glutamate and AVA activation. We show that these oscillations are mediated by the presynaptic metabolizable glutamate receptor mgl-2/mGluR5, and that mutations in mgl-2 suppress glt-1-mediated repetitive reversals. Loss of murine astrocyte-expressed GLT1 has been shown to produce repetitive grooming, and inhibition of murine mGluR5 ameliorates repetitive behavior in an Obsessive Compulsive Disease (OCD) model. Our studies, therefore, suggest conserved roles for glutamate clearance by glia in controlling repetitive behavior, a feature of some human neuropsychiatric disorders.

178 Plasticity of the electrical synapse network under environmental stress. Abhishek Bhattacharya1,2, Oliver Hobert1,2 1) Department of Biological Sciences, Columbia University, New York, NY; 2) Howard Hughes Medical Institute.

Extrinsic and intrinsic stress signals influence nervous systems to undergo plastic changes, including alteration of gene expression, modulation of synaptic structure and ultimately leads to behavioral adaptation. Under adverse environmental conditions, nematodes develop into hibernation-like diapause states like, the dauer and the L1-diapause states, which show altered responsiveness to various environmental cues and a strikingly different locomotory behavior, suggesting a wide scale rewiring of the nervous system. However, a system wide understanding of the circuit in the diapause state or the molecular mechanisms responsible underneath the possible circuit plasticity are lacking. I focused my study on the electrical synapse or gap-junction network.

I took an entire nervous system wide approach to understand the dynamics of innexin (inx) gene expression, the family of 25 genes that encodes the building blocks invertebrate electrical synapses. Using fosmid-based fluorescent transcriptional reporter
transgenes, I identified several of the inx genes show altered expression in the dauer nervous system. Among them, inx-6 showed the most striking expression plasticity in dauer and in L1-diapause states. Under favorable condition, inx-6 is expressed only in a subset of pharyngeal muscle cells. In diapause states, inx-6 expression is additionally turned on in the interneuron pair AIB, which regulates reversals and turns. inx-6 expression in AIB is reversible. As animals exit the diapause states, inx-6 expression turns off in AIB. I found that a transcriptional activator-repressor combination provides the cellular specificity of expression while integrating the environmental information. This diapause specific INX-6 expression in AIB leads to de-novo gap-junction formation between CO2-sensing sensory neuron BAG and AIB. INX-6 acts in a heterotypic gap junction complex with CHE-7, another innexin that is expressed in BAG, albeit constitutively. INX-6 expression in AIB also leads to formation of auto-synapses between AIBL and AIBR.

Nematodes show opposing chemotaxis preference towards CO2 in dauer state. While non-dauer animals are repelled by CO2, dauers are attracted to CO2. I found animals in L1-diapause are also attracted by CO2. Using an AIB-specific inx-6 mutant allele, generated by CRISPR/Cas9 mediated genome editing, I identified that the loss of INX-6 activity in AIB leads to defects in CO2-attraction in diapause states. Loss of che-7 also has similar effects on CO2-attraction. INX-6 activity in AIB is also required to control locomotion speed and locomotory quiescence, specifically in diapause stage. My studies provide novel insights into the process of stress induced nervous system plasticity at different levels of gene regulation, circuit plasticity and behavior.

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Saturday, June 24  8:30 AM – 11:30 AM
De Neve Auditorium
Development


**179  An automated method for analyzing 4D high content imaging data to profile the gene set controlling C. elegans embryonic development.**  R. Khalilullin1,2, S. Ochoa1,2, Z. Zhao1,2, S. Wang1,2, J. Hendel1,2, R. Biggs1,2, A. Gerson-Gurwitz1,2, A. Desai1,2, K.* Oegema1,2, R.A.* Green1,2  1) Ludwig Institute for Cancer Research; 2) University of California, San Diego.

An important challenge is to systematically define the contributions of the ~2600 genes that control embryogenesis to specify cell fate and position and drive the formation and morphogenesis of multi-cellular tissues. To address this challenge, we developed methods to film embryogenesis following RNAi-based gene knockdown in two engineered strains expressing fluorescent markers that: (1) mark nuclei in cells in the three germ layers (endoderm, ectoderm and mesoderm) in three different colors, and (2) mark epithelial cell junctions and the surface of a subset of neurons in green and red (morphogenesis strain). Developing an automated method to analyze this 4D data and compare the complex phenotypes resulting from gene knockdowns presents a significant challenge. To tackle this problem, we manually scored ~7000 individual embryo time-lapse datasets for a pilot set of 500 genes to generate a data set that identified the spectrum of embryonic developmental defects and served as a guide for the development of custom automated algorithms. Automated algorithms were developed that: (1) monitor the increase in the number of nuclei in each of the three germ layers over time and (2) measure the distribution of nuclei in the germ layer strain and the fluorescent signals in the morphogenesis strain over time by measuring their relative center of mass and moment of inertia around orthogonal axes bisecting the embryo. To evaluate the phenotypic similarity between RNAi conditions, we measure the distance between phenotypes in n-dimensional space, where n is the number of measured parameters. To correct for the fact that the Euclidean distance between genes increases non-uniformly as phenotypes become more severe, we measure the angle between the average phenotypes for the two conditions in n-dimensional space (phenotypic angle of deviation; PAD). This automated method is highly effective in identifying groups of genes that yield similar phenotypes, suggesting that they function together in specific developmental pathways. Our library of developmental data and predicted functional groupings for each essential gene will be made publically available as a resource to the scientific community. This work represents the first fully automated high-content screen of an intact developing organism and is the most complex morphological profiling effort to date.


Cell identification is a ubiquitous task in C. elegans research, allowing researchers to exploit the power of the stereotyped cell lineage uniquely available in the worm. However, cell identification from still or video microscopy images is a notoriously difficult, time consuming, and error prone process, more an art than a science and best done by experienced experts in the field.

We introduce an automated, community-driven, machine learning based cellular identification system we term a probabilistic cell atlas and demonstrate its use on C. elegans adult hermaphrodite pan-neuronal fluorescent calcium imaging recordings. We demonstrate use of the system on both stills of static fluorescent GFP and mCherry markers and videos of dynamic GCaMP markers, and combinations of these imaging modalities.

The distinguishing features of our approach vis à vis prior approaches include: (1) generation of probabilistic guesses at cell ID
rather than single best-guesses for each cell found in the test data, (2) flexible/optimal utilization of multi-modal training data, including cell position, morphology, color, and, notably, activity through time, and (3) iterative Bayesian improvement of the probabilistic model of cell features (i.e. the probabilistic atlas). Because of this design, the accuracy, confidence, and input flexibility of the system progressively improve with each dataset uploaded by users.

The system is free for use, open source and available online. We encourage fellow worm researchers to upload their datasets they wish to ID and thereby contribute to the collective value of the system. We hope this system becomes another long-term shared resource for the worm community.

181 Rotating and elongating embryos: SPIM microscopy reveals how planar polarity could be established during morphogenesis. Xinyi YANG¹, Teresa FERRARO¹, Julien PONTABRY², Nicola MAGHELLI³, Loïc ROYER³, Stephan GRILL³, Gene MYERS³, Michel LABOUESSE² ¹) Institut de Biologie Paris-Seine, Paris, FR; ²) Institut de Genetique et de Biologie Moleculaire et Cellulaire, Illkirch, France; ³) Max Planck Institute of Molecular cell Biology and Genetics, Dresden, Germany.

The process of morphogenesis in C. elegans embryos is largely driven by epidermal cells. Unlike Drosophila and zebrafish, no cell division or cell rearrangement is involved in C. elegans morphogenesis. Epidermis shape changes, which are characterized by junction lengthening along the anterior/posterior (A/P) direction, play a key role in this process. The nature of junction lengthening and planar polarity establishment, as well as the cellular mechanisms involved in these processes during C. elegans embryonic elongation are the main objectives of this project. Our lab observed that junction elongation along the A/P direction increases after muscle becomes active, and fails in muscle defective embryos. To better understand which role muscles play in polarized junction lengthening, we examined the global and local movement patterns of embryo using Single Plane Illumination Microscopy, focusing on epidermal adherens junctions and muscle nuclei. We found that wild-type embryos rotated strongly soon after muscle became active, and equally frequently to an outward or inward direction. However, muscle defective and Rho-kinase mutant embryos, which stop elongation at the 2-fold stage, scarcely rotated, suggesting that rotations are important for embryo elongation. By comparing the changes of seam cell aspect ratio, we observed that the head, body and tail mechanically behaved as partially independent entities. We next sought to understand how such movements could account for the polarized junction lengthening, keeping in mind that C. elegans embryos are radially symmetric. By measuring the distance between two dorsal or ventral muscle nuclei, respectively, we found that dorsal and ventral muscles mostly contract alternatively, accounting for embryo rotations. Intriguingly, analysis of junction roughness showed that during embryo rotations, junctions along the A/P direction were stretched when seam cells were positioned outwards. Laser ablation targeting DLG-1::GFP results further discovered that these junctions were under higher tension when stretched. These results suggest that asymmetric muscle activity defines the source of polarity in C. elegans embryo and provides the local driving force for epidermis stretching. To study how this tension impacts polarized junction lengthening, we are investigating the insertion of new E-cad molecule into junctions during embryo rotations by single molecule imaging. Altogether, our results suggest that C. elegans embryos extend in a ratchet mode due to the alternating muscle contractions.

182 PCP and SAX-3/Robo pathways cooperate to regulate convergent extension-based nerve cord assembly. P.K. Shah¹, M. Tanner¹, I. Kovacevic¹, A. Rankin¹, Z. Bao², A. Colavita³ ¹) Neuroscience Program, Ottawa Hospital Research Institute, Ottawa, ON; ²) Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON; ³) Developmental Biology Program, Sloan Kettering Institute, New York, NY; 4) equal contribution; 5) co-senior authors.

At hatching DD, DA and DB motor neurons are stereotypically ordered relative to each other and distributed as a single track along the anterior-posterior (AP) axis. During embryogenesis, these neurons arise from left and right lineages, move towards the midline and intercalate to assemble the presumptive VNC. This morphogenetic process, in particular how neurons intercalate to assemble the VNC, has not been well characterized. We performed 3D fluorescence time-lapse imaging to show that intercalation of VNC neurons into a linear structure that extends the length of the AP axis involves a rosette-mediated convergent extension (CE) process. The planar cell polarity (PCP) pathway plays a highly conserved role in CE-based movements. Using the 6 DD neurons which are evenly spaced along the AP axis and therefore a convenient marker of VNC assembly, we found that mutations in the core PCP components vang-1/Vang Gogh and prkl-1/Prickle caused DD neurons to be anteriorly displaced in newly hatched worms. In the embryo, these mutants displayed defects in cell neighbor exchange and rosette resolution. Interestingly, we found that mutations in sax-3/Robo, a gene better known for roles in axon guidance, displayed similar defects in DD positioning and embryonic cell-neighbor exchanges. VANG-1 and SAX-3 are localized to contracting edges and rosette foci. Exploring further, we found that PCP/sax-3 double mutants display a significantly shortened, anteriorly displaced distribution of VNC neurons compared to single mutants. This simultaneous loss of both PCP and sax-3/Robo correlates with a severe disruption of CE in the embryo suggesting that these pathways act in parallel during VNC assembly. Our results indicate that rosette-based convergent extension is conserved in central nerve cord assembly and reveals a novel role for sax-3/Robo in this process.

183 A “bHLH code” for sexually dimorphic form and function of the C. elegans somatic gonad. H. Littleford¹, M. Sallee¹,3, I. Greenwald¹,3 ¹) Biology, Columbia University, New York, NY; 2) Genetics and Development, Columbia University, New York, NY; 3) Biochemistry and Molecular Biophysics, Columbia University, New York, NY.

In C. elegans, sexual dimorphism in gonad shape reflects the apportionment of different roles to three “regulatory cells” of the somatic primordium that forms in the L2 stage: in hermaphrodites (somatic females), the U-shaped gonad is formed by outgrowth of two gonad arms led by the two hermaphrodite distal tip cells (hDTCs), while the single anchor cell (AC) remains central and connects to the vulva; the male J-shaped gonad is formed by posterior outgrowth of a sole linker cell (LC)-led gonad.
arm, which also connects to the cloaca, while the two male DTCs (mDTCs) remain at the fixed distal end (Kimble and Hirsch 1979). The basic Helix-Loop-Helix (bHLH) transcription factor HLH-2, the sole C. elegans ortholog of the E proteins mammalian E2A and Drosophila Daughterless, is critical for development of each regulatory cell type (Karp and Greenwald 2004, Chesney et al. 2009), and functions as a homodimer for both specification and the terminal features of the AC (Sallee and Greenwald 2015). We present our evidence for a “bHLH code” for regulatory cell identity by showing that distinct sets of bHLH genes are expressed in each non-AC regulatory cell type, which partner with HLH-2 to specify their fate or terminal features. We also show that prospective regulatory cell fate can be directly reprogrammed simply by loss or ectopic expression of bHLH genes, in direct accordance with the inferred code. Sexual identity and positional transformations suggest that that the “bHLH code” is embedded in a genetic network with bow-tie regulatory architecture (Nelson et al 2011), wherein sexual, positional, temporal, and lineage inputs connect through bHLH genes to diverse outputs for terminal features (Felix and Sternberg 1996, Matson et al 2012). Our results provide a plausible mechanism for the evolutionary plasticity of nematode gonad form, and more broadly suggest how functions of specialized cells can be grouped into genetic modules which may facilitate reassignment during evolution.


Cells in developing organisms must robustly assume the correct fate in order to fulfill their specific function. At the same time, cells are strongly affected by molecular fluctuations, so-called molecular noise, leading to inherent variability in individual cells. During development, some thought to exploit such molecular noise to drive stochastic cell fate decisions, with cells randomly picking one cell fate out of several possible ones. Yet, how molecular noise drives such decisions is an open question. We address this question by a novel quantitative approach, studying one of the genetically best-understood stochastic cell fate decisions: the AC/VU decision in C. elegans gonad development. Here, two initially equivalent cells, Z1.ppp and Z4.aaa, interact, so that one cell becomes the anchor cell (AC) and the other a ventral uterine precursor cell (VU). It is thought that the symmetry is broken when small molecular fluctuations are amplified via a positive feedback loop in the Notch signaling pathway (Seydoux and Greenwald 1990). To identify the noise sources that drive the AC/VU decision, we study the Notch ligand lag-2, using 1) a novel time lapse technique to follow expression dynamics in live animals and 2) smFISH to quantify gene expression with single mRNA resolution. We find not only that birth order biases the decision outcome, with the first-born cell typically assuming VU fate, but that both the strength of this bias and the speed of the decision decrease as the two cells are born at more similar times. Moreover, we find that lag-2 exhibits strongly stochastic expression already in the two mother cells, Z1.pp and Z4.aa. The strong asymmetry in lag-2 levels inherited by the daughter cells, Z1.ppp and Z4.aaa, might be responsible for symmetry breaking when both cells are born at similar times. Together, our results suggest that two independent noise sources, birth order and stochastic lag-2 expression, are exploited to help amplify the molecular differences in Z1.ppp and Z4.aaa into AC or VU fate and thereby ensuring a rapid and robust decision.

185 A real-time biosensor for MPK-1/ERK activity reveals signaling dynamics during C. elegans cell fate specification. C. de la Cova¹, R. Townley¹, S. Regot², I. Greenwald¹ 1) Dept of Biological Sciences, Columbia University, New York, NY; 2) Dept of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD.

Genetically encoded fluorescent activity sensors, such as Kinase Translocation Reporters (KTRs) that convert kinase activity into a nucleocytoplasmic shuttling equilibrium, have illuminated single cell signaling dynamics in cultured cells. Here, we have successfully adapted the first generation KTR for extracellular signal-regulated kinase (ERK) to allow easy implementation in vivo. This new sensor, called “ERK-nKTR,” allows ERK activity to be assessed qualitatively or quantitatively by analysis of individual nuclei in living animals; the improvements we describe for KTR design and image analysis will be generally relevant to the extension of this technology. In C. elegans, we show that the ERK-nKTR sensor faithfully reports activity of the ERK ortholog MPK-1 in several cell types and different developmental processes: Vulval Precursor Cell (VPC) fate specification, Sex Myoblast migration, AWC and ASE sensory neurons, and germ line development. Our in-depth analysis of ERK activity over time in the Vulval Precursor Cells (VPCs), a well-characterized paradigm of EGFR-Ras-ERK signaling, has identified dynamic signaling features not evident from analysis of developmental endpoints alone. The toolkit described here will facilitate studies of ERK signaling in other C. elegans contexts, and the design features will enable implementation of this and other KTR-based kinase activity sensors in multicellular organisms.

186 LET-60/Ras recruits its 2°-promoting effector, RGL-1/RalGEF, specifically to the apical plasma membrane of presumptive 2° cells. Hanna Shin, David Reiner Institute of Biosciences and Technology, College of Medicine, Texas A&M University, Houston, TX.

ERG from the anchor cell (AC) induces the vulval precursor cells (VPCs) to assume the highly reproducible 3°-3°*-2°-1°-2°*-3° pattern of cell fates. Two potentially competing models describe this event. In the morphogen gradient model, position of VPCs in the EGF gradient dictates cell fates. In the sequential induction model, LET-23/EGFR in the VPC closest to the AC triggers the canonical Ras-Raf-MEK-ERK MAP kinase cascade to promote 1° fate, which in turn synthesizes DSL ligands to activate LIN-12/Notch in the two neighboring VPCs to induce 2° fate. We reconciled these models by identifying a signaling mechanism by which the EGF gradient promotes 2° fate. We found that in vulval patterning, LET-60/Ras dynamically switches effectors, from the canonical Raf to the poorly understood RalGEF-Ral, which promotes 2° fate in support of the main LIN-12/Notch signal. Ras-RalGEF-Ral is required for 2° cells to respond fully to the 2°-promoting EGF-EGFR signal, and ectopic constitutively active RAL-1/Ral promotes 2° fate (see Zard et al., 2011). We are also investigating the signal downstream of Raf (see poster by Shin/Kaplan). LET-23/EGFR::GFP shows general plasma membrane localization in VPCs. Mammalian Ras is uniformly expressed at the plasma membrane, where upon activation it is thought to recruit effectors. Traditional heterologous means of
expressing proteins run the risk of missing regulatory sequences or, through over-expression, swamping delicate mechanisms of subcellular trafficking. We therefore used CRISPR to tag the N-terminus of endogenous RGL-1/RalGEF with mNeonGreen (mNG). Prior to induction, mNG::RGL-1 was diffuse and cytosolic in VPCs. After induction, mNG::RGL-1 was concentrated at the apical plasma membrane in presumptive 2° - but not presumptive 1° - VPCs, and not to basolateral membranes. Our observations raise the intriguing possibility of spatial compartmentalization of potentially conflicting signals, 1°-promoting LIN-45/Raf vs. 2°-promoting RGL-1/RalGEF, as a novel mechanism of interpreting the EGF gradient. Consequently, to complement our data with mNG::RGL-1, we are tagging endogenous LET-23/EGFR, LET-60/Ras and LIN-45/Raf to observe spatial regulation of signaling activity over time. We intend to have these new tools ready to present preliminary data.

187 Deconstructing eutely: mechanisms of phenotypic robustness in the seam. Dimitris Katsanos¹, Sneha Koneru¹, Lamia Mestek Boukhitar¹,², Nicola Gritti³, Ritobrata Ghose¹, Peter J. Appleford⁴, Maria Doitsidou⁵, Alison Woollard⁶, Jeroen van Zon³, Richard J. Poole⁷, Michalis Barkoulas¹ ¹) Department of Life Sciences, Imperial College, London SW7 2AZ, United Kingdom; ²) Centre for Translational Omics – GOgene, Genetics and Genomic Medicine, UCL, Institute of Child Health, London WC1N 1EH, UK; ³) AMOLF, Science Park 104, Amsterdam 1098 XG, The Netherlands; ⁴) Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK; ⁵) Centre for Integrative Physiology, University of Edinburgh, Edinburgh, EH8 9XD, UK; ⁶) Department of Cell and Developmental Biology, University College London, London WC1E 6BT, UK.

Biological systems constantly face perturbations both in the form of environmental changes and intrinsic stochasticity. Nevertheless, they are still capable of producing constant phenotypic outcomes. Development in particular is a remarkably robust process ensuring the reproducible generation of key phenotypic traits, such as tissues with the correct cell number. However, very little is known about the mechanisms governing developmental robustness. We use the epidermal stem cells of C. elegans, known as seam cells, as a model to identify factors modulating cell number variability. We employ a pipeline consisting of chemical mutagenesis, phenotype selection and mapping by next generation sequencing to identify genes modulating seam cell number variance. To establish a proof of concept, we present here that mutations in the Hes-related bHLH transcription factor lin-22 increase the variability of terminal seam cell number. Using time-lapse imaging of post-embryonic seam cell divisions, we pinned down the developmental basis of phenotypic variability. We show that stage and lineage-specific gain or loss of cell fate symmetry in stem cell daughters underlies the variable phenotype and that these developmental errors occur within the same animal or even a single cell lineage in a stochastic manner. We provide evidence by smFISH and genetics that LIN-22 acts in the epidermis to antagonise Wnt signalling. In contrast to the seam, lin-22 mutants also demonstrate loss of stochasticity when it comes to P3,p division frequency, emphasising the context-specific manifestation of phenotypic variability within a whole organism. Taken together, our work identifies a factor involved in phenotype construction as a modulator of developmental robustness and highlights the feasibility to map genes influencing developmental variability.

188 Controlling a stem cell division pattern in the C. elegans epidermis. S.E.M. van der Horst¹, J. Anselmo Cravo¹, J. Teapal¹, A. Woollard², S.J.L. van den Heuvel¹ ¹) Developmental Biology, Utrecht University, Utrecht, NL; ²) Dep of Biochemistry, University of Oxford, South Park Road, Oxford, UK.

The development from fertilized egg to adult requires forming the correct type cells in the right numbers. Switching between symmetric and asymmetric cell divisions is a critical part of this process. Symmetric cell divisions increase the cell numbers, while asymmetric divisions support the generation of cell diversity and maintenance of tissue-specific stem cells. The C. elegans seam cell lineage provides an attractive model in which to study the developmental control over symmetric versus asymmetric cell division.

Seam cells are stem cell-like precursor cells in the C. elegans epidermis, which form two epithelia at the lateral sides of the animal. These cells switch between symmetric and asymmetric divisions during larval development, dependent on conserved regulatory mechanisms. For instance, Wnt/beta-catenin signaling controls the seam cell fate. This involves the TCF/LEF-related transcription factor POP-1, which functions in association with the Groucho-related co-repressor UNC-37 to induce differentiation of seam daughter cells. Asymmetric localization of Wnt/beta-catenin pathway components during cell division facilitates the simultaneous formation of differentiating and self-renewing seam daughter cells. Interestingly, we observed that Wnt signaling components still localize asymmetrically during symmetric seam cell divisions. Our experimental evidence suggests that the Wnt/beta-catenin asymmetry pathway is bypassed during the L2 stage when seam cells switch to proliferative symmetric divisions. This bypass appears to be mediated by the Runx family transcription factor RNT-1, in association with the co-factors BRO-1 CBFβ and UNC-37 Groucho. These regulators are thought to have dual roles in controlling seam proliferation and differentiation. We observed that the combined induction of rnt-1 and bro-1 predominantly suppresses seam cell differentiation, thereby increasing the seam cell population at the expense of differentiated progeny. Our results indicate that antagonizing Wnt and Runx signals determine whether seam cells undergo asymmetric division or switch to a symmetric division mode.

189 Turning a “killer” into a developmental timing regulator: Coupling N-end rule E3 ubiquitin ligase UBR-1 to CED-3 caspase promotes recognition and degradation of LIN-28. B.P. Weaver¹, Y.M. Weaver¹,², M. Han¹,² ¹) MCD, CU Boulder, Boulder, CO; ²) Howard Hughes Medical Institute.

Loss of ced-3 caspase is well-known for defective cell death without other obvious defects. However, recent findings from several labs, including ours, have identified important non-apoptotic functions for canonical apoptotic regulators across diverse metazoans including nematodes, flies, and mammals, suggesting that the non-apoptotic activities of caspases critically contribute to the robustness of various cellular functions. We previously reported the CED-3 caspase had a critical non-apoptotic
activity negatively regulating the expression of LIN-28 in the developmental timing pathway thereby limiting supernumerary seam cell divisions (Weaver, et al. 2014). Mechanisms responsible for altered caspase client substrate interactions to yield apoptotic or non-apoptotic functions are currently unknown. We report a coupled mechanism between CED-3 caspase and the Arg/N-end rule pathway to inactivate the LIN-28 pluripotency factor in seam cells, thereby ensuring proper temporal cell fate patterning. Using genetic and biochemical methods, we find that the caspase and the E3 ligase execute this function in a non-additive manner. We find that CED-3 caspase and the Arg/N-end rule E3 ubiquitin ligase UBR-1 form a physical complex that couples their in vivo activities. Upon testing multiple human caspases, we find that human caspase 8 strongly interacts with human UBR2 (homologous to C. elegans UBR-1). Interestingly, human Caspase 8 has been implicated in non-apoptotic functions of monocyte differentiation and trophoblast fusion. Our findings imply that UBR-type E3 ligase/Caspase interactions may be conserved throughout metazoan evolution. The inter-dependence of these proteolytic activities provides a paradigm for non-apoptotic caspase-mediated protein inactivation. Our findings suggest the CED-3/UBR-1 complex likely serves three purposes: (1) promotes recognition of the non-apoptotic target, LIN-28; (2) facilitates rapid degradation of LIN-28; and (3) adds another level of regulation to ensure LIN-28 inactivation does not happen at inappropriate times.

**FACT is a reprogramming barrier in C. elegans and human cells.** Ena Kolundzic1,3, Andreas Ofenbauer1,3, Anne Sommermeier1,3, Gülkiz Baytek1, Stefanie Seelk1,3, Mei He1, Bora Uyar1, Altuna Akalin1, Scott Lacadie1, Sebastian Diecke1,2, Baris Tursun1  1) Berlin Institute for Medical Systems Biology BIMSB, Max Debrueck Center MDC, Berlin, DE; 2) Berlin Institute of Health Stem Cell Core Facility, Berlin, Germany; 3) Department of Biology, Humboldt University, 10115 Berlin, Germany.

Direct reprogramming of cellular identities by mis-expressing transcription factors (TFs) is limited in different tissue contexts. We previously identified the histone chaperone LIN-53 as a reprogramming barrier in C. elegans preventing the direct conversion of germ cells to specific neuron types (Tursun et al., Science 2011). Analogously, a recent study showed that the mammalian LIN-53/CAF-1p48-containing histone chaperone CAF-1 is a barrier for cellular reprogramming in mouse cells (Cheloufi et al., Nature 2015) suggesting that more barrier factors might be conserved among C. elegans and higher organisms. Therefore, we conducted genetic screens using transgenic animals expressing CHE-1, the ASE neuron fate-inducing TF, under the heat shock promoter together with ASE-neuron-specific reporter gcy-5:5::gfp and revealed that the histone chaperone FACT (FAcilitates Chromatin Transcription) is a novel reprogramming barrier. FACT protects different tissues from being directly reprogrammed into neuron-like cells in C. elegans. Moreover, we demonstrate that FACT depletion also enhances the reprogramming of human fibroblasts. FACT is predominantly known as a positive effector that regulates gene expression by facilitating transcription. Nevertheless, FACT can also act as a repressor in certain instances such as at the mating locus in yeast. We performed transcriptome analysis as well as chromatin profiling using ATAC-seq and found that loss of FACT results in transcriptional de-repression at specific genomic loci rather than disturbing chromatin organization globally. Furthermore, FACT depletion-mediated reprogramming requires the presence of the histone variant H2A.Z and Histone H3 phosphorylation by the Aurora Kinase. Hence, aberrant genomic H2A.Z and H3ph localization might facilitate gene activation leading to enhanced reprogramming. Our findings demonstrate the versatility of C. elegans as a discovery tool for identifying and understanding the role of epigenetic regulators in the context of safeguarding cellular identities.

**Decoding two Paths from Glia-to-Neuron.** M. Sammut1, K. Khambhaita1, R.C. Bonnington1, B. Kim2, S.J. Cook2, L. Molina-Garcia1, D.H. Hall1, S.W. Emmons2, A. Barrios1, R.J. Poole1  1) Cell and Developmental Biology, University College London, London, GB; 2) Albert Einstein College of Medicine, New York, USA.

We have recently characterised differentiated glial cells that divide to produce neurons, the mystery cells of the male (MCMs). Here we present a previously undescribed direct glia-to-neuron cell fate switch, revising the total number of male neurons to 387 and glia to 90. Studies of vertebrate neural development have revealed that differentiated glia can act as neural precursors, however the cellular and molecular mechanisms have not been fully determined. To identify the genetic factors that regulate these glia-to-neuron cell fate switches, we have generated a collection of no mystery cell (nom) mutants and assessed their role in both glia-to-neuron cell fate switches. In early L4, the male amphid socket (AMso) glial cells divide asymmetrically. We observe increased nuclear localization of the Wnt-signalling effector POP-1/TCF, in the anterior daughter, the self-renewed AMso than in the posterior MCMs. The proneural factor hlih-14/Ascl1 is then transiently expressed in the MCMs, prior to neuronal differentiation. From a GFP-based forward genetic screen of 4000 genes we have isolated nine nom mutants in which the MCMs fail to be specified. We have identified mutants that affect AMso division, MCM neuronal differentiation and MCM neuronal subtype specification. Two AMso division mutants, nom-5 and nom-8 were mapped-by-sequence to the cdk-4 locus. CDK-4 is required for G1-S progressions in postembryonic blast cells but surprisingly, our alleles only appear to affect the AMso division. Moreover, no loss of glial fate or acquisition of neuronal characteristics was observed in the undivided AMso, suggesting that MCM specification requires DNA replication or the asymmetric segregation of neuronal factors.

Following Sulston's preliminary observations, we find that the male phasmid socket one (PHS01) glial cells become bona fide neurons during the L4 stage and name them phasmid neuron D (PHD). Importantly, this transition is direct, not requiring cell division. We establish their developmental lineage, identity as cholinergic neurons, connectivity and provide insight into their function during mating, (see poster by R.C. Bonnington). PHD specification is unaffected in cdk-4 mutants and the majority of nom mutants tested. This suggests that largely distinct genetic mechanisms regulate the two events. The male provides two glia-to-neuron cell fate switches that occur by seemingly independent cellular and genetic mechanisms. We are continuing to investigate the regulatory strategies that confer neurogenic potential to differentiated glia.
Mechanisms of larval P-cell nuclear migration through constricted spaces.

Ma,

Cellular migrations through constricted spaces are a critical aspect of many developmental and disease processes. This includes the regulation of mitotic entry, which is controlled by the spindle checkpoint. The MAD-1/MAD-2 complex is a key component of this checkpoint, and its activity is regulated by specific developmental cues to promote cell cycle progression. Inhibition of MAD-2 delays the cell cycle of larval germ cells at the G2 stage, reducing their proliferation rate. These data suggest that MAD-2 is activated by such cues to promote mitotic entry, independently of its established function in the spindle checkpoint.

Using Near-TIRF imaging, we find that GFP::POS-1 is present in fast-diffusing and slow-diffusing particles that frequently interconvert. GFP::POS-1 RNA-binding mutants are uniformly fast-diffusing, suggesting that RNA binding mediates the formation of slow-diffusing particles. MEX-5/6 stimulate GFP::POS-1 segregation by inhibiting the formation of slow-diffusing particles in the anterior cytoplasm, thereby driving their accumulation in the posterior cytoplasm. We propose that PLK-1 is recruited into slow-diffusing MEX-5/RNA complexes that concentrate in the anterior, thereby positioning PLK-1 to inhibit the association of POS-1 with RNA in the anterior. In the posterior cytoplasm, the relatively low concentration of MEX-5/6 and PLK-1 provides a permissive environment for POS-1 to bind RNA, which retards POS-1 diffusion and results in the formation of the posterior-rich POS-1 gradient.

The MAD-1/MAD-2 spindle checkpoint complex is repurposed in development to promote cell cycle progression. PLK-1 couples the MEX-5/6 and POS-1 cytoplasmic gradients in the C. elegans zygote.

During the asymmetric division of the C. elegans zygote, the PAR proteins orchestrate the partitioning of the cytoplasmic RNA-binding proteins MEX-5/6 and POS-1 along the anterior/posterior axis. The posterior kinase PAR-1 increases the diffusivity of its substrate MEX-5 in the posterior, leading to the preferential retention of MEX-5 in the anterior cytoplasm (Tenlen, 2008; Daniels, 2010; Griffin, 2011). In turn, MEX-5/6 act to increase the diffusivity of POS-1 in the anterior cytoplasm, resulting in the preferential retention of POS-1 in the posterior cytoplasm (Daniels, 2009; Wu, 2015). The reaction/diffusion mechanisms underlying the segregation of POS-1 in response to MEX-5/6 are not understood.

Using Near-TIRF imaging, we find that GFP::POS-1 is present in fast-diffusing and slow-diffusing particles that frequently interconvert. GFP::POS-1 RNA-binding mutants are uniformly fast-diffusing, suggesting that RNA binding mediates the formation of slow-diffusing particles. MEX-5/6 stimulate GFP::POS-1 segregation by inhibiting the formation of slow-diffusing particles in the anterior cytoplasm, thereby driving their accumulation in the posterior cytoplasm. MEX-5/6 bind and recruit PLK-1 kinase to the anterior cytoplasm (Nishi, 2008), and we find that this interaction is required for MEX-5/6 to stimulate GFP::POS-1 segregation. Importantly, we provide evidence that PLK-1 phosphorylation of GFP::POS-1 is both necessary and sufficient to inhibit the formation of slow-diffusing GFP::POS-1 particles. Because MEX-5/6 activity depends on its interaction with RNA, we propose PLK-1 is recruited into slow-diffusing MEX-5/RNA complexes that concentrate in the anterior, thereby positioning PLK-1 to inhibit the association of POS-1 with RNA in the anterior. In the posterior cytoplasm, the relatively low concentration of MEX-5/6 and PLK-1 provides a permissive environment for POS-1 to bind RNA, which retards POS-1 diffusion and results in the formation of the posterior-rich POS-1 gradient.

The mitotic phase of the cell cycle is controlled by Cyclin B, which activates the essential mitotic kinase Cdk1. Cyclin B accumulation in G2 promotes mitotic entry, whereas its degradation by the Anaphase-Promoting Complex/Cyclosome (APC/C) triggers anaphase onset and mitotic exit. Here we report that components of the spindle assembly checkpoint that inhibit APC/C activity during mitosis until all chromosomes connect to the spindle are repurposed in development to control mitotic entry. This conclusion initiated with the observation that deletion of the genes encoding two essential spindle assembly checkpoint genes, mad-2 (mdf-2) and mad-3 (san-1), give rise to dramatically different phenotypes. mad-2 null mutants exhibit severe embryonic and post-embryonic developmental defects that are not observed in mad-3 nulls, despite both being equally defective in the spindle checkpoint. Comparison of the phenotypic profile of MAD-2 removal in the germline to a prior screen of genes required for embryo production in the same tissue revealed similarity to components that are required for mitotic entry (Cyclin B, CDK-1/NCC-1 and CDC-25.1), leading to the hypothesis that MAD-2 promotes mitotic entry. In agreement with this, MAD-2 inhibition delayed the cell cycle of larval germ cells at the G2 stage and greatly reduced their proliferation rate. These data suggest that MAD-2 is activated by specific developmental cues to promote cell cycle progression, independently of its established function in the spindle assembly checkpoint. This function requires MAD-2 to form a complex with its partner, MAD-1, to undergo conformational conversion following asymmetric dimerization, and to inhibit the mitotic activator of the APC/C, CDC-20 (FZY-1), all of which are key features of the spindle checkpoint pathway. We propose that the ability of the MAD-1/MAD-2 complex to inhibit APC/C-CDC-20 has been adapted in a checkpoint-independent developmental context to promote progression into mitosis, most likely by allowing accumulation of Cyclin B to a level sufficient for mitotic entry. Based on phenotypic analysis, Cyclin B3 (CYB-3) appears to be the Cyclin B isoform whose accumulation requires MAD-1/MAD-2. Current experiments are directed at analyzing MAD-1/MAD-2 activation in specific developmental contexts. Overall, this effort has revealed how a core module of the spindle checkpoint has been repurposed to provide an essential function in organismal development, and should influence interpretation of phenotypes associated with checkpoint component inhibitions in metazoans as well as therapeutic approaches designed to target the checkpoint pathway in cancer.

Mechanisms of larval P-cell nuclear migration through constricted spaces.

Valdez, L. Herrera, C.R. Bone, L. Ma, D.A. Starr Mol & Cellular Biol, Univ California, Davis, Davis, CA.

Cellular migrations through constricted spaces are a critical aspect of many developmental and disease processes including...
After migration, P cells divide and give rise to hypodermal, vulval, and neuronal fates. Mutations in KASH (UNC-83) or SUN (UNC-84) proteins disrupt nuclear migration by altering interactions between nuclei and microtubules. P-cell nuclei use both dynein (primarily) and kinesin-1 (secondarily) to migrate toward the minus ends of microtubules in the ventral cord. Null mutations in unc-83 or unc-84 cause a partially penetrant, temperature-sensitive, P-cell nuclear migration phenotype, suggesting other mechanisms contribute to P-cell nuclear migration. We performed forward genetic screens for enhancers of the nuclear migration defect of unc-83/84 (emu). To date, we have used whole-genome sequencing to identify three emu loci. First, toca-1 (transducer of CDC-42 activity) is a predicted actin nucleator with an F-bar domain thought to bind curved membranes. Second, we identified the divergent filamin fln-2, which is predicted to crosslink or bundle actin filaments. Most recently, we identified the CDC-42 GEF, cgef-1. Our working model is that branched actin networks function to squeeze nuclei and aid in their migration through constricted spaces. We are currently tagging TOCA-1, FLN-2, and CGEF-1 with GFP using CRISPR/Cas9 genome editing. The third mechanism to move P-cell nuclei through constricted spaces involves the C. elegans lamin, LMN-1. GFP::LMN-1 acts as an enhancer of unc-84 in disrupting P-cell nuclear migration. Our working model is that three mechanisms work together to squeeze P-cell nuclei: 1) Microtubule motors are recruited to the nuclear surface by the KASH protein UNC-83 to move nuclei toward minus ends. 2) A branched-actin network applies force on the nucleus to flatten it for migration through the constricted space. 3) LMN-1 is rearranged to soften the mechanical strength of the nucleus during migration.

196 Neuronal Exophers: A Novel Mechanism for the Removal of Neurotoxic Cytoplasm Components. I. Melentijevic1, M. Arnold1, R. Guasp1, M. Toth1, G. Harinath1, K. Nguyen2, D. Taub3, C. Gabel1, D. Hall1, M. Driscoll1 1) Rutgers, Piscataway; 2) Albert Einstein College of Medicine, Bronx; 3) Department of Physiology and Biophysics, Boston University School of Medicine, Boston.

Combating late-onset neurodegenerative disease and age-associated functional decline in brain are major health challenges of our time. For the effective design of interventions that protect the nervous system from disease-induced and/or age-associated deterioration, we must fully understand endogenous mechanisms for neuronal protection and how they might fail to enable disease promotion. Recently, it has come to be appreciated that neurodegenerative disease proteins/aggregates can be found outside of mammalian neurons, and when outside can actually be taken up by neighboring cells. Transfer of offending molecules has been suggested to be a mechanism of pathogenesis spread for multiple neurodegenerative diseases, including the prevalent Alzheimer’s and Parkinson diseases. We discovered a novel capacity of young adult C. elegans neurons - neurons can extrude substantial packets of cellular contents, which can include aggregated human neurodegenerative disease proteins, mitochondria, or lysosomes, but no nuclear DNA1. We call these extrusions “exophers”. The ability to jettison cell contents is first evident about day 2-3 of adult life, coincident with the documented changes to adult proteostasis2,3, is then minimal until 9-10 days of adult life. Extrusion is increased when protein turnover or autophagy is inhibited. Moreover, exophers can selectively incorporate aggregation-prone proteins and oxidized mitochondria. Exoper contents can appear later in remote cells, and their clearance is mediated by the ced-1, ced-6, and ced-7 engulfment pathway. Neurons that have made exophers appear to maintain functionality longer than non-exopher producing neurons. Thus, this pathway may constitute a novel neuronal protection mechanism that serves to maintain protein/organelle homeostasis when other systems are compromised. We propose that the neuronal extrusion phenomenon constitutes a significant but currently unknown conserved pathway by which healthy neurons maintain their functions, and speculate that, in diseases, this pathway may malfunction to promote spread of pathology. We will present the basic characterization of neuronal exopher production, structural findings as revealed through EM, and our latest data on genetic influences on exopher generation and clearance.

1 Melentijevic, I. et al. C. elegans neurons jettison protein aggregates and mitochondria into the extracellular environment in response to proteotoxic stress, under revision.


197 Biosynthesis of modular ascarosides in lysosome-related organelles. Oishika Panda1, Allison Akagi2, Alexander Artuykha1, Joshua Judkins1, Henry Le1, Parag Mahanti1, Sarah Cohen2,5, Paul Sternberg2, Frank Schroeder1 1) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY; 2) Howard Hughes Medical Institute and Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA. C. elegans and other nematodes use simple building blocks from primary metabolism and a strategy of modular assembly to build a large diversity of signaling molecules, the ascarosides, which function as a chemical language regulating development, lifespan, and many aspects of behavior. In the ascarosides, the dideoxysugar ascarylose serves as a scaffold to which diverse building blocks from conserved primary metabolism are attached. However, the mechanisms that underlie the highly specific assembly of ascarosides are not understood. Screening for candidate genes selected based on predicted sub-cellular localization, we found that worms mutant for the predicted acyl-CoA synthetase ACS-7 are defective specifically in the biosynthesis of osas#9 and icas#9, which mediate aggregation and starvation-dependent avoidance, respectively, whereas abundances of all other ascarosides are wildtype-like. We found that ACS-7 localizes to the acidic lysosome-related organelles (LROs), and further show that mutants lacking acidic LROs are defective in the production of all 4-modified ascarosides.
Additional evidence indicates that autophagy contributes to ascaroside biosynthesis in the acidic LROs. These results highlight the lysosomes, known as the waste disposal system of the cell, as a major source of signaling molecules. Taken together, our results indicate that in *C. elegans*, homologs of genes from canonical metazoan metabolism act in different cell compartments to produce modular specialized metabolites.

### Sunday, June 25 9:00 AM – 11:54 AM  
Royce Hall  
Plenary Session 5

**198 Quantitating Quality of Life with Age.** 
*C. Murphy* Princeton University.  
no abstract submitted

**199 Proteolytic activation of SBP-1/SREBP-1 is linked to membrane-lipid induced changes in the GTPase ARF-1.** 
Lorissa Smulan, Wei Ding, Amy Walker Program in Molecular Medicine, UMASS Medical School, Worcester, MA.

Balancing lipid levels within membranes is important for cell structure, organelle function and signaling. Preservation of membrane homeostasis occurs through multiple steps; cells sense changes in lipid ratios, communicate though molecular mechanisms to transcriptional activators, then genes supporting lipid synthesis are expressed. This homeostasis is disrupted when levels of the membrane phospholipid phosphatidylcholine (PC) fall. Rather than restoring PC, cells overproduce triglycerides (TAGs). Correlations between low PC and excess TAG have been noted in human fatty liver disease, mice, and in genetic models such as *C. elegans* and *Drosophila*. However, it is not clear why changing levels of an abundant membrane component should increase stored fats. We previously discovered that a master regulator of lipogenic transcription, SBP-1 (ortholog of sterol regulatory element binding protein, SREBP-1) is proteolytically activated in low PC, stimulating fat storage in *C. elegans*, murine liver and human cell lines.

In cholesterol-dependent SREBP maturation, cholesterol-chaperone interactions keep SREBP precursors in the ER; in low cholesterol, SREBP/chaperone complexes move to the Golgi, where proteases release the transcriptionally active portion. Using *C. elegans*, we identified a conserved feedback loop linking PC synthesis to SBP-1/SREBP-1 maturation. This feedback loop is distinct from cholesterol-dependent maturation and specifically targets SREBP-1 in the ER, as activating proteases loose Golgi-specific localization. Using subsequent screens in *C. elegans*, we have identified two new players, *lipin-1* and *arf-1*. Placing these genes in our model advances our understanding by identifying additional lipids linked to the mechanism and provides an explanation of why the Golgi-specific localization of SREBP proteases may be disrupted. First, we found that *lipin-1* knockdown activated SBP-1 in *C. elegans* and SREBP-1 mammalian cells and was necessary for low PC activation of SBP-1/SREBP-1. Lipins dephosphorylate phosphatidic acid (PA) in the PC synthesis pathway and comparative lipidomics suggest that the PA to PC ratio is the critical lipid signal in this mechanism. Second, we found a similar requirement for *arf-1.2*. *ARF-1.2/ARF-1.1/ARF-1.2/ARF-1* is a GTPase whose activity regulating Golgi to ER traffic depends on membrane interactions. Importantly, we found that blocks in PC synthesis or *LIPIN1* siRNA deplete levels of active, GTP bound ARF1 in human cells, mechanistically linking the two hits of our screen and suggesting that ARF1 is a mechanistic key to SREBP-1 activation when PA/PC ratios fall. Thus, these explore how abundant lipids can have specific regulatory functions, linking membrane dynamics to signaling and transcriptional pathways.

**200 Bacterial metabolism affects the *C. elegans* response to cancer chemotherapeutics.** 
Aurian P. García González1, Ashlyn D. Ritter1, Shaleen Shrestha1, Erik C. Andersen2, L. Safak Yilmaz3, Albertha J.M. Walhout1 1) Program in Systems Biology and Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA, 01605, USA; 2) Department of Molecular Biosciences, Northwestern University, Evanston, IL 60208, USA.

The human microbiota greatly affects physiology and disease. However, the contribution of bacteria to the response to chemotherapeutic drugs remains poorly understood. *Caenorhabditis elegans* and its bacterial diet provide a powerful system to study host-bacteria interactions. Our group previously demonstrated that two bacterial diets, *E. coli* OP50 (*E. coli*) and *C. aquatica* DA1877 (*Comamonas*) elicit differences in life history traits: *C. elegans* fed a diet of *Comamonas* develop faster, have fewer offspring and have reduced lifespan than animals fed *E. coli*. Furthermore, we used genetics in both bacteria and *C. elegans* to identify vitamin B12 as a key nutrient provided by *Comamonas*, but not *E. coli*, which explains several of the differences in life history traits. Here, we use this system to study how *E. coli* and *Comamonas* affect the *C. elegans* response to chemotherapeutics. We find that different bacteria can increase the response to one drug yet decrease the effect of another. For example, animals fed *Comamonas* require ~100-fold more 5-fluoro-2'-deoxyuridine (FUDR) than those fed *E. coli* to elicit embryonic lethality. In contrast, animals fed *E. coli* require ~1.5-fold more camptothecin (CPT) than animals fed *Comamonas* to elicit embryonic lethality. Interestingly, we find that bacterial metabolism is required for the effect on drug efficacy of both FUDR and CPT. To identify the mechanisms in bacteria that affect *C. elegans* drug efficacy, we perform genetic screens in the two bacterial species using three chemotherapeutic drugs, 5-fluorouracil (5-FU), FUDR and CPT. We find numerous bacterial nucleotide metabolism genes that affect drug efficacy in *C. elegans*. Surprisingly, we find that 5-FU and FUDR act through
bacterial ribonucleotide metabolism to elicit their cytotoxic effects in *C. elegans*, rather than by thymineless-death or DNA damage. Our study provides a blueprint for characterizing the role of bacteria in the host response to chemotherapeutics.

**201** **Metabolic superbugs regulate the effect of drugs and diet on host health and lifespan.** R. Pryor¹, P. Norvaisas¹,², W. De Haes³, L. Maier⁴, K. Bala-Krishnan¹, K. Bryson⁵, A. Typas⁴, L. Temmerman³, F. Cabreiro¹

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Animals are typically colonised by a diverse community of commensal and symbiotic microorganisms. Recent studies have linked perturbations to the gut microbiota with several nutrition-associated pathologies including obesity, type-2 diabetes and potentially ageing. Therefore, in order to fully understand the physiology of an organism, it is important to consider both the host and its microbiota as a unique metabolic entity-collectively referred to as the holobiont. Metformin has been found to extend lifespan in *C. elegans* by altering the metabolic profile of the gut bacteria. Similarly, a role for the microbiota in mediating the therapeutic effect of the drug has been uncovered in both mice and humans. Nevertheless, precisely how the microbiota mediates the effects of metformin on the host remains unclear. To this purpose, we have created 35 biguanide-resistant bacterial strains which regulate the effects of metformin on *C. elegans* lifespan. To systematically elucidate which bacterial mechanisms confer resistance against metformin we utilised a chemical genomics approach and screened a genome-wide overexpression and deletion library in *E. coli*. This strategy allowed us to identify novel bacterial genes regulating the effects of metformin on the host. In order to establish how bacterial-dependent effects of metformin regulate host health, we have employed a combination of diverse “omic” techniques at the holobiont level using *C. elegans* and germ-free mice. Utilising metformin-sensitive and resistant bacterial strains we have disentangled the bacteria-dependent effects of metformin from those that result from direct action of the drug in *C. elegans*. This has led to the identification of a unique pro-longevity signature mediated by the effects of metformin on bacterial metabolism. Finally, we have discovered a "legacy effect" whereby microbial exposure to metformin triggers long-lasting metabolic rearrangements that have beneficial or detrimental consequences in the host in a nutrient-dependent manner. Ultimately these findings underscore the importance of microbes as molecular integrators of the effects of drugs and diet on host physiology.


**202** **Counteracting components determine the sexual identity of *C. elegans* pheromones.** E. Aprison, I. Ruvinsky Molecular Biosciences, Northwestern University.

Sex pheromones facilitate reproduction by attracting potential mates and altering their behavior and physiology. In *C. elegans*, the pheromones produced by males and hermaphrodites consist of similar blends of multiple molecules. However, several molecules are present in different relative concentrations, notably ascr#3, which is more abundant in hermaphrodites and ascr#10, which is more abundant in males. It is not currently known how the compositional differences of the sex-specific pheromones lead to dramatic difference in their effects on behavior and physiology. We report two key elements of the mechanism that allows hermaphrodites to recognize the male pheromone and to respond accordingly. First, ascr#3 counters the activity of ascr#10. This antagonism lowers the magnitude and decreases the sensitivity of the hermaphrodite response to the male pheromone, restricting it to situations, in which the presence of a male could be inferred with high confidence. Second, worms determine the ratio of ascr#10 and ascr#3. Blends are recognized as male if the concentration of ascr#10 is higher than that of ascr#3, provided that an absolute threshold is exceeded. Despite the compactness of the *C. elegans* nervous system and the similarity of the two molecules, the ascr#10 and ascr#3 signals are processed by distinct sensory mechanisms. Several phenomena we discovered are shared by sex pheromones of multiple species, suggesting that diverse organisms perform particular neuronal computations using similar general principles.

**203** **Single-cell transcriptome profiling in *C. elegans* daf-2 mutants identifies tissue-specific expression of daf-16 target genes.** J. Preston, J. Willis, P. Phillips Institute of Ecology and Evolution, University of Oregon, Eugene, OR.

Aging is a complex, highly-regulated process involving cross-talk among many cell types. In order to fully understand the genetic mechanisms underlying the regulation of the aging process, it is crucial to be able to understand the behavior of individual cells. However, previous work in *C. elegans* has largely utilized whole-organism transcriptional profiling for functional analysis. Recently, single-cell transcriptomics has emerged as a powerful tool for resolving the heterogeneity of gene expression, which may be helpful for identifying important regulatory networks and biomarkers involved in the aging process.

We used a droplet-based approach to individually barcode ~30,000 single cells across eight samples, including three daf-2 mutant strains, from adult *C. elegans* hermaphrodites on day one of adulthood. We performed 3’ mRNA counting to characterize the cellular transcriptomes and grouped the cells into clusters based on similar gene expression patterns using unsupervised hierarchical clustering.

We found that *C. elegans* single cells cluster into remarkably consistent, well-defined groups across the various samples, with
each cluster exhibiting the co-expression of a distinct set of marker genes. We have identified many tissue-specific changes in the transcriptomes of the *daf*-2 mutants compared to the wild type worms, including altered regulation of several *daf*-16 target genes. In particular, the cells of the muscle, pharyngeal, germline, and neuronal tissues show consistent patterns of altered gene expression of metabolic and stress-response pathways. Many of the genes identified as cell cluster markers and tissue-specific *daf*-16 targets are not well-studied, yet their distinct expression patterns highlight the possibility that they may have important developmental and regulatory functions.

204 Spatiotemporal coupling and decoupling of gene transcription with DNA replication origins during embryogenesis in *C. elegans*. Ehsan Pourkarimi, James Bellush, Iestyn Whitehouse Memorial Sloan-Kettering Cancer, New York, NY.

During DNA replication, half of the genome is duplicated discontinuously on the lagging strand in the form of Okazaki Fragments (OFs). Despite many years of research, the precise location of replication origins, efficiency, and abundance in metazoa remains elusive. We have recently isolated and sequenced OFs from *C. elegans* embryos and developing larva, and have generated a first comprehensive genome wide map of DNA replication in multicellular organism.

Aligning sequence reads of OFs to the *C. elegans* genome reveals the complementary enrichment of Okazaki fragments to either the Watson or Crick strands, which is the hallmark of a replication origin. Importantly, we found that replication origins are associated with transcriptionally active regions of chromatin and replication start sites are strongly associated with histone modifications that define genetic enhancers such as H3K27 acetylation. By mapping DNA replication origins in various embryonic stages, we have demonstrated that origins are defined prior to the onset of zygotic transcription. However, at the time of gastrulation, when zygotic transcription initiates, it does so in proximity of pre-defined replication origins. Replication origins and transcription are correlated until the last wave of embryonic cell division. When cell division ceases, this association breaks down and transcription shifts away from replication initiation sites and anti-correlates with replication origins in differentiated cells.

Whilst majority of embryonic cells are terminally differentiated at the time of hatching, 53 somatic blast cells in newly hatched L1 larvae divide post embryonically to generate additional 403 cells in *C. elegans* hermaphrodites. The majority of the somatic blast cell lineage generate vulva, muscle, hypodermal, and neuronal cells. We have shown that the position and the efficiency of somatic replication origins are distinguishable from the embryonic origins yet remain associated with somatic H3K27 acetylation. Interestingly, genes located in proximity of somatic replication start sites are functionally related to vulva, muscle, hypodermal and neuronal cells.

Our data strongly suggest that in rapidly dividing embryonic cells, replication is a fundamental regulator of gene activity, while during notably slower post embryonic cell division, transcriptional activity shapes the DNA replication profile. Currently we are investigating the causal link between transcription and somatic replication.


205 Two miRNAs are sufficient for morphologically-normal embryogenesis of *C. elegans*. Philipp Julian Dexheimer, Luisa Cochella Research Institute of molecular Pathology, Vienna, Austria.

miRNAs are essential regulators of gene expression during animal development, as exemplified by the drastic consequences of removing the miRNA biogenesis machinery. However, the biological function of most annotated miRNA genes remains unclear. To this day, only few existing examples link the presence or absence of a given miRNA to a striking phenotype. Thus, it has been proposed that bulk miRNAs act as fine-tuners of gene expression, conferring robustness to biological systems. An alternative model that has gained support lately, is that few miRNAs engage in processes essential to viability, while most seemingly non-essential miRNAs are expressed with high spatial or temporal specificity, allowing for development of highly specific traits that give rise to the phenotypic complexity observed in present-day organisms.

In *C. elegans*, two miRNA families have been shown to be necessary for embryonic development, the *mir-35* and *mir-51* families. Here, we asked whether these two families are also sufficient for embryonic development. To do so, we use a Pasha temperature-sensitive allele to show that in the absence of canonical miRNA processing activity, embryogenesis is arrested around the onset of morphogenesis. This fully-penetrant embryonic lethality can be rescued by expression of just a *mir-35* and a *mir-51* family member via the Drosha/Pasha independent mirtron pathway.

Our experiments demonstrate that two miRNAs are sufficient for morphologically-normal embryogenesis of *C. elegans*, something that leads to two important conclusions. First, there are no other miRNAs or miRNA combinations that are necessary for morphologically-normal development that have been missed so far. Second, these results strongly argue that the essential requirement for Pasha is due to its function in miRNA biogenesis and not due to other proposed miRNA-independent functions. In addition, the Pasha mutant, mirtron-rescued larvae lack all other miRNAs, allowing us to assess the contribution of “non-essential” miRNAs to the proper specification of various cell-types. Further characterization of these animals will help shape our idea of global miRNA function during early animal development.

206 Regulation of programmed cell death by miRNAs and RNA-binding proteins. R. Sherrard, S. Luehr, H. Holzkamp, K. McJunkin, N. Memar, B. Conradt. 1) Center for Integrated Protein Science Munich – CIPSM, Department Biology II, Ludwig-Maximilians-University Munich, Planegg-Martinsried 82152, Germany; 2) Program in Molecular Medicine, RNA Therapeutics Institute, University of Massachusetts Medical School, Worcester, Massachusetts 01606, USA.

Programmed cell death is an important process in animal development and requires strict control to avoid the unwanted death
of cells. In *C. elegans*, the pro-apoptotic BH3-only gene *egl-1* is the most upstream factor of the core apoptotic pathway, and its activity is thought to determine the life-versus-death decision during development. Previous studies have identified regulatory modules that control the transcription of *egl-1* in a lineage-specific manner; however, investigations into the post-transcriptional regulation of *egl-1* are largely absent. We find that the *mir-35* family and *mir-58 bantam* family of miRNAs directly target the 3'UTR of *egl-1* mRNA and act cooperatively to suppress its expression. This suppression affects both mRNA copy number and translation efficiency, and is crucial to prevent the precocious and collateral death of mothers and sisters of cells programmed to die. Using single-molecule RNA FISH, we show that *egl-1* is already transcribed in mothers of cells programmed to die and that *mir-35* family and *mir-58 bantam* family miRNAs prevent their precocious death by keeping the copy number of *egl-1* mRNA below a critical threshold. These miRNAs also act to dampen the transcriptional boost of *egl-1* that occurs specifically in the daughter that is programmed to die, but they are generally not required for the degradation of *egl-1* mRNA in the daughter that is programmed to survive. We propose that these miRNAs function to adjust lineage-specific differences in *egl-1* transcriptional activation and thereby ensure that *egl-1* expression reaches a level sufficient to trigger death only in the daughters that are programmed to die. Finally, we investigate the role of PUF (Pumilio/FBF) RNA-binding proteins (RBPs) in the post-transcriptional regulation of *egl-1*, whose 3'UTR contains two FBF binding elements. Mutations in these elements mediate increased reporter expression in both the germline and early embryos, suggesting that *egl-1* is directly regulated by FBF during development. Although miRNAs and RBPs can function independently, recent studies have found these two pathways can interact in the regulation of shared targets, and we speculate that *egl-1* may be one such target.

207 Transgenerational silencing in *C. elegans* can be triggered by mating. S. Devanapally, S. Allgood, A.M. Jose  Cell Biology and Molecular Genetics, University of Maryland, College Park, MD.

Silencing of foreign DNA sequences is a significant obstacle to genome engineering and has been interpreted to be the result of the organism mounting a non-self response through studies in the worm *C. elegans*. This hypothesis does not explain how some sequences apparently defy recognition as foreign by the organism and remain stably expressed for many generations.

Here, we report a phenomenon where the expression of a foreign sequence in some sequences apparently defy recognition as foreign by the organism and remain stably expressed for many generations. Sibling progeny from a mating differ in their ability to initiate and transmit silencing across generations, suggesting that the phenotype of an animal can depend on the ancestral progeny selected from a mating. Dissection of this can dictate phenotype in response to ancestral mating.

Closing Remarks. no abstract submitted

Cell Biology - Cell Death

209A Novel functions of Poly(ADP-ribose) glycohydrolase in the repair of double-strand DNA breaks in *Caenorhabditis elegans*. W. Bae, H. Koo  Department of Biochemistry, College of Life Science and Biotechnology, Yonsei University, Seoul 03722, Republic of Korea.

Poly(ADP-ribose) (PAR) chains are rapidly synthesized at DNA break sites, which recruit proteins mediating checkpoint activation and DNA repair. Poly(ADP-ribose) polymerases (PARPs) synthesize PAR chains, which are degraded by poly(ADP-ribose) glycohydrolases (PARGs) later. There has been extensive research carried out on more than ten PARP enzymes in mammals. In contrast, relatively much less is known about the only one PARG enzyme in mammals, which is essential for survival at the organismal and cellular levels. In the model animal *C. elegans*, there are two paralogs of PARG, PARG-1 and PARG-2, of which double deficiency, but not either single deficiencies, causes lethality under normal conditions. Single mutations of the two genes induced hypersensitivity of germ cells to gamma rays as well as to other types of DNA damage. In accord with the increased sensitivity to double-strand DNA breaks, the dissipation of RAD-51 foci, not their formation, was slower in the mutant germ cells than in wild-type cells, suggesting defects in the repair via homologous recombination. However, it was very peculiar that the focus formation of RPA-1 (the large subunit of replication protein A) and its dissipation were accelerated in the single mutant cells after gamma ray treatment. The rapid accumulation of RPA-1 at double-strand DNA breaks is thought to have resulted from changes in the chromatid structure.

210B Regulation of Cell Extrusion by Protein Ubiquitination in *C. elegans*. Vivek K Dwivedi1,2, Dan Denning1,2, Bob Horvitz1,2  1) HHMI; 2) Dept. of Biology, MIT, Cambridge, MA 02139.

Cell extrusion, or shedding, is a cell elimination process that occurs in metazoans ranging from sponges to mammals. In humans, approximately 10^11 cells are extruded from the small intestine epithelium each day. Dysregulation of cell extrusion is deleterious, as inadequate extrusion or excessive extrusion can lead to hyperplasia or a defective epithelial barrier, respectively. Despite the importance of cell extrusion in physiology and relevance to disease, little is known about how extrusion is regulated or what molecules are involved in the process.

We previously identified cells in *C. elegans* that are normally eliminated during embryogenesis by caspase-mediated apoptosis but are alternatively eliminated by cell extrusion in mutants defective in caspase-mediated apoptosis. When both
Caspase-mediated apoptosis and cell extrusion are disabled, these cells survive. For example, such cells survive in mutants with loss-of-function mutations in both the gene ced-3, which encodes the caspase required for programmed cell death, and the gene pig-1, which encodes a homolog of the protein kinase MELK (Denning et al., Nature (2012)). One such cell, ABplapapp, when it survives in pig-1 ced-3 double mutants divides to generate an extra excretory cell, resulting in the two excretory cell (Tex) phenotype.

We mutagenized ced-3 mutants and screened for mutants with the Tex phenotype to identify additional genes involved in cell extrusion and identified the gene C45G7.4. C45G7.4 encodes a RING/B-box protein with putative E3 ubiquitin ligase function. Consistent with a role of the ubiquitin proteasome system in extrusion, knockout or knockout of ubc-25, which encodes an ubiquitin-conjugating enzyme and homolog of the mammalian UBE2Q1 and UBE2Q2 proteins, also produces the Tex phenotype in a ced-3 mutant background.

We are currently performing rescue and epistasis experiments to confirm the role of ubc-25 in cell extrusion and to determine whether it functions in the same pathway as C45G7.4 to promote cell extrusion. With the assumption that C45G7.4 functions as an E3 ligase, we plan to use the UBAIT technique (O’Connor et al., EMBO Rep. (2015)) to covalently trap C45G7.4 targets and identify them by immunoprecipitation followed by mass spectrometry. We will subsequently test the identified targets for a role in cell extrusion.

Our study reveals that the ubiquitin proteasome system is involved in cell extrusion and suggests that ubiquitination of an unknown factor is a critical step in the mechanism of cell extrusion.

211C RAB-35 Promotes the Robustness of Apoptotic Cell Clearance. R.C. Haley, Y. Wang, Z. Zhou Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX.

In metazoans, apoptotic cells are swiftly engulfed by phagocytes and degraded inside phagosomes through phagosome maturation. Defects in this process can cause inflammatory responses and autoimmune diseases. In the nematode C. elegans, small GTPases known as Rabs function in vesicle trafficking, the maturation of developing phagosomes, and the degradation of apoptotic cell corpses. Utilizing an RNAi screen, we discovered that knockdown of rab-35 causes a moderate Ced (cell death defective) phenotype, indicative of defects in apoptotic cell clearance. Because the human ortholog of Rab-35, Rab35, has been recently identified as an oncogene and implicated in other biological processes such as innate immunity, proper brain function, endocytosis, and receptor recycling, we sought to thoroughly characterize the role of Rab-35 in apoptotic cell clearance, both to broaden the knowledge base of our field and to provide a solid foundation for future mammalian studies. By visualizing apoptotic cell clearance using time lapse microscopy, we uncovered that Rab-35 localizes to the nascent phagosome. Additional experiments suggest that Rab-35 must cycle between its GDP-bound and GTP-bound states to be properly recruited to the phagosome; consequently, we have identified potential GEFs and GAPs for Rab-35. Epistasis analysis indicates that rab-35 represents a novel engulfment pathway. Rab-35 was also found to act upstream of rab-5 and rab-7 in phagosome maturation. These results are indicative of the role of Rab-35 as a robustness factor and serve as a novel link between defects in engulfment and early maturation and subsequent defects late in degradation.

212A The CISD-1 iron-sulfur protein impacts apoptosis in the germline of C. elegans. Skylar D. King, Chipo F. Gray, Luhua Song, Ron Mittler, Pamela A. Padilla Department of Biological Sciences, University of North Texas, Denton, TX.

The recently discovered CISD proteins (mitoNEET/CISD1, NAF-1/CISD2, CISD3) belong to a class of iron-sulfur (Fe-S) proteins that are localized to the mitochondria and/or ER and involved with several human pathologies including diabetes, neurodegeneration, and Wolfram syndrome 2. Phylogeny studies indicate that gene duplication occurred in vertebrates resulting in the distinct CISD1 and CISD2 genes. In C. elegans the cisd-1 gene has homology to the CISD1 and CISD2 genes in vertebrates and cisd-3.1 and cisd-3.2 has homology to vertebrate CISD3. In mammals the CISD proteins are thought to have a role in the regulation of iron, reactive oxygen species, autophagy, and redox metabolism. The CISD1 and CISD2 protein levels accumulate in human epithelial breast cancer cells; suppression of the these proteins in cancer cells result in significantly reduced cell proliferation and tumor growth, decreased mitochondrial function, uncontrolled accumulation of iron and ROS in mitochondria, and activation of autophagy. Furthermore, the NAF-1/CISD2 was found to interact with BCL-2 leading to activation of apoptosis. Although these phenotypes are of interest the specific molecular function of the CISD genes is not well understood especially in the context of a whole organism. Here we used C. elegans to examine the function of cisd-1. The cisd-1(tm4993) mutant and cisd-1(RNAi) animals exhibit a reduction in fecundity and abnormal germline morphology. Using CRISPR technology we generated a cisd-1 transcriptional GFP reporter. The pcsiD-1::GFP reporter exhibited GFP expression in the germline and other tissues including intestine. To test the hypothesis that cisd-1 has a functional role in apoptosis we used the programmed cell death (PCD) markers (CED-1::GFP, ACT-5::YFP) to examine PCD in the germline. The cisd-1(tm4993) mutant displayed a significant increase in apoptotic cells in the adult germline relative to controls. Knock-down of cisd-3 function suppressed the increase of apoptotic cells within the cisd-1(tm4993) animal. These data suggest that cisd-1 has a role in PCD within the C. elegans germline. We are conducting additional experiments to determine the functional role cisd-1 has in C. elegans.

213B Competitive phagocytosis: a novel mechanism for clearance of the non-apoptotically dying linker cell. Lena Kutscher, Wolfgang Keil, Shai Shaham Laboratory of Developmental Genetics, The Rockefeller University, New York, NY.

Programmed cell death and subsequent cell clearance are important in the development and maintenance of organisms and tissues. Defects in cell clearance can result in inappropriate inflammatory responses and autoimmune disorders. Although much is known about apoptotic cell corpse removal, our understanding of mechanisms driving the clearance of cells dying by non-apoptotic means is rudimentary. The C. elegans linker cell dies non-apoptotically through a death process termed LCD (Linker Cell-type Death), which has been molecularly characterized in our lab. This male-specific cell is born in the L2 larval stage,
leads elongation of the developing gonad, and then dies in a programmed manner independently of known apoptosis, autophagy, or necrosis genes. Importantly, linker cell corpses are robustly engulfed in mutants carrying lesions in genes required for engulfment of apoptotic bodies, suggesting a novel engulfment program. Recently, we imaged the migration, death, engulfment, and degradation of the linker cell over a 20h period, through the L4-to-adult transition, using a new three-channel microfluidic imaging device we built, which allows for long-term imaging of C. elegans larval development. Using this device, we uncovered the precise sequence of events leading to linker cell demise and degradation. The linker cell leads the gonad to the cloaca, and plugs the distal gonad to prevent inappropriate leakage of gonadal contents. Near the cloaca, the cell is ensheathed, but not yet engulfed, by the two U.I/rp cells. Nuclear crenellation, a LCD hallmark, then become evident. Both U.I/rp cells next actively attempt to engulf the dying linker cell, eventually breaking it into two parts of unequal volume. This competitive phagocytosis occurs in 60/64 wild-type animals imaged. Once the cell is engulfed, the caspase ced-3 and its upstream activator ced-4, which are not required for LCD, act in the linker cell to facilitate its degradation. The small GTPases RAB-35 and ARF-6 function antagonistically in the engulfing cells for efficient linker cell degradation. Together, our results demonstrate the utility of long-term imaging of C. elegans larva at high spatiotemporal resolution, uncover components of a novel cell degradation mechanism, and reveal previously unknown roles for caspases in cell corpse degradation during caspase-independent cell death.

214C Nucleotide excision repair is the main pathway to repair interstrand DNA crosslinks in C. elegans. S. Oh, H. Koo Department of Biochemistry, College of Life Science and Biotechnology, Yonsei University, KKR.

Interstrand DNA crosslinks (ICLs) that block the progression of replication forks, are predominantly repaired by the Fanconi anemia (FA) pathway in mammalian cells. The FA pathway converts ICLs to one-ended double-strand DNA breaks, which are subsequently processed by homologous recombination. Unlike the case in S phase, nucleotide excision repair (NER) is the main pathway of ICL repair, which involves translesion DNA synthesis (TLS). In the model organism C. elegans, only a half of 16 FA proteins in mammals, including FANC D2, FANC M, and FANC Q (XPF), are conserved. We have tested the relative importance of the two ICL repair pathways in C. elegans, which are the FA pathway in combination with homologous recombination and the NER pathway accomplished by TLS. Any mutations of NER proteins such as XPA, XPC, and XPF increased the sensitivity to ICLs more than ten-fold, in comparison with the FANC D2 mutation. In addition, deficiencies of TLS polymerases such as Pol zeta and REV1 greatly increased the sensitivity to ICLs as well. Therefore, in the proliferating germ cells of C. elegans, the NER pathway appears to be much more potent than the FA pathway to repair ICLs. We are now examining the repair kinetics in the germ cells defective in either one or both of the ICL repair pathways, to establish a relationship between the two pathways.

215A Autophagy and the degradation of apoptotic cells. O. Peña-Ramos, X. Liu, L. Chiao, T. Yao, L. Gao, Z. Zhou Biochemistry & Molecular Biology, Baylor College of Medicine, Houston, TX.

The autophagy pathway generates autophagosomes, double-membrane structures that are responsible for the degradation of protein aggregates, intracellular organelles, and other cellular components. This intracellular trafficking process is essential for cell stress response that converts the degraded material into an energy source. Components of the autophagy pathway have previously been linked to phagosome maturation in a process called LC3-associated phagocytosis (LAP). In this process, autophagy machinery is proposed to conjugate LC3 directly onto the phagosomal membrane to promote lysosome fusion. During C. elegans embryonic development, 113 somatic cells undergo apoptosis and are engulfed and degraded by their neighboring cells. We have been studying the involvement of autophagy in the clearance of apoptotic cells. Using time-lapse microscopy, we found that LC3-labeled puncta generated in the engulfing cell are actively recruited to the surface of the phagosomes containing engulfed apoptotic cells and subsequently fuse to phagosomes, depositing their contents into the phagosomal lumen. This fusion pattern suggests that the puncta are of a double-membrane nature instead of being the single-membrane vesicles labeled with LC3 proposed for the LAP process. We have obtained multiple lines of evidence that indicate that canonical autophagosomes are incorporated in phagosomes during the apoptotic cell degradation process. We further found that autophagosomes made an important and specific contribution to the degradation of apoptotic cells. The autophagosomes that fuse to phagosomes can be split into two separate sub-groups, those that are labeled with LGG-1 and those that are labeled with LGG-2. LGG-1 and LGG-2 are both homologs of mammalian LC3. Lacking either group of autophagosomes significantly delays the degradation of apoptotic cells. In addition, we found that the small GTPase RAB-7 and its effector complex are critical for the fusion of autophagosomes to phagosomes; however, they do not play a role in the recruitment step.

Our findings have revealed that in addition to the LAP reported in mammalian phagocytes, canonical autophagosomes play an important role in the clearance of apoptotic cells. These findings add autophagosomes to the collection of intracellular organelles that contribute to the phagosome maturation and have revealed a new and important function of the autophagy pathway. Currently, we are exploring the upstream signaling system that initiates the recruitment and fusion of autophagosomes to phagosomes.

Cell Biology - Cell Division


The nuclear envelope (NE) is critically involved in numerous biological processes, including control of nucleocytoplasmic
transport, genome organization and DNA replication, repair and transcription. Reflecting the diversity and complexity of these processes several hundreds different proteins are reported to accumulate at the NE. However, the interactions between NE components are incompletely mapped, which hinders full understanding of NE biogenesis and functions.

Phosphorylation of NE proteins is a prerequisite for NE breakdown at entry to mitosis. We previously identified VRK-1 (Vaccinia-Related Kinase 1) as the kinase responsible for mitotic phosphorylation of BAF-1, which is involved in anchoring of chromatin to the nuclear lamina via the NE proteins LMN-1, EMR-1 and LEM-2. VRK-1 is also expressed in postmitotic cells and to identify novel interaction partners we immunoprecipitated VRK-1 from C. elegans and human cells followed by mass spectrometry analysis. The top hits from these experiments include several NE and chromatin-associated proteins, including BAF-1 and EMR-1, which validates this approach.

To verify the interactions we are employing a variety of methods, such as yeast two-hybrid assays and surface plasmon resonance technology. In addition, we have developed a bicistronic system to study protein-protein interactions in vivo by protein fragment complementation assays based on BiFC and NanoLuc technologies. This system enables us to characterize VRK-1 interactions at physiological expression levels and subcellular resolution, but the system can be adapted to any other protein pair.

217C  Kinetochores involvement in bipolar spindle maintenance in the 2nd division of C. elegans embryos.  A. Bondaz1, E. Zanin2, P. Meraldi1, M. Gotta1 1) Department of Cell Physiology and Metabolism, Faculty of Medicine, University of Geneva , Geneva, Switzerland; 2) Biozentrum der LMU München Department Biologie II , Germany.

During mitosis, the spindle must assemble in a bipolar configuration in order to properly segregate chromosomes into two daughter cells. In most animal cells, the kinesin Eg5, which pushes apart the two centrosomes, plays a dominant role for bipolar spindle assembly. However, when centrosomes separation is delayed, kinetochores also contribute to bipolar spindle assembly (Toso et al., 2009).

In C. elegans the Eg5 homologue BMK-1 does not contribute to bipolar spindle assembly, facilitating the identification of additional factors required for this process. We find that in embryos depleted of Ga (in which centrosome separation is delayed), co-depletion of the Kinetochores NuL-1 or 2 (KNL-1 or 2) proteins, results in defects in bipolar spindle maintenance. We observe the formation of a transient spindle that rapidly collapses. Spindle collapse does not occur in single depletion (Ga or knl-1 or 2 depleted embryo) indicating that in both mammalian cells and C. elegans embryos, kinetochores become essential for bipolar spindle assembly when centrosomes separation is delayed.

Since the kinetochore has two functions, first building up the microtubule attachment platform and second direct the formation of the central spindle (Maton et al., 2015), we have started to dissect which function of the kinetochore was required for bipolar spindle maintenance. Our data suggest that central spindle formation rather than the microtubule attachment function of kinetochores is required for bipolar spindle assembly and maintenance in the 2nd division of C. elegans embryo.

218A  Cytokinesis and actomyosin regulation in early Caenorhabditis elegans embryos.  B. Chapa-y-Lazo, M. Hamanaka, M. Mishima, M.K. Balasubramanian  Centre for Mechanochemoical Cell Biology, Division of Biomedical Sciences, Warwick Medical School, University of Warwick, Coventry, United Kingdom.

Caenorhabditis elegans early embryos are an ideal model for studying cytokinesis and cytoskeletal dynamics thanks to their translucence and the availability of strains expressing various fluorescently tagged proteins. We have begun to explore how the actomyosin cytoskeleton is regulated during the cell division cycle in this model by using a pharmacological approach. To this end, we have established a system in which we render the embryo’s eggshell permeable to small molecules and simultaneously deplete a protein of interest by means of RNAi. With this system, we have obtained evidence suggesting that the cell cycle regulates myosin II (NMY-2) dynamics independently of actin, and are currently investigating the molecules regulating this phenomenon. Preliminary observations indicate that this cell-cycle-dependent but actin-independent regulation may not be exclusive to myosin but inclusive of other cytoskeletal proteins, and we aim to dissect the regulatory networks that apply in each case.

This system is also allowing us to test the importance of actin turnover and myosin II activity for cytokinetic actomyosin ring constriction, as well as for the homeostasis of the actomyosin cytoskeleton. In this sense, we have results that suggest F-actin turnover is important not only for cytokinesis progression but also for a proper interaction of the actin network with the plasma membrane.

219B  Genetic analysis of a sas-6 mutant suggests an instructive role for the mother centriole in centriole assembly.  N.E. DeVaul1, P. Sankaralingam1, G. Fabig1, D. Streichert2, T. Mueller-Reichert2, S. O’Rourke3, B. Bowman3, K. O’Connell1 1) NIDDK, National Institutes of Health, Bethesda, MD; 2) Technische Universität, Dresden, Germany; 3) IMB, University of Oregon, Eugene, OR.

Centrioles are highly stable barrel-shaped microtubule-based structures that exist as mother-daughter pairs. In mitotic cells, centrioles recruit pericentriolar material to form centrosomes, which serve as microtubule-organizing (MTOCs) that help to establish the poles of the spindle. In interphase centrioles serve as basal bodies that direct the formation of cilia and flagella. Centrioles are assembled from a set of conserved core proteins. The coiled-coil protein SPD-2 recruits the kinase ZYG-1, which then recruits the SAS-5/SAS-6 complex to form a central scaffold. SAS-4 is the last to be recruited and is required for the addition of microtubules to the outer wall. SAS-6 is a stable component of the C. elegans centriole and plays a key structural role that helps define the nine-fold symmetry of this structure. We have identified a new mutation in the SAS-6 N-terminal globular domain that results in a novel phenotype. The sas-6(or1167) mutation causes a single amino acid substitution (D9V) that appears to affect centriole stability and duplication. Unlike RNAi-mediated depletion of SAS-6, which simply blocks centriole
duplication, the mutant protein can only block duplication when it is both present in the mother centriole and present in the embryonic cytoplasm. This suggests some type of molecular communication between SAS-6 molecules in the mother and those being recruited to form the daughter centriole. Interestingly, centrioles assembled in this mutant also appear unstable and over time lose SAS-6 and other components such as SAS-4. Further, by electron microscopy, the sas-6 mutant centrioles appear structurally abnormal. Together, our data suggest that SAS-6 is not just required for the assembly of centrioles, but also functions in maintaining their long-term stability. Our data also indicate that SAS-6 in the mother centriole directs the assembly of daughter centriole SAS-6.

220C The role of DAF-18/PTEN in nutritional regulation of primordial germ cell quiescence. A. Fry, E.J. Hubbard Developmental Genetics, NYU School of Medicine, New York, NY, NY.

DAF-18 is required for this cell cycle arrest of the two stem cells and cancer cells in response to nutritional signals. In a striking parallel, the C. elegans PTEN homolog, DAF-18, suppresses proliferation of primordial germ cells (PGCs) in response to food deprivation. When hatched in the absence of food, C. elegans development is arrested at the first larval stage (L1). During L1 arrest, the primordial germ cells (PGCs) and somatic cells halt proliferation and remain quiescent until food is encountered. DAF-18 is required for this cell cycle arrest of the two PGCs in the absence of food. Previous studies have shown that AKT acts downstream of DAF-18, while TOR signaling is either acting in parallel or partially downstream to regulate PGC quiescence [1]. Additionally, DAF-16/FoxO, which promotes somatic cell quiescence during L1 arrest, is not required for PGC quiescence [2]. Several avenues are open for exploration, including the identification of relevant factors downstream of DAF-18, the tissue in which DAF-18 acts to regulate PGC quiescence. DAF-18-dependent processes within PGCs, and key environmental cues. To examine PGCs in vivo, we have generated a fluorescent germ cell reporter. By tightly synchronizing L1 animals, we show that daf-18 mutant PGCs divide within several hours of hatching in the absence of food, and also divide prematurely in the presence of food. We have also found that zygotic daf-18 is sufficient to restore PGC quiescence in starved L1s. Currently, we are determining the tissue(s) in which DAF-18 acts to regulate PGC quiescence. Additionally, we are investigating the DAF-18-dependence of specific modifications within PGCs that coincide with proliferation. Thus, we are using the powerful system of C. elegans PGC proliferation to gain insight into nutritionally-regulated mechanisms of PTEN control over quiescence.


221A The strength of the spindle assembly checkpoint is determined by cell size and PAR protein-mediated cell fate determination during C. elegans embryogenesis. A.R. Gerhold1, J.-C. Labbé1, P.S. Maddox2 1) Institute for Research in Immunology and Cancer, University of Montreal, Montreal, Quebec, CA; 2) Department of Biology, University of North Carolina, Chapel Hill, Chapel Hill, North Carolina.

The fidelity of mitotic chromosome segregation is maintained by the spindle assembly checkpoint (SAC), which monitors kinetochore-microtubule attachments and prevents anaphase onset until all chromosomes are stably bi-oriented on the metaphase plate. Perturbing kinetochore-microtubule attachments prevents cells from satisfying the SAC, leading to prolonged mitotic delays. The duration of SAC-dependent mitotic delays are commonly used as an indication of the strength of the SAC. By this metric, SAC strength varies widely between cell types, yet how this variation is achieved is poorly understood. As a major class of chemotherapeutics (spindle poisons, e.g. Taxol) relies on SAC activity to perturb mitosis in cancer cells and a weakened SAC can lead to aneuploidy and/or tumor development, understanding the basis for variation in SAC activity is both fundamentally and clinically relevant. We have previously reported that adult germline stem and progenitor cells exhibit longer SAC-dependent mitotic delays than early embryonic blastomeres. Here we have used live imaging and an inducible monopolar spindle assay to show that a stronger SAC is also a feature of germline-fated cells during embryonic development. In agreement with a recent report, we find that the strength of the SAC scales with cell size during embryogenesis, with smaller cells experiencing longer SAC-dependent mitotic delays. However, SAC strength also depends on cell lineage, with germline precursors showing a stronger SAC across all cell sizes. In 2-cell stage embryos, we find that cell size and PAR protein-mediated cytoplasmic asymmetries have separable effects on differential SAC activity between the somatic AB and germline P1 blastomeres, with approximately equal contributions from both. Our results are consistent with a model in which PAR-1-dependent posterior sequestration of a checkpoint-enhancing factor(s) increases the strength of the SAC in germline cells. Altogether, our results indicate that size scaling of SAC activity is modulated by cell fate and reveal a novel interaction between asymmetric cell division and the SAC.

222B The role of the HOX protein LIN-39 in the maintenance of VPC proliferation. S. Heinze, A. Hajnal Institute of Molecular Life Science, University of Zurich, Zurich, CH.

hox genes are a large and conserved gene family encoding homeodomain-containing transcriptional factors. HOX proteins regulate a wide range of developmental processes, such as cell-fate specification, tissue morphogenesis and cell proliferation. Moreover, de-regulated hox gene expression has been observed in various types of human tumor cells. The Caenorhabditis elegans vulva is an excellent model to study how hox genes coordinate cell fate specification and cell proliferation. During the first larval stage (L1) a WNT signal secreted by a group of tail cells induces basal expression of lin-39 in six of the twelve epidermal Pn.p cells in the mid-body region (P3.p-P6.p), which become the vulva precursor cells (VPCs). The expression of lin-39 prevents the VPCs from fusing with the surrounding hypodermis (hyp?) by repressing the expression of the fusogen eff-1. In addition, LIN-39 maintains the VPCs in the G1 cell cycle phase keeping them competent to proliferate at a later
stage. At the beginning of the L3 stage, an inductive epidermal growth factor (EGF) signal from the anchor cell combined with lateral DELTA/NOTCH signaling determines the primary and secondary cell fates of the three proximal VPCs (P5.p-P7.p). The proximal VPCs will proliferate in order to generate 22 fully differentiated vulva precursor cells. By contrast, the distal VPCs (P3.p, P4.p and P8.p) divide only once and then fuse with hyp7. LIN-39 is a central player in a regulatory network coordinating VPC differentiation and proliferation (Roiz et al., 2016).

One question that has not yet been studied is how the exit of the vulval cells from the cell cycle is regulated after three—or in the case of VulD- two rounds of cell divisions. We hypothesize that LIN-39 is necessary to maintain the VPCs proliferating by regulating the expression of the two cell-cycle components cye-1 and cdk-4. According to this model, the VPCs arrest to divide as soon as the levels of LIN-39 drop below a certain threshold during the L4 stage. We used the CRISPR-Cas9 system to tag the lin-39 gene with GFP and with a ZF1 degradation tag, allowing us to rapidly deplete the LIN-39 protein after inducing the expression of the E3 ubiquitin ligase substrate-recognition subunit ZIF-1 (Armenti et al., 2014). We can now induce LIN-39 degradation at different stages of vulval cell proliferation and observe if cells lacking LIN-39 prematurely exit the cell cycle. Moreover, we will monitor the expression of cell cycle components in presence or absence of LIN-39. Together, these experiments will permit us to test if LIN-39 is necessary to maintain vulval cell proliferation.

223C  Role of sgo-1 in preventing centriole separation in spermatids and sperm.  Anthony James, Saul Betesh, Mara Schvarzstein  Biology, City University of New York, Brooklyn College, Brooklyn, NY.

In addition to providing a haploid genome at fertilization, the oocyte and sperm each provides complementary components of the centrosome. During fusion of gametes the centriole pair that together with the oocyte’s pericentriolar material make the first centrosome of the zygote. Accurate inheritance of a centriole pair by the sperm is therefore crucial to ensure a normal first mitotic division of the embryo. Centriole separation (disengagement) licenses their duplication. Therefore, centriole pairs must remain together (engaged) during the second meiotic division and in the sperm until after fertilization, to prevent abnormal extra duplications of the centrioles and multipolar first mitosis of the embryo. A module of genes including the separase protease (sep-1), the meiosis specific cohesin component rec-8, and the HORMA domain containing genes htp-1/2 and him-3 is involved in centriole engagement in the second meiotic division of spermatocytes in addition to regulating sister chromatid cohesion in the first division. Recently we found that the C. elegans shugoshin, sgo-1, also plays a vital role in maintaining centrioles engagement, but the mechanism by which this happens is unclear. Unlike other organisms, C. elegans sgo-1 does not play a role in protecting sister chromatid cohesion.

With the aim of further understanding the role of sgo-1 in preventing centriole disengagement we are performing comparative phenotypic analysis of these mutants individually and in combination. For example we have generated sep-1(dm);sgo-1 double mutants to assess whether SGO-1 functions to prevent centriole disengagement by protecting centrioles from SEP-1 protease. Our preliminary fecundity assays suggest a genetic interaction between these two genes in C. elegans. We will present this and other phenotypic analysis of the double mutant and each single mutant, as well as our interpretation of what these findings might mean regarding the control of centriole disengagement during male meiosis.

224A  Towards understanding the importance of the unequal first division in C. elegans embryos.  R. Jankele, Z. Spiró, P. Gónczy  Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, Swiss Federal Institute of Technology (EPFL), 1015 Lausanne, Switzerland.

Development of C. elegans begins with PAR protein-mediated polarization of the zygote along an anterior-posterior (A-P) axis. As a result, fate determinants are distributed asymmetrically into the two daughter cells. Moreover, the first division is physically unequal and generates a larger anterior AB cell and a smaller posterior P1 cell, corresponding to ~60% and ~40% of the initial volume, respectively. Such unequal first division is conserved throughout the Rhabditidae genus, suggesting functional relevance. However, the importance of having an unequal first division for C. elegans development has not been addressed. To investigate this, we used a temperature sensitive (ts) allele of lin-5, a component of the ternary complex that anchors the microtubule-dependent molecular motor dynein on the cell cortex, and which is essential for asymmetric spindle positioning and unequal cell cleavage. Transient upshifts of lin-5(ev571ts) embryos inactivates LIN-5 solely during the first cell division, resulting in the two blastomeres of similar sizes. We find that lethality of such manipulated embryos increases as the first cleavage becomes more equal. Furthermore, embryos in which P1 is larger than AB always fail to develop, together suggesting that the characteristic unequal division is somehow important for robust development.

To understand what causes developmental failure in near-equalized embryos, we monitored development at subsequent embryonic stages. Importantly, we observed that embryos with near-equal cell sizes almost invariably exhibited defects in gastrulation, with the two intestinal precursors Ea and Ep dividing precociously on the embryo periphery instead of following their normal fate characterized by a slower cell cycle and mitosis happening only after ingestion into the blastocoel. Ea and Ep cells in these embryos not only divide precociously but are also larger than in the wild-type. We are currently implementing approaches for simultaneous tracking of cell fates and volumes to unravel the impact of altered cell sizes on the embryonic development. Together our data to date suggest that the unevenness of the first cell division needs to be tightly regulated to ensure proper timing and spatial arrangement of blastomeres during subsequent embryonic development of C. elegans.

225B  Hyperglutamylation of microtubules causes a reduction in brood size.  S. John, A Heperker, J Dominguez, N Peel  TCNJ, Ewing, NJ.

Microtubule glutamylation is a reversible covalent modification of the microtubules, which can regulate microtubule function. The addition of glutamate to the polymerized microtubule is catalyzed by the tubulin tyrosine ligase like (TTLL) enzymes and its removal is carried out by the cytosolic carboxypeptidase (CCPP) family of enzymes. A mutation in C. elegans ccpp-1 results in
tubulin hyperglutamylation and ciliary fragmentation, suggesting an essential function for ccpp-1 in maintenance of microtubule stability in the cilia (O'Hagan et al., 2011). This neuronal phenotype parallels defects observed in mice when the murine homolog, CCP1, is lost. Interestingly these murine CCP1 mutants also show a reduction in female fertility, leading us to investigate the function of CCP-1 in the C. elegans germline. We have found that mutations in ccpp-1 lead to a temperature sensitive reduction in brood size, although embryonic viability is unperturbed. Breeding mutant hermaphrodites with wild type males did not restore the normal brood size and ccpp-1 males were able to sire a normal brood when mated with wild-type hermaphrodites. Thus the reduced fertility can be attributed to a defect in oogenesis. To further investigate the underlying cause of this fertility defect, we first examined the gross morphology of the ccpp-1 mutant germline, but found no overt problems with germline size or organization. We are now undertaking an analysis of whether increased apoptosis leads to a reduction in gamete production. This work will ultimately allow us to better understand the function of tubulin glutamylation in the worm.

226C  AMPK regulates germline stem cell quiescence and integrity through effects on a small RNA pathway(s) during energy stress. P. Kadekar, R. Roy  Department of Biology, McGill University, Montreal, CA.

In response to unfavourable growth conditions, C. elegans larvae undergo a global developmental arrest following the execution of a diapause-like state called ‘dauer’. Germline stem cell (GSC) quiescence in the dauer state requires the function of at least two protein kinases: LKB1 (PAR-4) and AMPK. PAR-4 and AMPK[AV1] . In the absence of either of these protein kinases germ cells undergo extensive hyperplasia and can generate sperm in an untimely manner. Although wild type dauer larvae recover from this developmentally quiescent state with no negative impact on their reproductive fitness, AMPK mutant animals exhibit complete sterility after dauer recovery, suggesting a loss of germ cell integrity. Furthermore, these animals show severe defects in germline organization and fail to make functional gametes. We were able to partially rescue this observed sterility by compromising the function of two of major effectors of the RNA interference pathway (dcr-1 and rde-4) as well as the primary argonaute protein ergo-1. Disruption of these gene activities also partially suppressed the uncontrolled germ cell proliferation in AMPK mutant dauer larvae. Small RNAs have been shown to affect gene expression at the chromatin level by regulating histone modification. Consistent with this role, we found that in AMPK mutant dauer larvae, the H3K4me3 and H3K9me3 chromatin marks are both increased and aberrantly distributed throughout the dauer germline. The abnormal levels of H3K4me3 and H3K9me3 marks persist beyond the dauer stage and are not resolved in the post-dauer adult germline. Our data therefore suggests that in AMPK mutants a small RNA pathway that ensures appropriate GSC quiescence and integrity becomes misregulated in the dauer stage resulting in the establishment of inappropriate histone modifications that eventually compromise germline integrity and subsequent fertility. We are currently in the process of identifying AMPK targets that mediate its effects on the small RNA pathway(s) to better understand the relationship between the small RNA repertoire and the chromatin landscape during AMPK-mediated adaptation to acute energetic stress.

227A  Microtubule glutamylation is dispensable for embryonic development. J. Lee, DG Chawla, R Shah, P Suri, KE Badecker, M Brewster, N Peel  TCNJ, Ewing, NJ.

Glutamylation, the covalent attachment of glutamic acid to tubulin in the polymerized microtubule, is enriched on long-lived microtubules, and is proposed to contribute to centriole stability, cilia motility and axon function. Glutamylation of the microtubules is catalysed by a family of tubulin tyrosine ligase like (TTLL) enzymes. Comprehensive in vivo analyses of the function of tubulin glutamylation have proved challenging because of the existence of a large family of TTLL enzymes in many species. To investigate the function of tubulin glutamylation we have generated a quint mutant that lacks all five C. elegans glutamylating TTLL enzymes. The quint mutant shows normal embryonic viability and brood size indicating that the centrioles are functional and the microtubules of the spindle are competent for cell division. Recent evidence suggests that in human cells glutamylation modulates microtubule severing by spastin and katanin. To investigating whether a similar regulatory mechanism also exists in worms we have combined our quint mutant germline, but found no overt problems with microtubule function in the absence of glutamylation.

228B  Studying Ran-GTP regulation of cytokinesis. Karina Mastronardi, Daniel Beaudet, Brittany Williams, Imge Ozugerin, Alisa Piekn Department of Biology, Concordia University, Montreal, Quebec, CA.

Cytokinesis physically divides a cell into two daughters at the end of mitosis and must be highly robust to avoid aneuploidy and cell fate changes. A RhoA-dependent actomyosin contractile ring ingresses to divide the appropriate genetic and cell fate determinants into each daughter. The prevailing dogma in the field is that the mitotic spindle regulates contractile ring assembly and ingestion. However, microtubule-independent mechanisms also regulate cytokinesis, and may be crucial in polarized cells. During metaphase, a Ran-GTP gradient forms around chromatin where it mediates mitotic spindle assembly by releasing importins α/β complexes from microtubule-binding proteins and motors. Our data suggests that the Ran-GTP gradient persists in early anaphase where it affects the localization of contractile proteins for cytokinesis in human cells. Importantly, one of the Ran targets may be anillin, which is a scaffold that binds to and coordinates actin, myosin, RhoA and its upstream regulators, microtubules, and phospholipids. We found that human anillin contains a C-terminal nuclear localization signal (NLS) that binds to importin-β. Point mutations in the NLS delay anillin’s recruitment to the cortex, and the contractile ring oscillates and subsequently fails to ingress in a subset of cells rescued with this mutant. Interestingly, mutant anillin’s localization is strongly demarcated by astral microtubules, suggesting that the mitotic spindle dominantly regulates cytokinesis in these cells. We
hypothesize that the Ran-GTP regulation of cytokinesis is conserved among metazoans, and the strength of this pathway is proportional to ploidy, cell geometry and/or cell volume. To test this, we are determining if the Ran pathway regulates cytokinesis in early *C. elegans* embryos. Studies showed that there is a negative correlation between the rate of furrow ingression and cell volume as cells decrease in size through the first four rounds of divisions (Carvalho et al., 2009). We are determining how perturbing the Ran pathway alters the rate of furrow ingression and the localization of contractile proteins during these divisions. Also, we are determining if ANI-1 (*C. elegans* anillin), which is highly conserved with human anillin, contains an NLS that may function in the same way as human anillin. We also will determine if the Ran pathway regulates other well-known cytokinesis components.


229C Genetic suppressors of mutant separase may elucidate membrane trafficking role of *C. elegans* separase. M. Melesse, A. Peden, K. Gosine, Q. Caylor, J. Bembenek Biochemistry & Cellular and Molecular Biology, University of Tennessee, Knoxville, TN.

Successful cell division depends on coordinated regulation of chromosome segregation and cytokinesis. Chromosome segregation requires equal partitioning of sister chromatids duplicated in S-phase and held together by the cohesin complex during mitosis. Separase regulates multiple processes during mitotic exit and cytokinesis. The canonical role of separase, the cysteine protease, is to cleave the kleisin subunit of cohesin, allowing chromosome segregation during mitotic and meiotic anaphase. Separase has also been shown to have a non-canonical role in regulating cortical granule exocytosis (CGE) during meiotic division in *Caenorhabditis elegans* embryos. A temperature sensitive separase mutation, *sep-1(e2406)*, results in a single missense mutation (C450Y) within the N-terminal domain of SEP-1, that does not localize to cortical granules and is unable to promote exocytosis but results in minimal chromosome segregation defects.

We have performed a genetic suppressor screen to identify separase regulators that rescue lethality of *sep-1(e2406)*. We found multiple intragenic suppressors that alter residues in the N-terminus of SEP-1. These residues are likely to affect structure stability and binding to other proteins. Consistent with previous observations, we have also identified a substantial number of pp5-5 mutant suppressors. These suppressor mutations are found both in the TPR and phosphatase domain of the highly conserved phosphatase PPH-5 and may provide insight into the phosphoregulation of separase function during exocytosis. We have also identified multiple novel *sep-1(e2406)* suppressors which belong to independent complementation groups, greatly expanding the potential for elucidating separase regulation during membrane trafficking.

230A A worm, a bridge, a circle: How the cell cycle in body wall muscles might be influenced by a nuclear-envelope bridge. A. Ofenbauer, M. Hano, W. Keil, R. Schnabel, B. Tursun 1,2, 1) Berlin Institute for Medical Systems Biology (BIMSB), Berlin, DE; 2) The Max Delbrueck Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, DE; 3) Technical University Braunschweig, Braunschweig, DE; 4) Center for Physics and Biology, The Rockefeller University NYC, NY, USA; 5) Laboratory of Developmental Genetics, The Rockefeller University NYC, NY, USA.

From a forward genetics EMS screen, we isolated the mutant *bar18* showing additional muscle nuclei at the posterior end of the pharynx based on a *myo-3* reporter. These additional nuclei appear during the L2 to L3 larval transition, at a stage where body wall muscle development is normally finished. We performed laser ablation studies and found that ablating the D-lineage suppresses the increased number of nuclei in the *bar18* mutant. Thus, a part of the D-lineage appears to undergo at least one additional round of cell division during the L2 to L3 larval transition, which is unexpected since the D-lineage normally stops dividing during embryogenesis (before Comma stage).

To identify the relevant mutated locus, we used whole genome sequencing and identified a premature STOP in the KASH-domain gene *unc-83*. We could recapitulate the phenotype using other *unc-83* mutant alleles as well as by using a mutant allele of *UNC-84*, a SUN-domain containing protein that interacts with UNC-83. UNC-83 and UNC-84 belong to a nuclear membrane LINC complex that bridges the nuclear lamina with the cytoskeleton. SUN and KASH domain proteins are conserved in mammals and mutations in the corresponding genes have been implicated in cancer. However, so far the UNC-83/UNC-84 LINC complex has not been implicated in muscle development. In *C. elegans*, the UNC-83/UNC-84 nuclear envelope bridge is known to be necessary for nuclear migration in certain tissues during embryonic development. For mutated alleles of these genes, nuclear migration defects of P cells, hyp-7 hypodermal precursors and intestinal polarization defects during embryogenesis have been reported. Currently, we are performing automated time-lapse imaging to capture the additional nuclear divisions as well as suppressor and enhancer screens with known cell-cycle regulators and members of specific pathways such as the Wnt signaling pathway. Additionally, Co-IP experiments are ongoing in order to identify potential unknown interaction partners. By combining these different approaches, we hope to elucidate the mechanisms, by which the LINC complex can re-initiate the cell cycle in fully differentiated body wall muscle cells.

231B Chromosome features regulating genome partitioning and spindle organization in meiosis. K.A. Rivera Gomez1, G. Fabig2, A. Villeneuve2, T. Muller-Reichert2, M. Schwarze21 1) Biology Dept, Brooklyn College, Brooklyn, NJ; 2) Dresden University of Technology, Dresden, Germany; 3) Stanford University, Stanford, CA.

Aberrations in chromosomal partitioning gives rise to aneuploidy. Aneuploid gametes result in cellular arrest, miscarriage, developmental and congenital disorders. Meiosis is primarily concerned with the production of haploid gametes through replication of maternal and paternal chromosomes, recombination, and two subsequent equatorial genome partitions. During recombination, homologous chromosomes exchange information from one chromosome to the other through a temporary connection between the homologous chromosomes. This temporary connection is essential for proper partition of the genome in meiosis. In the first meiotic division, homologous chromosomes separate to opposite spindle poles, the following meiotic division
separates sister chromatids away from each other to give rise to haploid gametes. When homologous chromosomes fail to recombine, they remain unlinked and are known as univalents. REC-8 cohesin protein is necessary for sister chromatids segregation to the same spindle pole during meiosis one. Severson et al. (2009) suggested that meiotic mutants that do not experience a recombination event segregate in one of two ways depending on the lack or presence of REC-8 during oocyte meiosis. Mutants that did not have REC-8 segregate sister chromatids in meiosis one, whereas mutants that had REC-8 were expected to segregate sister chromatids as a unit randomly toward a spindle pole in the first meiotic division. Our live imaging of meiotic mutants defective at different steps leading to recombination revealed that meiotic mutants containing REC-8 such as: him-3, syp-1, syp-1 and spo-11 did not segregate chromosomes during the first division. In these mutants, univalents remain together until the second division and form transient multipolar or connected spindles that yield four aneuploid spermatids. SPO-11 is necessary for DNA double-strand breaks (DSBs) necessary for initiation of crossover recombination. Analysis of spo-11 mutant suggests that the presence of one single bi-oriented homologous chromosome allows suppression of abnormal spindle pole formation in these meiosis mutants. By inducing about one single exogenous DSB per nucleus abnormal spindle formation is suppressed in these mutant spermatocytes. We are investigating the mechanisms by which a single homologous chromosome might allow normal bi-polar spindle organization and thus normal segregation of meiotic mutant chromosomes that show inability to segregate in the first meiotic division.

232C Investigating redundancy among tubulin glutamylating enzymes. P. Shah, DG Chawla, RV Shah, ZK Barth, JD Lee, M Chakraborty, N Peel TCNJ, Ewing, NJ.

Microtubule glutamylation is an important modulator of microtubule function and has been implicated in the regulation of centrosome stability, neuronal outgrowth and cilia motility. Glutamylation of the microtubules is catalyzed by a family of tubulin tyrosine ligase-like (TTLL) enzymes. Analysis of individual TTLL enzymes has led to an understanding of their specific functions, but how activities of the TTLL enzymes are coordinated to spatially and temporally regulate glutamylation remains relatively unexplored. We have undertaken an analysis of the glutamylating TTLL enzymes in C. elegans. We find that although all five TTLL enzymes are expressed in the embryo and adult worm, loss of individual enzymes does not perturb microtubule function in embryonic cell divisions. Moreover, normal dye-filling, osmotic avoidance and male mating behavior indicate the presence of functional amphid cilia and male-specific neurons. Redundancy between the TTLL enzymes has been observed in other species and we are therefore constructing combinations of ttll mutations to explore potential redundancy between the enzymes. We have found that a ttll-4(tm3310); ttll-11(tm4059); ttll-5(tm3360) triple mutant shows reduced male mating efficiency due to a defect in the response step, suggesting that these three enzymes function redundantly, and that glutamylation is required for proper function of the male-specific neurons. We are currently investigating whether further redundancies exist.

233A Molecular mechanisms of developmentally controlled polyploidization in the C. elegans intestine. L.M. van Rijnberk1, D. Morgan2, M. Galli1 1) Hubrecht Institute, Utrecht, Utrecht, NL; 2) University of California, San Francisco, USA.

Polyploid cells, which contain more than two duplicates of each chromosome, arise in many organisms in a regulated manner during development. Two cell cycle variations are known to generate polyploid cells, namely endoreplication and endomitosis. During an endoreplicative cycle, cells skip M phase completely, whereas endomitotic cells enter M phase but bypass many mitotic processes, such as cytokinesis. Although a lot is known on the regulation of endoreplication, the molecular factors that control endomitosis are largely unknown. In particular, it is unclear what determines how far cells progress through mitosis in an endomitotic cycle, and if there is a master regulator responsible for initiating endomitosis. Moreover, it is unknown what the functional difference is between polyploid cells arising from either endomitosis or endoreplication. The C. elegans intestine provides an ideal system to study these questions, as transitions between canonical, endomitotic and endoreplicative cycles all take place at known moments during development within a single tissue. In an attempt to understand how endomitotic cells are able to skip cytokinesis, we imaged intestinal endomitoses using DNA and tubulin markers. Our analysis revealed that endomitotic cells lack the antiparallel microtubule bundles of the central spindle, a structure known to be important for cytokinesis signaling. Using single molecule FISH and endogenous GFP protein tags we found that mRNA and protein levels of the essential central spindle components zen-4 and cyk-4 are downregulated in intestinal endomitotic divisions, suggesting that endomitosis is regulated on a transcriptional level. To identify the transcriptional regulators of endomitosis, we are currently setting up an unbiased RNA sequencing approach in which we purify intestinal cells at specific cell-cycle stages. For this, we developed and characterized a cell cycle marker consisting of a fluorescently tagged cyclin B1 destruction box, which is marked for degradation by the APC/C during late M and G1 phase, but stabilized during S and G2 phases. Visualization of this marker has allowed us to precisely define S phase timings of intestinal endomitotic and endoreplicative cycles. Moreover, we have combined this cell cycle marker with an intestine-specific GFP-NLS marker, allowing us to purify intestinal cells in S/G2 phases of canonical and endomitotic cycles using FACS of single cell suspensions. Together, these approaches will allow a systematic analysis of transcriptional changes that occur during endomitosis and endoreplication, providing insights into how tissues specify cell cycle programs and what the importance is of specific forms of polyploidization.

234B Dissecting the function of the mitotic histone H3 kinase haspin and its paralogs in C. elegans. E. Pianin2, D. Das2, L. Drubel2, O. Dawson2, R. Parker2, S. V. Ganapa2, D. J. Wynne2,1 1) Department of Biology, University of Portland, Portland, OR; 2) Department of Biology, The College of New Jersey, Trenton, NJ.

Haspin is a protein kinase known to phosphorylate histone H3 at threonine 3 (H3T3ph) during mitosis. This histone mark (H3T3ph) aids in centromeric recruitment of Aurora B kinase, which is essential for regulation of kinetochore microtubule attachment, sister chromatid cohesion, the mitotic checkpoint and cytokinesis. However, haspin's functions outside of mitosis remain less well understood. Haspin was identified due to its high expression in mouse testis, but its role in this tissue has never been characterized (Tanaka et al. 1999). Its inhibition affects chromosome condensation in mouse oocytes and
asymmetric histone segregation in the *Drosophila* germline (Nguyen et al. 2014, Xie et al. 2015), and haspin protein remains throughout interphase, when overexpression can halt progression through G1. Despite these various roles and cell types, only a single substrate of haspin, H3T3ph, has been identified. How this mark leads to the variety of haspin functions, or whether there are other substrates remains unclear. Further, the activity of haspin’s atypical C-terminal kinase domain has been shown to be regulated by residues in the N-terminus in human and frog haspin (Ghenou et al. 2014), but the N-terminal domains are poorly conserved, so it is not clear if this auto-regulatory mechanism is common. Unlike most eukaryotes, which have a single haspin gene, *C. elegans* has had a dramatic expansion to form a haspin gene family that includes two genes, hasp-1 and hasp-2, with closest homology to mammalian haspin, and over 10 other paralogs with lower homology (Higgins 2001). This expansion of the gene family raises two interesting possibilities. First, some haspin-like genes in *C. elegans* may have roles independent of phosphorylation of H3T3ph. Second, *C. elegans* haspins may have undergone differentiation and sub-functionalization such that individual paralogs control subsets of haspin functions. We aim to understand the function and regulation of haspin kinases by systematically examining the localization and loss-of-function of haspin paralogs. We confirmed that an existing putative-null allele of hasp-1 is sterile due to a germline proliferation defect, consistent with its expected role in mitotic progression. In addition, we have generated two loss-of-function alleles in hasp-2 using CRISPR/Cas9. hasp-2 homozygotes show no dramatic loss of oocyte or sperm fertility, and H3T3ph is still present on mitotic and meiotic nuclei. However, the mutants have a slight viability defect, which we are currently investigating further. The cause of viability defects in hasp-2 mutants, and the extent to which hasp-2 function is redundant with other haspin paralogs, may lead us to new roles for this important family of kinases.

**Cell Biology - Cell Polarity**

**235C Investigating the role of heterochronic genes in the polarity of DA9 neurons.** Maria Armakola1,2, Gary Ruvkun1,2 1) Molecular Biology, MGH, Boston, MA, USA; 2) Department of Genetics, Harvard Medical School, Boston, MA, USA.

Neuronal polarity is established early in development of the nervous system and is subsequently maintained throughout the adult life. Understanding how molecular mechanisms that regulate development could interfere with biological mechanisms by which neurons maintain their polarity and synapses is important to design therapeutics. Using RNA interference, we previously tested how progeric gene inactivations might affect the formation of synapses in DA9 motor neurons in *C. elegans*. We used confocal microscopy to test the localization of synaptic vesicle associated protein RAB-3 (GFP::RAB-3) in DA9 neurons. A set of progeric inactivations caused the formation of ectopic dendritic puncta and changed the localization of RAB-3::GFP puncta in the dorsal side. Here we focus on how heterochronic genes (*lin-29, blmp-1, lin-14, dre-1*) change the localization of presynaptic puncta in adult stage of DA9 neuron. We show that a *pqm-1* lof mutation causes ectopic dendritic localization of RAB-3::GFP puncta in 100% of adult animals tested. This ectopic dendritic localization of RAB-3::GFP puncta appears even at early larval stages but is *lin-29* dependent. PQM-1 is a zinc transcription factor that synergizes with DAF-16/FoxO to mediate the transcriptional changes triggered by disruption of insulin signaling. The effect of *pqm-1* lof mutation is reversed in the *daf-16(mu86)* genetic background. We also measured the thrashing activity of these mutants and their effect on locomotion. We are continuing to characterize how genetic pathways that regulate developmental timing and neuronal polarity intersect.

**236A Polo-like kinase links cytoplasmic RNA-binding protein gradients in the *C. elegans* zygote.** B. Han, M. Rutigliano, E. Griffin 1) Biological Sciences, Dartmouth College, Hanover, NH.

Intracellular protein gradients underlie essential cellular and developmental processes, but the mechanisms by which they are established are incompletely understood. During the asymmetric division of the *C. elegans* zygote, the RNA-binding protein MEX-5 forms an anterior-rich cytoplasmic gradient that causes the RNA-binding protein POS-1 to form an opposing, posterior-rich gradient. We provide evidence that MEX-5 targets the polo-like kinase PLK-1 to POS-1, which in turn phosphorylates POS-1 to increase its diffusivity in the anterior cytoplasm. Both the interactions between PLK-1 and MEX-5 and between MEX-5 and RNA are required for regulation of POS-1 diffusivity. Additionally, we demonstrate that the retention of POS-1 in the posterior depends on POS-1 RNA-binding, suggesting that PLK-1 phosphorylation inhibits the formation of slow-diffusing POS-1/RNA complexes in the anterior. Our findings elucidate a novel reaction/diffusion mechanism that targets polo-like kinase to RNA in one cytoplasmic domain, thereby partitioning the cytoplasm.

**237B GPA-16 Affects Asymmetric Cell Division and Learning and Memory.** M. Mersha1, P. Han1, H. Dhillon1, A. Klar2 1) Delaware State University, Dover, DE; 2) National Cancer Institute, Frederick, MD.

Animal body plans tend to display external symmetry; however, their internal organs can be anatomically and/or functionally asymmetrical. Visceral organs such as liver, heart, pancreas etc. show clear anatomical asymmetry in a bilateral fashion. The right and left cerebral hemispheres are functionally asymmetric and apparently small deviations have been correlated with pathologies such as schizophrenia and bipolar disorders. The bilateral asymmetry is genetically and developmentally defined as a third axis as are the other two axes: anterior/posterior and dorsal/ventral. The lab model *Caenorhabditis elegans* is particularly suited to study left/right (L/R) asymmetry. Like most other animals, *C. elegans* shows predominantly bilaterally symmetric external anatomy, but clear bilateral asymmetry in the viscera, a key feature being the placement of the anterior gonad towards the right. In addition to anatomical asymmetry certain neuronal pairs such as AWL-C and AWL-R also display functional asymmetry. The anatomic bilateral asymmetry is discernible during the initial cell divisions of the fertilized egg. Previous studies have suggested that PAR proteins along with Ga proteins associated with spindle positioning that play a role in anterior/posterior and dorsal-ventral are likely to underlie the first symmetry-breaking step as well. The absence of gpa-16, a Ga protein, has been shown to yield up to 50% sinistral worms. On the contrary, wild type N2 animals invariably lead to dextral embryos. We have investigated the direct effects of disrupted asymmetry on embryonic lethality and adult behavior. Here, we show that the
absence of gpa-16 results in not only sinistral embryos but also randomly dividing embryos. Surviving adults with the gpa-16 mutation are impaired in both, associative and non-associative learning. We are examining if the reversed asymmetry manifests its functional effects on behavior due to potentially atypical neuronal circuitry and looking at synaptic connectivity of gpa-16 mutants with the goal of unraveling anatomically atypical circuits.

238C Regulation of ERM proteins in cortical membrane remodeling.  J.J. Ramalho1, M. Harterink2, M. Boxem1  1) Developmental Biology, Utrecht University, The Netherlands; 2) Cell Biology, Utrecht University, The Netherlands.

Dynamic remodeling of specific cortical domains occurs in most polarized cells and is an essential process for animal development and body homeostasis. Membrane remodeling requires interaction between the plasma membrane and the underlying cytoskeletal actin network. Proteins of the conserved Ezrin-Radixin-Moesin (ERM) family interact with membrane components through an N-terminal FERM domain as well as with F-actin via the C-terminal domain. In different model systems, ERM linker activity has been associated with formation of dynamic actin-based structures such as microvilli, axonal growth cones, or the leading edge of migratory cells. Activity of ERM proteins depends on a conformational change that turns an inactive cytoplasmic form into an active membrane- and actin-bound form. In vitro data suggests an activation model dependent on PIP$_2$-binding followed by phosphorylation of a conserved C-terminal residue. However, in vivo data supporting this model is scarce and contradictory. We use the single C. elegans ERM ortholog, erm-1, as a model to study the contribution of different regulatory sites for ERM protein activity and tissue morphogenesis in vivo. Using CRISPR/Cas9 gene editing and quantitative live-cell imaging we show that PIP$_2$-binding, but not phosphorylation of the C-terminal T544 residue, is critically required for ERM-1 function. erm-1 mutants unable to bind PIP$_2$ mimic the null phenotype. In contrast, mutants that either constitutively lack or mimic T544 phosphorylation are viable, with defects whose severity differs between tissues. Both erm-1(T544A) and erm-1(T544D) mutants show severe defects in excretory canal development, and our data suggests that ERM-1 T544 phosphocycling is necessary for canal lumenogenesis. In the intestine, phosphocycling appears to regulate ERM-1 stability and trafficking of apical cargo. However, the effects of T544A and T544D mutations on the apical actin ACT-5 differ. Expression of the unphosphorylatable ERM-1 T544A variant decreases the levels and stability of ACT-5, while the phosphomimicking ERM-1 T544D mutation induces formation of additional luminal membrane, but does not affect ACT-5 levels or stability. We are currently investigating how these mutations affect ERM-1 dynamics, and how they affect the intestine at the ultrastructural level. Unraveling the regulatory mechanisms behind ERM protein function will contribute to our understanding of membrane remodeling events during development and disease.

239A The essential centrosome components γ-TuRC and AIR-1/Aurora A are not required to organize non-centrosomal microtubules in the developing intestine.  Maria Sallee, Taylor Skokan, Jenny Cade, Jessica Feldman  Dept. of Biology, Stanford University, Stanford, CA.

Microtubule organization is critical for cell function. Nearly all dividing animal cells use the centrosome as a microtubule organizing center (MTOC), where microtubules tether chromosomes to the spindle poles to facilitate the correct segregation of DNA between daughter cells. By contrast, differentiated cells organize their microtubules in a wide variety of patterns, and establish specific noncentrosomal locations as MTOCs to achieve these microtubule arrangements. While much is known about how centrosomes organize microtubules, little is known about the composition of noncentrosomal MTOCs (ncMTOCs), how these sites are designated, or how they organize microtubules. A simple hypothesis is that an ncMTOC is essentially the MTOC features of a centrosome targeted to a different cellular location. Our studies suggest that the ncMTOC that forms at the apical surface of the polarized C. elegans intestine is in fact different in its composition and protein requirements for microtubule organization than the centrosome. We have examined the localization of several CRISPR-tagged microtubule- and MTOC-associated proteins in the intestinal ncMTOC, and observe three classes of proteins: (1) only at the centrosome (SPD-2/Cep192, SPD-5), (2) only at the ncMTOC (PTRN-1/CAMSAP, NOCA-1/Ninein), and (3) whichever location is the active MTOC (?-TuRC proteins, AIR-1/Aurora A). To test the requirement of several factors in intestinal ncMTOCs, including AIR-1 and the ?-TuRC components GIP-1 and Mozart, we have optimized the ZIF-1/ZF protein degradation system to allow for the removal of early essential proteins from tissues of interest. We find evidence that the microtubule nucleation complex ?-TuRC must be intact for the recruitment of ?-TuRC components to the ncMTOC. Surprisingly, microtubules are still made and correctly localized to the apical ncMTOC even when ?-TuRC and AIR-1 are compromised, and we are currently analyzing the dynamics of these microtubules. These results suggest that differentiated cells use novel mechanisms to create a functional ncMTOC that organizes their microtubules.

240B Local and global remodelling of cortical myosin by the mitotic kinase Aurora-A.  P. ZHAO1,2, S.K. SZE3, F. MOTEGI1,2,3  1) Temasek Life Sciences Laboratory, Singapore, SG; 2) Department of Biological Sciences, National University of Singapore, Singapore, SG; 3) Mechanobiology Institute, Singapore, SG; 4) School of Biological Sciences, Nanyang Technological University, Singapore.

The development of multicellular organisms involves the ability to coordinate cell proliferation with cell fate diversification. To proceed, each cell acquires spatial asymmetry to facilitate asymmetric cell divisions. Asymmetric cell division requires spatially biased remodelling of the actomyosin cytoskeleton in response to intrinsic or extrinsic inputs. However, the nature of the mitotic signalling that coordinates the remodelling of actomyosin network to support both cell polarization and cell division remains poorly understood.

We present evidence that the mitotic kinase, Aurora-A, dictates local and global remodelling of cortical actomyosin to facilitate asymmetric cell divisions. In Caenorhabditis elegans zygotes, anterior-posterior asymmetry is induced by asymmetric
contraction of cortical actomyosin network, which drives transient flows of the cell cortex. During early prophase, Aurora-A accumulates at centrosomes to locally disassemble cortical myosin at the proximal cortex. Notably, induction of actomyosin flows is independent of a role of Aurora-A in centrosome maturation. During late prophase, Aurora-A in cytoplasm stimulates global disassembly of cortical myosin. Loss of Aurora-A caused sustained activation of RhoA GTPase throughout the cortex during prophase, suggesting Aurora-A as a local and global inhibitor of RhoA.

We thus propose a mechanism by which cell cycle progression is coordinated with actomyosin remodelling through the mitotic kinase Aurora-A. To identify the downstream targets of Aurora-A, we performed in vivo large-scale protein interaction studies complemented with in vitro kinase assay screen. We will present our current progress and discuss how Aurora-A controls two distinct phases of myosin remodelling for asymmetric cell division.

Cell Biology - Cytoskeleton and Migration

241C  Actin cytoskeleton dynamics in anchor cell invasion.  R. Caceres¹, N. Bojanala¹, L. Kelly², D. Sherwood², J. Plastino¹ ¹) Laboratoire Physicochimie Curie, UMR 168, Institut Curie, Paris, FR; ²) Department of Biology, Duke University, Durham, North Carolina, United States of America.

Basement membrane (BM) is a dense sheet of specialised extracellular matrix, composed principally of lamnin and type IV collagen polymers and glycoproteins that separate epithelia from underlying tissues. The invasion of cells through BM barriers is important during normal tissue development and in cancer metastasis. The breach in the BM is mediated by an actin-rich protrusion known as an invadopodium, and actin assembly dynamics in the invadopodia are essential for invasion. Much has been understood concerning the genetics and signalling of how holes are formed in the BM during invasion. However less is clear about the biophysics that is involved: how do cells exert mechanical forces via their actin cytoskeleton to make holes in BM barriers? To address this question, we study an invasion event in a developmental process, anchor cell (AC) invasion in Caenorhabditis elegans. We use mutants, RNAi treatments and dominant negative approaches to disrupt actin polymerization and interfere with actin binding proteins in the AC. Under these different perturbations, we evaluate the efficiency of invasion and observe the dynamics of the actin cytoskeleton during protrusion across the BM.

In addition to the known role of WASP in cancer cell invasion, previous studies on AC invasion have shown the importance of the Arp2/3 complex activator WASP/WSP-1: WASP-null worms, wsp-1 (gm324), display only 18% full invasion at a developmental stage where WT worms show 100% invasion (Lohmer et al. 2016). Alternately, we expressed a dominant-negative form of an Arp2/3 complex activator specifically in the AC, which led to a complete block in invasion, confirming the importance of Arp2/3 complex-based actin polymerization for AC invasion.

242A  Mutations in the CDC-42 GEF cgef-1 enhances unc-84 nuclear migration defects in larval P cells.  Leslie Herrera, Daniel A. Starr  Molecular and Cellular Biology, University of California, Davis, CA.

Nuclear migration is essential for cellular migration. A cell’s ability to successfully migrate through a constricted space is limited by the ability of the large and rigid nucleus to deform during migration. Caenorhabditis elegans larval P-cell nuclei (~3-4 um in diameter) must migrate through a constricted space between body wall muscles and the cuticle (~125 nm) while moving from the lateral to the ventral side of the animal. If the nuclei are unable to squeeze through this constriction, migration fails and P-cells die, which leads to uncoordinated (Unc) and egg-laying deficient (Egl) adult animals. C elegans raised at 25°C with an unc-84 null mutation have severe P-cell nuclear migration defects, but develop normally when raised at 15°C. We hypothesize there is an enhancer pathway acting parallel to the UNC-84/UNC-83 microtubule-based nuclear migration pathway. We performed a forward genetic screen to find mutants in the enhancer of the nuclear migration defect of unc-84 (emu) pathway. To date, we have isolated 8 emu lines from this screen and used a whole genome sequencing approach to clone emu genes. We previously found that toca-1 and fln-2 are in the emu pathway, suggesting the mechanism of nuclear migration is actin based. Here we report that one of our emu mutations, yc3, is a G-to-A substitution causing a premature stop codon in amino acid Trp (345) of cgef-1. To confirm that cgef-1 is an enhancer of the unc-84 nuclear migration defect, we injected fosmids WRM0625dG08 and WRM0622bA03 into the UD285 (yc3; unc-84(n369) otxls12 X) ?strain for a rescue experiment. We count GABA neurons labeled with Punc-47::GFP to assay P-cell nuclear migration defects. Wild-type C elegans have 19 GABA neurons, unc-84 (null) animals have 17.4 ± 0.2 (mean ± SEM) and cgef-1(yc3); unc-84 (null) 11.7 ±0.3 GABA neurons at 15°C. The fosmids WRM0625dG08 and WRM0622bA03 rescue yc3; the rescued strain had 18.1 ± 0.1 GABA neurons at 15°C. To further investigate cgef-1 and the interactions with other hypothesized emu genes, we are in the process of using CRISPR/Cas9 to make a CGEF-1::GFP fusion protein.

243B  A novel myosin light-chain kinase regulates myosin-dependent contraction in the C. elegans spermatheca.  Charlotte A Kelley, Erin J Cram  Biology, Northeastern University, Boston, MA.

Biological tubes, such as our airways and vasculature, rely on a balance of contraction and relaxation to either maintain homeostatic tone, or to constrict or dilate in response to a stimulus. We use the spermatheca, a single layer tube of smooth muscle-like cells and the site of fertilization of Caenorhabditis elegans, as a model biological tube to study how constriction is controlled. Smooth muscle contractility depends on the pool of phosphorylated myosin, which can form bipolar filaments, bind actin, and has ATPase activity. Previous work from our lab shows that contraction requires PLC-1 to generate IP₃, which in turn mediates Ca²⁺ release from the endoplasmic reticulum. We therefore hypothesized that a calmodulin-regulated myosin light chain kinase may be phosphorylating myosin within the spermatheca. We performed an RNAi screen of candidate genes and
identified a new myosin light-chain kinase, ZC373.4/mlck-1, that is necessary for constriction in the C. elegans spermatheca and shares significant homology with human MYLK within the kinase domain. DIC time lapse imaging shows that depletion of mlck-1, either with a hypomorphic allele mlck-1(tm4159) or with RNAi, slows or stalls oocyte transit through the spermatheca. mlck-1 worms have a small brood size, which seems to be the result of maternal damage to oocytes as they pass through the spermatheca. In addition to its kinase domain, MLCK-1 has two putative calmodulin binding domains. Calmodulin is activated upon calcium binding and can inhibit myosin light-chain kinases, leading to contraction. We find that cmd-1, a calmodulin, is required for transit through the spermatheca. This work identifies and characterizes a new myosin light-chain kinase in C. elegans.

**244C** A Nima-kinase pathway controls Cdc-42 activity and actin organization in the epidermis.  
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During molting the epidermal apical extracellular matrix (cuticle) is extensively remodeled. We have shown that the NimA-related kinases, NEKL-2/NEK8 and NEK-3/NEK6/NEK7, as well as their conserved ankyrin-repeat partners, MLT-2/ANKS6, MLT-3/ANKS3 and MLT-4/INVS, are essential for molting. Both NEKLs and MLTs are expressed in the epidermal syncytia and are specifically required in hyp7 for normal molting. Our studies indicate that NEKLs and MLTs act primarily within two subcellular domains. To understand how the NEKL–MLT network controls molting, we identified suppressors of neki mutant molting defects. Our screens identified CDC-42, a highly conserved Rho-family GTPase, and its upstream regulator, KIN-25/SID-3. CDC-42 is important for the establishment of cell polarity and for organization of the actin cytoskeleton. KIN-25 is the ortholog of Activated CDC42 Kinase 1, which binds to CDC42 and inhibits its GTPase activity, thereby maintaining CDC42 in an active state. Notably, molting has been proposed to require extensive reorganization of actin within the epidermis. Specifically, actin is reorganized at each molt to form a series of circumferential bundles along the apical surface of hyp7, however, the mechanisms underlying this process are unknown.

We found that inhibition of NEKL–MLT activities leads to defects in the pattern of apical actin and failure to form molting-specific actin bundles. Furthermore, expression studies indicate that components of the NEKL–MLT network colocalize with actin in specific regions of the epidermis. In addition, normal localization of CDC-42 in the epidermis depends on presence of NEKL–MLT proteins, and CDC-42 partially colocalizes with MLT-2. Our findings suggest that the NEKL–MLT network may negatively regulate CDC-42 activity and that partial inhibition of CDC-42 may thus reduce defects in nekl–mlt mutants. Interestingly, we also observed that downregulation of cdc-42 on its own can lead to molting defects, suggesting that there is a tight balance between NEKL–MLT and CDC-42 activities in the epidermis.

Notably, studies in mammalian cells have implicated the MLT-4 ortholog, Inversin, in the regulation of CDC42 activity and apical actin organization. In addition, it was independently shown that the mammalian orthologs of NEKL-3, NEK6 and NEK7, physically associate with CDC42, however no functional connection between these proteins has been described. For the first time, our data provide in vivo evidence for a functional link between the NEKL–MLT network and regulation of the actin cytoskeleton through the CDC42 pathway.

**245A** Using NemaFlex to investigate the contribution of specific muscle genes to muscle strength.  
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*Caenorhabditis elegans*, a well-known research animal model, has also become a robust model for studying muscle structure and function in vivo. Their body wall muscles are functionally analogous to vertebrate muscle, as they include dense bodies and M-lines, the former being functionally equivalent to vertebrate Z-lines. The number of proteins known to be located in dense bodies and M-lines is increasing and the role of many are not well understood, particularly with regards to the development and maintenance of muscular strength. *C. elegans*, along with our NemaFlex apparatus that enables muscle strength measurements, offers an extremely powerful platform to study genes impacting muscle strength.

We have studied mutations of specific genes that encode proteins known to localize to each muscle structural category: dense body, M-line, and dense body and M-line together. In our initial investigation, we have chosen unc-82 and unc-89 mutants (strains CB1220 and CB1460, respectively) that have defects in M-line structure, uig-1 mutants (strain RB978) that affect the dense body and zyx-1 mutants (strain VC299) that affect both the dense body and the M-line. Strength measurements were performed using the NemaFlex, which consists of force-sensing micropillars. Our results show that some of the mutants were weaker in strength, but others interestingly did not show discernible strength difference. unc-89 and zyx-1 animals showed a significant decrease in strength, while uig-1 and unc-82 did not. unc-89 animals were the weakest likely due to the fact that these mutants are reported to be missing the M-line. On the other hand, mutations in uig-1 and unc-82 did not result in strength discernible from wild-type indicating that they may not have a key role in force generation. This could be due to the fact that the protein products of these genes are reported to have a signaling function instead of a structural role in muscles. These initial studies lay the groundwork for a high-throughput screen of muscle-related genes that can help identify the key muscle proteins impacting strength. This knowledge can help identify targets for disease therapies involving muscle dysfunction including muscular dystrophy, cachexia and dynapenia.

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Autosomal dominant polycystic kidney disease (ADPKD) is a human ciliopathy that affects more than one in every 1000...
individuals and is primarily caused by defects in either PKD1 or PKD2, genes with the nematode orthologs lov-1 and pkd-2 respectively. We recently found that a mutation in the C. elegans ortholog of human mitogen activated kinase-15 (mapk-15) results in defective functions of ciliary sensory neurons (CSNs) in the tail of worms, including dye-filling defects (Dyf) in hermaphrodite phasmid CSNs and PKD-2 localization defects (Cil) in male ray CSNs (Piasecki et al., in review). To identify genes in the same molecular pathway as mapk-15, a screen was conducted to identify suppressors of the mapk-15 Dyf phenotype. Animals harboring a large deletion at the mapk-15 locus (gk1234) were exposed to N-ethyl-N-nitrosourea (ENU), a potent mutagen effective at inducing point mutations. Following ENU exposure, worms with a 40-50% increase in dye-filling efficiency were isolated and their progeny were subjected to additional screening to confirm the stable inheritance of this phenotype. Currently, backcrossing of four suppressed strains to the original mapk-15 mutant strain are in progress to obtain near isogenic suppressed strains with mutations at extragenic loci. Whole-genome sequencing will be used to identify mutations. The ability of each of these strains to suppress Dyf and Cil phenotypes alone and in different combinations will be determined. These results should reveal genetic interactions occurring in the mapk-15 pathway and may provide insights into ADPKD etiology.

247C Interactions between SUN and KASH proteins in vivo during nuclear migration, nuclear anchorage, and the developmental switch between moving and stationary nuclei.  N. Cain, H. Hao, D.A. Starr  Mol & Cellular Biol, Univ California, Davis, Davis, CA.

LINC complexes, consisting of SUN proteins in the inner nuclear membrane and KASH proteins in the outer nuclear membrane, transfer forces between the cytoskeleton and the nucleoskeleton. One role of LINC complexes is to mediate nuclear positioning. The canonical C. elegans SUN protein UNC-84 interacts with two different KASH proteins—UNC-83 (a functional homolog of mammalian Nesprin-4 and KASH5) to move nuclei via microtubule motors and ANC-1 (the ortholog of the giant mammalian Nesprin-1 and -2) to anchor nuclei to actin. It is not known how UNC-84 chooses between binding UNC-83 to move nuclei or binding ANC-1 to anchor nuclei. Based on structural data of human SUN-KASH complexes, we first hypothesized that key conserved residues in the SUN domain of UNC-84 are required to interact with the C-terminus of KASH proteins. Both UNC-84(S1034E) and UNC-84(C994E) failed to recruit UNC-83 to the nuclear envelope and nuclear migration was blocked. Second, the structure predicted that the length of the C-terminus of KASH proteins is limited by the size of the binding pocket in the SUN domain. The addition of a single alanine to the end of UNC-83 completely blocked UNC-83 localization and nuclear migration in vivo. Finally, the structure identified an intermolecular di-sulfide bond between conserved cysteine residues in SUN and KASH proteins. The conserved cysteine at position -23 of KASH proteins is present in ANC-1, but not in UNC-83. This led us to hypothesize that cysteine bonds are dispensable for nuclear migration, but required for nuclear anchorage. To test this hypothesis, we used CRISPR/Cas9-mediated genome editing to mutate conserved cysteines in SUN and KASH proteins. Hypodermal nuclei expressing UNC-84(C953A) migrated normally, but clustered unevenly in the adult syncytium. Similar anchorage defects in the adult animal were observed after mutating the conserved cysteine in the KASH domain of ANC-1, or replacing the KASH domain of ANC-1 with the KASH domain of UNC-83, which does not have a cysteine. Based on these results, our model is that di-sulfide bonds between SUN proteins and various KASH partners could be a component of a molecular switch between actively moving and anchored nuclei. Interestingly, careful quantification of the nuclear anchorage phenotype shows that unc-84 mutants are not as severe as anc-1 mutants, suggesting ANC-1 has activities independent of UNC-84. We are currently testing potential ANC-1 partners to see if they enhance nuclear anchorage defects of unc-84.


Microtubules (MTs) are composed of a- and ß-tubulins, which are encoded by multiple genomic loci in most organisms. Despite implications of distinct properties of tubulin isotypes, whether and how they contribute to diverse MT functions in vivo remains unclear. In this study, we aimed to comprehensively analyze null phenotypes and expression patterns/levels of all tubulin isotypes (nine a-tubulins and six ß-tubulins) in C. elegans, using CRISPR/Cas9-mediated genome editing. Firstly, we focused on two a-tubulins (TBA-1 and TBA-2) and two ß-tubulins (TBB-1and TBB-2) that are expressed in early embryos. Among ß-tubulins, TBB-2 was expressed two times higher than TBB-1. Substitution of isotype coding regions by CRISPR/Cas9 demonstrated that, under the same concentration, TBB-1-composed MTs had higher switching frequency between growth and shrinkage than TBB-2-composed MTs. The two a-tubulins distinctively affected growth rates of TBB-1- and TBB-2-composed MTs. These results demonstrated that each isotype has distinct properties for MT dynamics, and alteration of ratio and concentration of isotypes distinctively modulates MT dynamics in vivo. Next, using GFP-insertion lines, we analyzed expression patterns and levels of each tubulin isotype in adult tissues. TBA-1, TBA-2, TBB-1, TBB-2 were ubiquitously expressed at a high level, whereas TBA-4 was expressed in many tissues except germline at a low level. On the other hand, MEC-12 (a), MEC-7 (ß) and TBB-4 were expressed in specific sets of neurons (as previously reported) at a much lower level than the expression levels of ubiquitous tubulin isotypes, and TBA-7 was expressed in intestine. MEC-12 and MEC-7 are known to be required for the function of touch receptor neurons and generating 15-protofilament microtubules. Thus, these results implicate that MTs are mainly constructed by ubiquitous tubulin isotypes and only a small fraction of tissue-specific tubulin isotypes (such as MEC-12 and MEC-7) can affect MT dynamics to confer tissue-specific MT behaviors. To further understand specific properties of tubulin isotypes, we plan to artificially alter isotype composition and examine their effects on MT dynamics.

249B The C. elegans spermatheca is a model contractile tube for elucidating the roles of actin interacting proteins in tissue contractility.  A. C. E. Wirshing, E. J. Cram  Biology, Northeastern University, Boston, MA.

Actin cytoskeleton organization and composition determine the mechanical properties of cells and tissues. There is evidence
that disruption of the actin cytoskeleton underlies the pathophysiology of contractile tissue diseases including asthma and hypertension. Elucidating the mechanisms for organization of the actin cytoskeleton in contractile tissue is challenging due to the presence of numerous actin interacting proteins and the dynamic nature of actin regulation. Much of our current understanding comes from in vitro and ex vivo experiments. In this work, we use the C. elegans spermatheca as a novel in vivo model for identifying and characterizing genes involved in actin organization and tissue contractility. The spermatheca is a sack-like organ of the reproductive system that houses the sperm. During ovulation, the oocyte is propelled from the proximal gonad, through the spermatheca, where it is fertilized, and into the uterus. This process occurs approximately 150 times per gonad arm requiring robust regulation of contraction. To identify actin interacting and regulatory genes required for gonad contractility, we used a candidate RNAi screen of more than 200 genes. We have identified actin nucleators, crosslinkers, and motor proteins required for spermathecal contractility. Here we highlight the role of actin motor protein non-muscle myosin II, nmy-1, in not only spermathecal contractility, but also organization of the spermathecal actin network. Using 4-D confocal microscopy to observe spermathecal actin in live animals during ovulation, we show that prominent parallel actomyosin bundles develop from a loose meshwork during the first ovulation. Bundle development requires active myosin and bundle periodicity, tortuosity, connectivity, and orientation are regulated by myosin activity. We conclude that tight spatiotemporal control of myosin activity is required for formation and maintenance of the functional spermathecal actin network.

Cell Biology - Germline, Meiosis


RNA-binding proteins are essential regulators of gene expression that act through a variety of mechanisms to ensure the proper post-transcriptional regulation of their target RNAs. ETR-1, a highly conserved ELAV-Type RNA-binding protein belonging to the CELF/Bruno protein family, is canonically known for its involvement in C. elegans muscle development. Animals depleted of ETR-1 have been previously characterized as arresting at the two-fold stage of embryogenesis. We show that ETR-1 is expressed in the hermaphrodite somatic gonad and germ line, and that ETR-1 depletion results in reduced hermaphrodite fecundity. Detailed characterization of this fertility defect indicates that ETR-1 is required in both the somatic tissue and the germ line to ensure wild-type reproductive levels. Additionally, the ability of ETR-1 depletion to suppress the published WEE-1,3-depletion infertility phenotype is dependent on ETR-1 being depleted in the soma. Within the germline of etr-1(RNAi) hermaphrodite animals, we observe reduced oocyte size and an increase in the number of germline apoptotic cell corpses as evident both by an increased number of CED-1::GFP positive early apoptotic cells and acridine orange positive committed apoptotic germ cells. Transmission Electron Microscopy (TEM) studies confirm the significant increase in apoptotic cells in ETR-1-depleted animals, and reveal a delay of the somatic gonadal sheath cells to properly engulf dying germ cells in etr-1(RNAi) animals. Through investigation of an established engulfment pathway in C. elegans, we demonstrate that co-depletion of CED-1 and ETR-1 suppresses both the reduced fecundity and the increase in the number of apoptotic cell corpses observed in etr-1(RNAi) animals. Combined all this data suggests that ETR-1 functions in the CED-1 engulfment pathway. We have identified a novel role for ETR-1 in hermaphrodite gametogenesis and in the process of engulfment of germline apoptotic cell corpses.

251A The CUL-4 E3 Ligase complex plays two roles in meiotic prophase I. Benjamin Alleva, Sean Clausen, Sarit Smolikove Biology, University of Iowa, Iowa City, IA.

Meiosis is the specialized cellular division that starts from a diploid meiocyte leading to the formation of 4 haploid gametes. The first meiotic division uses DNA damage repair to pair homologous chromosomes for proper segregation. Once paired, the Synaptosomal Complex (SC), a tripartite proteinaceous structure, assembles along homologous chromosomes to provide pairing stabilization. Stabilization allows for recombination to fix the DNA damage and create crossovers between homologs. The SC disassembles leaving the crossover holding together homologs up to their segregation. Previously, our lab has shown that the CSN/COP-9 complex plays a crucial role in SC assembly. CSN/COP-9 has deubiquitination activity which is specific to Cullin E3 ligases. E3 ligases are activated by the addition of NED-8 and deactivated by the release of NED-8. Mutants of the complex, as well as NED-8 knockdown, lead to SC polycross formation. Due to uncompleted synapsis, DNA damage accumulates in these nuclei leading to a decrease in overall crossovers. We have previously shown that the CUL-4 E3 ligase is the likely target of CSN/COP-9, but further investigation of the CUL-4 complex was lacking. Here we show that the CUL-4 complex plays a role in both SC assembly and DNA damage repair. Mutants of the CUL-4 complex have SC polycross formation throughout pachytene. These mutants also have defects in DNA damage repair as RAD-51 immunofluorescent staining shows high levels within nuclei. We also show that not all canonical components of the CUL-4 complex are required for SC assembly. Through SYP-1 immunofluorescent staining, we found that DDB-1, a protein that connects CUL-4 to an adapter protein, and GAD-1, the adapter protein, are both involved in SC assembly. Interestingly, the RING proteins, RBX-1 and RBX-2, docking sites for E2 conjugating enzymes, are not required for SC assembly. During Cullin E3 ligase cycling, CAND-1 binding prevents reactivation until it’s released which allows for reactivation. We found that loss of CAND-1 had no effect on SC assembly, suggesting that cycling but not sequestering of the inactive complex is necessary for SC assembly. The function of the Cullin E3 ligases are to ubiquitinate targeted proteins either for degradation or other functions such as signaling. We show that in csn-5 mutants, there is an increase in the localization of LYS-48 ubiquitination to nuclei, the residue associated with degradation. Whereas in cul-4 mutants, there is a lower nuclear signal of LYS-48 as compared to csn-5 mutants. This suggests that over-ubiquitination of target proteins or over-self-ubiquitination of CUL-4 is occurring in these
mutants. Altogether, we suggest that the CUL-4 E3 ligase complex functions in both SC assembly and DNA damage repair through its ubiquitination activity.

252B Sex-specific kinetochore features in sperm meiosis. Christopher Black, Vanessa Cota, Thais Cintra, Jui-ching Wu, Diana Chu Biology, San Francisco State University, San Francisco, CA.

Chromosome segregation in sperm has unique features that make it different from oocyte meiosis. Oocytes undergo two rounds of asymmetrical chromosome segregation without centrosomes, while sperm have symmetrical divisions and utilize centrosomes to organize microtubule attachment to chromosomes. This attachment is mediated by a protein complex called the kinetochore, but the role of the kinetochore during sperm meiosis remains poorly understood. We report here that sperm-specific phosphatases GSP-3/4 may regulate kinetochore-microtubule connection to organize sister chromatid orientation from segregating to the same pole in meiosis I to opposite poles in meiosis II. gsp-3/4 mutant sperm exhibit delayed resolution of a univalent lagging X chromosome and sisters fail to separate. Therefore, I hypothesize that sperm uniquely require kinetochore-microtubule attachment/detachment and GSP-3/4 regulate detachment. Interestingly, GSP-3/4 localize in a kinetochore-like pattern, which suggests these phosphatases may regulate kinetochore function. Sperm also have distinct outer kinetochore localization patterns (KNL-1, KNL-3, and NDC-80), compared to oocytes. For example, similar to oocyte meiosis, KNL-1 and KNL-3 localize in a cup shape on chromosomes, but NDC-80 surrounds chromosomes only in sperm. A bigger difference between oocyte and sperm meiosis is kinetochore presence at anaphase. In oocytes, immunofluorescence results depicted the kinetochore disassembles from chromosomes during anaphase I. However, the kinetochore is retained during anaphase in sperm suggesting kinetochore importance for chromosome segregation. The question still remains, do kinetochores mediate the connection between chromosomes and microtubules in sperm? Recent super-resolution images of immunostained sperm in anaphase I show there is a gap between chromosomes and microtubules where NDC-80 localizes, indicating the kinetochore functions in sperm meiosis to connect chromosomes to microtubules during anaphase separation. Further, live imaging of H2B labeled DNA and GFP labeled tubulin show that sperm meiosis exhibits pole-chromosome shortening (anaphase A) in anaphase I, which contrast to oocyte meiosis and mitosis, which rely on anaphase B mechanisms. This result taken together with the gsp-3/4 mutant phenotypes of delayed lagging X resolution and sister separation failure, support GSP-3/4 role in detaching microtubules from kinetochores. GSP-3/4-regulated detachment would allow X resolution and sisters to shift orientation towards opposite poles. Microtubule detachment from kinetochores may be unique in sperm to reconcile the combination of centrosome organized microtubules and chromosomes going through two rounds of segregation.

253C Regulation of male meiotic divisions in C. elegans. Shang-Yang Chen, Yi-Shiu Lin, Jui-Ching Wu National Taiwan University, Taipei, TW.

Different from mitotic cells, spermatocytes and oocytes divide twice after one round of chromosome duplication. Therefore, it is intriguing whether the two divisions are regulated by the same principles as in mitosis. Using time-lapse recording, we followed the kinetics of chromosome-associated division regulators during male meiotic divisions. We found that while inner kinetochore persists throughout the two divisions, outer kinetochore is released from chromosomes during first chromosome segregation event and recruited back before second division. This suggests that a functional kinetochore is disassembled and then re-assembled between first and second divisions. Because the kinetochore also plays essential roles in spindle assembly checkpoint (SAC), we examined if a functional checkpoint is also re-established after first division. Interestingly, securin, the downstream target of SAC, though is degraded during first chromosome separation, is not recruited back to chromosome for the second division. These results suggest the second male meiotic division is not controlled through timely degradation of securin, which is dependent on APC/C and proteasome. Consistent with this, temperature-sensitive APC/C mutant adult males do not show accumulation of arrested secondary spermatocytes when shifted to non-permissive temperature. To further explore how the two division events are regulated differently, we examined localization of Aurora B kinase in dividing spermatocytes. During first division, Aurora B kinase is specifically localized in between homologous but not sister chromosomes. This localization pattern is dependent on PP1 phosphatase GSP-2: Aurora B kinase spreads to sister chromosomes in the absence of GSP-2. Consistent with Aurora B kinase promotes cohesion removal, gsp-2 mutant spermatocytes exhibit premature sister chromatid separation. These results support that during first meiotic division GSP-2 protects sister cohesion from being degraded by separase, which is regulated through the SAC signals. Taken together, we hypothesize that the two male meiotic divisions are regulated through different mechanisms. The first division is regulated by canonical SAC signaling and dependent on APC/C activity. Contrarily, the second division is independent of APC-proteasome activity. Initiation of chromosome separation in second division might rely on release of sister cohesion that is protected from degradation during first division by PP1 phosphatases GSP-2.

254A ZYG-9 and TAC-1 are involved in early meiotic spindle assembly in C. elegans oocytes. Chien-Hui Chuang, Bruce Bowerman University of Oregon, Eugene, OR.

Oocytes of many animals form bipolar spindles in the absence of centrosomes, and how these acentrosomal spindles establish bipolarity remains unclear. In C. elegans oocyte meiosis I, spindle microtubules first form a cage-like structure peripheral to the chromosomes, with multiple small and peripheral spindle poles appearing as assembly progresses. These small poles later merge together and ultimately form two mature poles on the opposite sites of the aligned chromosomes (Wolff et al., 2016). Previous studies have documented defects in oocyte meiotic spindle structure in ZYG-9 or TAC-1 depleted oocytes (Matthews LR et al., 1998; Bellanger JM and Gonczy P, 2003; Yang et al., 2003), but when these assembly defects first appear is not known. Using live imaging, we are investigating the temporal requirements for ZYG-9 and TAC-1 during meiotic spindle assembly. Here we show that ZYG-9 and TAC-1 are required very early in spindle formation, with defects that then further interfere with the establishment of spindle bipolarity.
Using GFP and mCherry fusions to mark microtubules and chromosomes, respectively, in zyg-9 (-) oocytes, we have detected defects very early in meiotic spindle assembly. Rather than forming a cage-like structure with all microtubules restricted to the periphery, we instead observed a cage-like structure with some microtubules extending across the interior, projecting through the volume occupied by chromosomes. This abnormal microtubule scaffold subsequently assembled into a multi-polar spindle network, with individual bivalents sometimes surrounded by discrete small bipolar spindles. Ultimately chromosomes often segregated toward more than two poles. Similarly, using GFP fused to ASPM-1 to mark spindle poles, we found that wild-type oocytes had two stable ASPM-1 foci established by metaphase, while zyg-9 (-) oocytes had multiple ASPM-1 foci that dynamically coalesced and dispersed throughout most of meiosis I. ZYG-9 and TAC-1 form a complex that promotes microtubule assembly (Bellanger JM and Gonczy P, 2003; Le Bot N et al., 2003; Srayko M et al., 2003), and TAC-1 depleted oocytes showed similar phenotypes to the zyg-9 (-) oocytes. We are currently investigating the localization of the ZYG-9/TAC-1 complex during early meiotic spindle assembly to further advance our understanding of how these proteins contribute to the acentrosomal assembly of bipolar spindles during oocyte meiotic cell division.

References:

255B HIS-35: a novel histone H2A protein involved in fertility. Criszel Corpuz, Monet Jimenez, Rodrigo Estrada, Margaret Jow, Diana Chu Biology, San Francisco State University, San Francisco, CA.

Infertility can arise when DNA accessibility is misregulated. This regulation is tightly controlled by core histones: H2A, H2B, H3, and H4 and the replacement with histone variants throughout germ line development. In C. elegans, three H2A variants were identified: HTZ-1, HTAS-1 and HIS-35. HTZ-1 is a known regulator of developmental genes while HTAS-1 is a unique component of sperm. However, the role of HIS-35, which differs from core H2A by only one amino acid is unknown. Preliminary data revealed HIS-35 enrichment in sperm and oocytes, thus I hypothesize that HIS-35 participates in germ line development to maximize fertility. To determine HIS-35 effects on fertility, we developed a mutant strain containing a deletion of the his-35 gene. Our results reported a 35% reduction in progeny, indicative of a role for HIS-35 in fertility. Furthermore, his-35; htas-1 double mutants are sterile, which is a phenotype more severe than either individual mutant alone. These results suggest that these H2A variants may compensate for one another to maximize fertility. Since we have demonstrated that HIS-35 is an important fertility factor, we utilized Crispr/Cas9 mediated repair system to create HIS-35::GFP and assess dynamics within the germ line. Surprisingly, HIS-35::GFP is present in almost all tissues and as expected, HIS-35::GFP localizes in both male and hermaphrodite germ lines. In addition, HIS-35::GFP is retained within mature sperm, oocytes and the newly-fertilized embryo. These findings raise the possibility that HIS-35 may be transferring or maintaining epigenetic states of genes needed throughout the developmental events of gametogenesis, fertilization and embryogenesis. Defining HIS-35 incorporation within these processes provides a basis for how H2A variants regulate DNA accessibility in different tissue types.

256C atz-1 promotes meiosis to maintain germline chromosomal integrity. J.A. Dawson, C Methven-Kelley, G.M. Davis School of Applied and Biomedical Sciences, Federation University, Melbourne, Victoria, AU.

Sexually reproducing species rely on the exchange of genetic information between homologous chromosomes in a process referred to as meiosis. This can be summarised as one round of DNA replication accompanied by two rounds of chromosome segregation to produce haploid gametes from diploid cells. This process involves tight coordination of a meiotic specific cohesion complex, the synaptonemal complex and DNA damage repair mechanisms. We have investigated an uncharacterised component of sperm. However, the role of HIS-35, which differs from core H2A by only one amino acid is unknown. Preliminary data revealed HIS-35 enrichment in sperm and oocytes, thus I hypothesize that HIS-35 participates in germ line development to maximize fertility. To determine HIS-35 effects on fertility, we developed a mutant strain containing a deletion of the his-35 gene. Our results reported a 35% reduction in progeny, indicative of a role for HIS-35 in fertility. Furthermore, his-35; htas-1 double mutants are sterile, which is a phenotype more severe than either individual mutant alone. These results suggest that these H2A variants may compensate for one another to maximize fertility. Since we have demonstrated that HIS-35 is an important fertility factor, we utilized Crispr/Cas9 mediated repair system to create HIS-35::GFP and assess dynamics within the germ line. Surprisingly, HIS-35::GFP is present in almost all tissues and as expected, HIS-35::GFP localizes in both male and hermaphrodite germ lines. In addition, HIS-35::GFP is retained within mature sperm, oocytes and the newly-fertilized embryo. These findings raise the possibility that HIS-35 may be transferring or maintaining epigenetic states of genes needed throughout the developmental events of gametogenesis, fertilization and embryogenesis. Defining HIS-35 incorporation within these processes provides a basis for how H2A variants regulate DNA accessibility in different tissue types.

257A GCNA-1 is a novel P granule protein that interacts with the SUMO pathway and promotes germline immortality. G.A. Dokshin, M.A. Carmell, H. Kim, C.C. Mello 1) RNA Therapeutics Institute, University of Massachusetts Medical School, Worcester, MA; 2) Howard Hughes Medical Institute, Chevy Chase, MD.

Germ cells employ specialized pathways, such as the piRNA pathway, to preserve genetic and epigenetic information essential for fertility and germline immortality. We recently reported the discovery of a novel family of germ cell proteins called GCNA (named after the founding member, the mouse germ cell nuclear antigen). GCNAs are broadly conserved and enriched in reproductive cells (or during the sexual cycle in single cell organisms). In C. elegans GCNA-1 predominantly localizes to P granules in the gonads and in the embryo. C. elegans gcna-1 mutants have reduced broods, produce males at an increased frequency (him phenotype) and become sterile upon continued propagation at 25 °C (mrf phenotype). Evolutionary analysis indicates that GCNA-1 is related to a yeast DNA replication-associated metalloprotease Wss1 (weak suppressor of smt-3 [sumo]). We show that like Wss1, gcna-1 can suppress a hypomorphic SUMO pathway mutant. In addition, we recently uncovered several genetic interactions between gcna-1 and other known germline pluripotency factors. We are currently exploring the potential role of GCNA-1 in genome maintenance and epigenetic mechanisms in the germline. Together our
findings suggest that gcna-1 may be a member or a broader network of germ-cell specific pathways that maintain the pristine genomic and epigenetic information necessary for fertility and germline pluripotency.

258B Epigenetic contributions to homologous chromosome recognition during meiosis. C. Doronio, W. G. Kelly Biology Department, Emory University, Atlanta, GA.

During meiosis, homologous chromosomes must correctly identify one another in order for proper alignment and recombination to occur. Improper pairing between chromosomes can lead to chromosomal rearrangements, aneuploidies, and improper gamete formation that can result in increased sterility and defective embryonic development. Currently, little is known about how homologs identify each other to the exclusion of the other chromosomes. There has been evidence supporting the role of DNA Double Strand Breaks (DSB) and strand invasion in homolog recognition. In humans and other organisms, DSBs stabilize interactions between homologs and are required for synapsis. However, organisms like C. elegans and mutants lacking the ability to form DSBs still have the ability to properly align homologous chromosomes suggesting that a DNA DSB independent mechanism exists. In C. elegans specific sites located near the ends of chromosomes, Pairing Centers (PC), initiate pairing between homologs. Despite their sequence specificity and requirement for synapsis, some PCs are shared between non-homologous chromosomes, such as ZIM-3 binding to chromosomes I and IV. Synapsis between chromosomes with similar PCs does not occur suggesting an additional PC-independent mechanism occurs for proper alignment. During meiosis distinct patterns of active transcription are produced on each chromosome and are associated with specific epigenetic modifications such as the methylation of Lysine 36 on Histone H3 (H3K36me). In humans, H3K36me is recognized by the chromodomain containing protein MRG15. The Nabeshima lab reported that homolog pairing defects were observed in C. elegans lacking normal function of the MRG15 homolog, mr-1 (Domecki et al., 2011). The specific role of MRG-1 in homologous chromosome pairing and recognition is unknown. Our hypothesis is that histone modifications that result from meiotic transcription, including H3K36me, provide an “epigenetic barcode” used to distinguish chromosomes during homolog searching, and is facilitated through the recognition of H3K36me by the chromodomain of MRG-1. We are examining the role of H3K36me in homolog recognition in germlines lacking mes-4 and met-1, the histone methyltransferases responsible for H3K36me. Our recent data demonstrates that germ cells lacking H3K36me exhibit increased sterility and synaptic delay, as can occur with defective pairing. Similar observations are seen in mr-1 mutants. These results suggest that epigenetic modifications such as H3K36me may play an important role in homologous chromosome recognition during meiosis.


Sex-linked genes are pressured to evolve due to environmental changes and the importance of fertility. This competitive nature drives certain regulatory genes towards novel roles in multiple processes. Two sex-linked genes that are essential in different fertility pathways are gsp-3 and gsp-4. These 98% identical genes arose from a duplication event and encode sperm-specific phosphatases that are orthologous to the PP1 gamma phosphatase in humans. We find that the GSP-3/4 phosphatases in C. elegans function sequentially in sperm meiosis, sperm motility, and the completion of oocyte meiosis. First, tracking of fluorescently-labeled chromosomes during sperm meiosis show gsp-3/4 mutants take longer to segregate in meiosis I and fail to segregate during meiosis II. In gsp-3/4 mutants, CLS-2, a central spindle protein that stabilized microtubules, is dumped into the mid-zone during meiosis I. We hypothesize that GSP-3/4 dephosphorylates CLS-2 as a regulatory switch to release the plus end of microtubules. This allows for microtubule de-polymerization dynamics during chromosome segregation. Second, sperm are less motile in gsp-3/4 mutants. Sperm motility is driven by actin independent pseudopodial locomotion, where MSP proteins form filaments to create movement. Immunostaining and live imaging show that GSP-3/4 localize towards the disassembly end of MSP filaments. We hypothesize that GSP-3/4 play a regulatory role in disassembling MSP to complete this process of motion. Thirdly, GSP-3/4 is delivered with the paternal DNA post-fertilization. It is hypothesized that GSP-3/4 thus act as a signal for the completion of oogenesis. Co-IP with mass spectrometry, along with global phosphorylation profiling will uncover interacting partners and targets that these phosphatases regulate. Understanding the regulatory roles of GSP-3/4 in their journey throughout spermatogenesis, motility, and oogenesis will lead to a better understanding of the major players essential for fertility.

260A Proteoglycans in stem cell development. S. GOPAL, R. POCOCK Monash University, Australia.

Proteoglycans are composed of a core protein and multiple glycosaminoglycan chains. At least 14 proteoglycans are encoded in the mammalian genome whereas Caenorhabditis elegans carry four. The proteoglycans present in C. elegans include, a syndecan (sdn-1), a perlecan (unc-52) and two glypicans (lon-2, gpn-1). These glycosylated proteins are involved in several cellular functions, including cell-cell/cell-matrix attachment, cancer and neuronal development. Besides being signaling molecules, proteoglycans are involved in the activation of other signaling molecules such as Wnts (Munoz et al., 2006) or Notch (Pisconti et al. 2010). In addition to developmental defects in C. elegans, the loss of the proteoglycan sdn-1 leads to reduced brood size, suggesting a function for SDN-1 in germline development. The germline houses the only stem cell population in C. elegans and our data suggest that proteoglycans play a role in stem cell proliferation and differentiation. In C. elegans, stem cells are regulated by a network of RNA-regulatory molecules and the Notch signaling pathway components to control differentiation and the production of eggs and sperm. Earlier studies in mammals also implicated syndecan-3 along with Notch in cell differentiation, though the underlying mechanism is poorly understood. In addition, syndecan-2, -3 and -4 were shown as regulators of stem cell development. We have recently identified a role for syndecans in the control of transient calcium changes in C. elegans. This syndecan-mediated calcium regulation is achieved through the mechanically sensitive, transient receptor
potential (TRP) channels. Transient calcium is an important factor in stem cell development where calcium fluxes impact the cell cycle. We have also identified germline specific defects in syndecan mutant worms, which is controlled by syndecan expression at specific domains. We hypothesize that syndecan is involved in germline maintenance by regulation of transient calcium through TRP channels. Our study currently focuses on the role of syndecans in stem cell development and differentiation through various factors including notch signaling, calcium metabolism and extracellular mechanical forces.

261B Do defects specific to crossover recombination inhibit the DNA damage response in C. elegans? Kevin Hagy1, Stefani Giacopazzi1, Barbara Conrad2, Needhi Bhalla1 1) University of California, Santa Cruz, Santa Cruz, CA; 2) Ludwig-Maximilians-Universität of Munich.

Diversity in the animal kingdom is established through mutations, gene flow, and sexual reproduction. During gamete production, genetic material is exchanged between maternal and paternal chromosomes via a process called crossover recombination. In C. elegans, recombination between homologs is essential for proper segregation during anaphase I. The steps of recombination occur as follows: 1. SPO-11 introduces DNA double-strand breaks (1), 2. RAD-51-dependent single-end strand invasion favors repair from homologous chromosomes (4,5), and 3. crossover(CO)-specific DNA repair is promoted by MSH-4, MSH-5 and ZHP-3 (2,3). Thus, loss of CO promoting factors, such as MSH-4, MSH-5 and ZHP-3, should lead to persistent DNA damage and an increase in activation of DNA damage induced apoptosis, a process dependent on the conserved apoptosis activator EGL-1. However, Silva et al showed that loss of these CO-promoting factors fails to activate DNA damage induced apoptosis. Further, these factors appear to be required for initiation of the DNA-damage checkpoint since apoptosis decreases in msh-4, msh-5, and zhp-3 mutants after IR radiation (6). We present an alternate explanation of these results: When we mutate a conserved upstream regulatory region of egl-1, msh-5 and zhp-3 mutants now exhibit an increase in apoptosis and this increase is dependent on the DNA damage checkpoint protein HUS-1. We hypothesize that when CO recombination is specifically disrupted, DNA damage induced apoptosis is inhibited, potentially to promote repair over removal. We plan to 1) identify the regulatory factor(s) that bind EGL-1’s regulatory region to limit apoptosis when CO recombination is defective and 2) test whether this inhibition of apoptosis is mechanistically linked to characterized meiotic feedback mechanisms that are activated by defects in recombination.

262C Investigating the role of early double strand DNA break repair dynamics on repair pathway and partner choices during meiosis. A. M. Harvey, D. E. Libuda Department of Biology, University of Oregon, Eugene, OR.

Meiosis is the specialized cell division utilized by sexually reproducing organisms to produce haploid gametes, such as sperm and eggs. During meiosis, programmed double strand DNA breaks (DSBs) are introduced in the genome of developing gametes and must be repaired with high fidelity to maintain genomic integrity as well as promote proper chromosome segregation. Repair of meiotic DSBs using homologous recombination is critical, as it establishes a crossover between each homolog pair. Interhomolog crossovers establish a physical link between chromosome pairs which ensures faithful chromosome segregation during the first meiotic division. Although each meiotic DSB undergoes both a template choice (homolog vs. sister chromatid) and a repair pathway choice (crossover vs. noncrossover) that are essential for achieving specific repair outcomes, the mechanisms underlying these meiotic DSB repair decisions are not well understood. The highly conserved recombinase RAD-51 is an early stage repair protein required for all meiotic homologous recombination events regardless of repair template or repair pathway choice. Cytological appearance of RAD-51 as a focus indicates the site of a DSB at an early stage of repair, while RAD-51 focus disappearance indicates progression of DSB further down a repair pathway. To observe early DSB repair dynamics, we created a functional GFP-tagged version of RAD-51 in Caenorhabditis elegans, where all of the stages of meiotic prophase can be visualized simultaneously in a single germ line. Live imaging of GFP::RAD-51 in whole worms revealed that RAD-51 forms two distinct classes of foci at DSB sites: 1) bright, static and long-lived; and, 2) flickering, transient, and short-lived. These data revealed that despite being at the same early stage of repair, DSBs can display distinct early DSB repair dynamics. Further, this finding suggests that DSB repair template and pathway decisions may be established at an early stage of repair. To determine whether these different classes of foci are associated with distinct repair template or pathway choices, our ongoing investigations are assessing the dynamics of RAD-51 foci: 1) specific mutant backgrounds that lack specific repair outcomes; 2) defined phases of meiotic prophase I that are known to utilize specific repair templates; and, 3) precise regions of the genome where we can induce a single DSB and track its repair outcome. Overall, these studies will elucidate the relationship between early DSB repair dynamics and repair template and pathway choices.


Cyclin-dependent kinases (CDKs) and their activating cyclin subunits play central roles in driving cell cycle progression in eukaryotes. The classical view was that CDK2 is essential for cell cycle progression by controlling the G1/S transition. Studies using CDK2 knockout mice, however, have revealed an unexpected role of CDK2 during meiotic prophase, while demonstrating that CDK2 is dispensable for mitotic cell cycle progression and proliferation. Consistent with its meiotic functions, CDK2 has been shown to localize to telomeres as well as crossover sites during mammalian meiosis. However, the role of CDK2 in the establishment and maintenance of crossover sites remains unknown. We found that CDK-2, a C. elegans homolog of CDK2, is localized to the crossover sites during meiotic prophase. Using super-resolution microscopy, we show that CDK-2 colocalizes with COSA-1, a cyclin-like protein that plays a conserved role in crossover formation, raising the possibility that CDK-2 might partner with COSA-1 to function as an active kinase. To determine the role of CDK-2 during meiosis, we used the auxin-inducible degradation system to rapidly deplete CDK-2 from the C. elegans germline. CDK-2-depleted germ cells exhibit normal homolog pairing and synaptonemal complex formation. However, germ cells progressively lose COSA-1 foci from the distal end and exhibit an increase in the number of univalents in diakinesis following auxin treatment, indicating that CDK-2 is required for...
the establishment of crossovers. Additionally, worms hatched on auxin plates exhibit distinct germline developmental defects, implying a role of CDK-2 in the germline development. These results suggest that CDK-2 promotes crossover formation and may play additional roles during C. elegans germline development.

264B Identification of a novel component of the meiotic double-strand break machinery.  Albert W. Hinman, Anne Villeneuve  Departments of Genetics and Developmental Biology, Stanford University School of Medicine, Stanford, CA.

Meiotic recombination performs two key functions: 1) to promote genetic diversity by reassorting traits and 2) to establish temporary attachments between pairs of homologous chromosomes (bivalents) necessary for their future segregation. Recombination is initiated by the introduction of DNA double-strand breaks (DSBs). Some DSBs are repaired to form crossovers (COs) between homolog pairs, and the rest are repaired as noncrossover products to restore genome integrity. Although DSBs are required for CO formation, they may lead to genomic instability if they are not repaired or are repaired erroneously. Thus, meiotic cells use surveillance mechanisms to ensure that enough DSBs are created to guarantee a CO on each homolog pair while limiting excess DSBs that may endanger the genome. Without appropriate DSB formation and repair, COs fail to form and univalents (unattached homologs) are observed at late meiotic prophase, leading to chromosome missegregation during the meiotic divisions and aneuploidy in resulting progeny.

Here I have identified a new component of the meiotic recombination machinery defined by the me6ts mutation. me6ts was previously isolated in a genetic screen as a temperature-sensitive mutant with elevated chromosome missegregation and univalents at the nonpermissive temperature. Based on several lines of evidence, I found that the primary defect in me6ts is in DSB formation. Nuclear localization of DSB-2 (a chromatin-associated protein required for efficient DSB formation) is greatly reduced in early meiotic prophase nuclei of the me6ts mutant. Further, cytological foci of RAD-51 (a DNA strand exchange protein that marks the sites of processed DSBs) are greatly reduced in number. Finally, introducing DSBs through irradiation is sufficient to restore CO formation. Complementation tests and mapping experiments suggest that the me6ts mutation is not within any currently known meiotic gene. Thus, I have used genomic sequencing of a backcrossed me6ts strain and have developed a sequence-analysis pipeline to identify homozygous candidate mutations. I am currently in the process of testing candidate mutations for me6ts-phenotype causality by RNAi, CRISPR/Cas9-mediated knock-outs, and complementation assays. My future experiments aim to identify how the product of the gene defined by me6ts facilitates DSB formation through cytological examination of DSB-associated proteins and their regulators in me6ts mutants.

265C top-2 is required for proper chromosome segregation during male meiosis in C. elegans.  Aimee Jaramillo-Lambert, Andy Golden  Laboratory of Biochemistry and Genetics, NIDDK/NIH, Bethesda, MD.

During sexual reproduction, haploid gametes (i.e. eggs and sperm) are generated from diploid precursors through the specialized cell division of meiosis. Meiosis reduces ploidy by following one round of DNA replication with two rounds of chromosome segregation (MI and MI respectively). In MI sister chromatids segregate from each other similar to mitosis; but in MI, it is the homologs that segregate, which requires pairing, synapsis, and recombination. Type II DNA topoisomerases are enzymes that play a crucial role in chromosome fidelity by disentangling topological problems that arise in double stranded DNA. Topo II is a large ATP-dependent, homodimeric enzyme. Each subunit breaks one DNA strand, passes a second unbroken strand through the break, and then reseals the break. Thus, during mitotic divisions, Topo II enzymes solve topological problems that arise during replication, transcription, sister chromatid segregation, and recombination. However, the exact role Topo II plays during meiosis has not been fully elucidated. Previously, we have shown that a novel allele of Topo II, top-2(it7ts), uniquely disrupts the segregation of homologous chromosomes during the meiotic divisions of spermatogenesis but not oogenesis. TOP-2 is expressed throughout the germ lines of both spermatogenic and oogenic germ lines, localizing along the lengths of chromosomes during meiotic prophase. In top-2(it7ts) mutants, localization of TOP-2 is disrupted in both spermatogenesis and oogenesis leading us to question why only meiotic chromosome segregation in spermatogenesis is affected. A major difference between spermatogenesis and oogenesis is that chromosome morphology differs after pachynema. We hypothesize that TOP-2 plays a role in chromosome remodeling/architecture during late meiotic prophase thus facilitating homologous chromosome segregation during spermatogenesis. Preliminary evidence evaluating the role of top-2 in chromosome architecture has found that meiotic chromosome axis components, the meiotic cohesins, COH-3 and COH-4, localize normally during spermatogenesis, however, the other meiotic cohesin, REC-8, may be prematurely removed from chromosomes. We are continuing to investigate the role of top-2 in chromosome structure through the examination of additional architectural components.

266A Ceramides localize to granules within the P cell of C. elegans embryos.  Skylar D. King, Jose Robledo, Kevin Schmidt, Pamela A. Padilla  Department of Biological Sciences, University of North Texas, Denton, TX.

Disruption of the ceramide biosynthesis impacts many biological processes including stress responses (e.g. O2 deprivation, apoptotic signals in the germline, mitochondrial surveillance system), lifespan, and fecundity. Ceramides, which are composed of sphingosine and a fatty acid, have a role in membrane structure (mitochondria, ER) and are important signaling molecules. In C. elegans the hyl-1, hyl-2, and lagr-1 genes code for ceramide biosynthetic enzymes. Simultaneous disruption of hyl-1 and hyl-2 results in lethality indicating that ceramide biosynthesis is an essential process. We used an anti-ceramide antibody (mAb15B4) to examine the localization pattern of ceramides in embryos. Surprisingly, the mAb15B4 recognized centrosomes (marked by anti-gamma tubulin) and granule-like perinuclear structures within the P cell. Ceramides, which impact programmed cell death in the germline and fecundity, are thought to colocalize with a mitochondrial marker (anti cox(V)) in oocytes. However,
to our knowledge there are no reports of the localization of ceramides to granule-like structures in the P cell. Thus, we tested the hypothesis that mAb15B4 recognizes ceramides that colocalize to the P granules. We used the MEX-3::GFP and PGL-1::GFP strains and determined there is overlap between the anti-GFP and mAb15B4 localization pattern. Additionally, the hyl-2 mutant shows a loss of mAb15B4 localization to the granule-like structures in the P cell, indicating that the mAb15B4 antibody is recognizing an antigen that is dependent on ceramide biosynthesis. The mAb15B4 localization to the granules in the P cell is restored in the fat-7(wa36);hyl-2(tm2031) double mutant demonstrating that alteration of lipid biosynthesis genes do impact granules recognized by the antibody. However, the P granules, as recognized by the K76 antibody (anti PGL-1) or MPMP2 antibody, remained intact in the hyl-2 mutant. These data suggest that ceramides localize to P granules but the alteration of ceramides do not alter PGL-1 localization. Given that ceramides are not only mitochondrial and ER structural membrane components but are also important signaling molecules we are investigating whether their presence in the P cell is for signaling.

267B Identification and Characterization of Genes Essential for C. elegans Sperm Guidance.  S.B. Legg, M.A. Miller  Department of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham, Birmingham, Alabama.

Fertilization is the fusion of two gametetes to initiate the development of a new organism. In internally fertilizing animals such as C. elegans, motile sperm must navigate through the female reproductive tract to locate and fuse with oocytes. Our lab has shown that C. elegans oocytes secrete F-series prostaglandins (PGFs) that help guide sperm to the spermatheca. These PGFs are synthesized independent of the canonical cyclooxygenase cascade, which is thought to be the sole enzymatic route for mammalian prostaglandin synthesis. However, we have recently shown that cyclooxygenase null mice and worms produce similar PGF isomers, suggesting that a second PGF biosynthesis pathway exists. We have also identified these PGF isomers in human ovarian follicular fluid. The goal of my project is to identify genes critical for C. elegans sperm guidance and in particular, PGF metabolism.

In order to identify genes essential for sperm guidance in C. elegans, we have devised an in vivo screening method using RNAi and a sperm guidance assay. As the female germline is the site of PGF synthesis, I am focusing on genes expressed in the adult germline (Reinke et al., 2004). N2 hermaphrodites are administered RNAi by the feeding method. L4 stage worms are added to the RNAi plates and kept at 25°C for 36-48 hours. RNAi hermaphrodites are anesthetized and incubated with wild-type males stained with MitoTracker Red CMXRos, which stains the sperm. Mated hermaphrodites are removed from males and allowed to rest for an hour. Then, fluorescent sperm distribution within the uterus is viewed on plates using a stereomicroscope. In controls, ~90% of sperm target the two spermatheca after an hour. In mutant hermaphrodites with sperm guidance defects, sperm are more evenly distributed throughout the uterus. To date, I have screened 223 germine genes on Chromosome II. Out of these 223 RNAi clones, I have identified 38 clones that cause strong sperm guidance defects. These RNAi clones correspond to genes implicated in diverse functions, including receptor-mediated endocytosis, protein secretion, calcium ion binding, and lipid storage. I am currently working on rescoring the 38 positive clones using DIC microscopy to eliminate those clones that disrupt germline development. I am particularly interested in RNAi clones that cause sperm guidance defects but do not cause defects in gonad morphology. I will then validate a subset of the remaining clones using mutational analysis. Liquid chromatography tandem mass spectrometry methods will be used to determine if the mutations impact PGF metabolism. My goal is to identify enzymes and other factors that modulate PGF levels in the germ line.

268C C. elegans BRC-1/BRD-1 regulates meiotic recombination differently during male and female meiosis.  Q. Li, P. Checchi, K. Lawrence, J. Engebrecht  Department of Molecular and Cellular Biology, University of California, Davis, Davis, CA.

During meiosis, homologous chromosomes pair, synapse and undergo crossover recombination initiated by SPO-11-dependent DNA double strand breaks (DSBs). Homologous recombination (HR) is the preferred pathway to repair DSBs during meiotic prophase, and RAD-51 assembly and disassembly serves as a readout of ongoing repair by HR. In mammalian cells, the breast cancer 1 gene (BRCA1) is required for HR in somatic cells. In mouse spermatogenesis, BRCA1 is required for successful meiotic sex chromosome inactivation (MSCI), the failure of which leads to pachytene arrest and apoptosis of germ cells. Consequently, the role of BRCA1 in meiotic recombination is unclear. The C. elegans ortholog, brc-1, has been reported to promote HR at a subset of DSBs in meiotic cells as visualized by altered pattern of RAD-51 foci in brc-1 hermaphrodite germline nuclei (Adamo et al., 2008). We discovered that in C. elegans males, BRC-1/BRD-1 does not participate in MSCI, which allowed us to investigate the role of BRC-1/BRD-1 in meiotic recombination during spermatogenesis. In contrast to what was observed in hermaphrodites, brc-1 and brd-1 mutant males had a reduction in the number of RAD-51 foci, which returned to wild-type levels when non-homologous end joining (NHEJ) was inhibited. Inactivation of NHEJ in brc-1 or brd-1 mutant hermaphrodites, in contrast, led to a further increase in the number of RAD-51 foci, suggesting that BRC-1/BRD-1 regulates DSB repair choice differentially in the sexes. We also discovered that BRC-1/BRD-1 is important for stabilizing the RAD-51 filament when the formation of a crossover-intermediate was disrupted in oogenesis but not spermatogenesis. In pairing center protein (zim-1, zim-2, him-8), sy-1 or rad-54 mutants, RAD-51 was precociously removed and reappeared in late pachytene in brc-1 mutant hermaphrodites. In contrast, in male brc-1 germ cells RAD-51 foci persisted in the mutants. Furthermore, GFP::COSA-1, which marks designated crossover sites, was also altered differentially in hermaphrodite versus male germ cells. Removal of brc-1 decreased the average GFP::COSA-1 foci in hermaphrodites, while in brc-1 males the average number of GFP::COSA-1 increased under conditions where chromosomes can not form a crossover (e.g., zim-1 mutant). Our data suggest that there are distinctive mechanisms in which BRC-1/BRD-1 participates in homologous recombination in the two different sexes of C. elegans.
The proper segregation of chromosomes during meiosis relies on a series of molecular events leading to the formation of crossovers (COs) between homologous chromosomes. The formation of COs initiates with programmed double-strand breaks (DSBs) in the DNA. After DSB induction, breaks are resected and channeled into homologous recombination (HR) repair rather than to other, more error-prone repair pathways including non-homologous end-joining (NHEJ), alternative NHEJ (alt-NHEJ) and single-strand annealing (SSA). While previous works suggest that NHEJ is prevented by early steps of resection, little is known about the mechanisms inhibiting the other pathways.

In *C. elegans*, meiotic HR relies on the recombinase RAD-51 to invade a homologous template for repair. In wild type worms, HR-mediated repair leads to the formation of 6 pairs of CO-attached homologs at the end of prophase I. When *rad-51* is mutated, DSBs are not repaired properly and massive chromosomal fusions occur. We discovered that depleting DSB-promoting factors, such as *him-5* or *dsb-2*, abrogates chromosomal fusions in *rad-51* mutants, independently from their role in DSB formation. The diakinesis nuclei of *rad-51;him-5* and *dsb-2;rad-51* contain 12 intact univalent chromosomes indicating repaired DSBs independently from HR. This data leads us to hypothesize that the DSB machinery has a role in DNA-repair pathway choice during meiosis.

We thus investigated the role of NHEJ, alt-NHEJ and SSA during oocyte formation in a *rad-51;him-5* double-mutant context. These three pathways are compatible with the use as template of the different DNA intermediates which are formed as a result of the processing of DSB ends. First, we observed that in the absence of *rad-51* alone, NHEJ and SSA are the two major repair pathways used by the cell, while alt-NHEJ does not contribute significantly to repair. By contrast, in the *rad-51;him-5* double mutant, NHEJ and alt-NHEJ are the major repair pathways. Thus, the absence of *him-5* switches the preferred repair pathways from SSA to alt-NHEJ. We propose that *him-5* promotes HR by favoring extended resection of the DSB ends, leading to the formation of a template for RAD-51-dependent HR or for SSA in a *rad-51* mutant.

This work provides insights on the regulation of meiotic DSB-repair pathway choice. In addition to its role in DSB formation, *him-5* appears to promote HR by preventing alt-NHEJ-mediated repair, revealing that components of the DSB-promoting machinery inhibit alternative repair to promote HR.

**270B** Zinc regulates germ line viability through dynamic zinc fluxes in *Caenorhabditis elegans*. A.D. Mendoza¹, S Vogt², T.K. Woodruff², S.M. Wignall¹, T.V. O’Halloran¹ ¹) Northwestern University; 2) Argonne National Laboratory.

Infertility affects 6.7 million women between the ages of 15 and 44 in the US. Our approach to solving the infertility problem is multidisciplinary; drawing from cell biology, chemistry, and physics to define how the transition metal zinc, maintains healthy female eggs. Zinc is critical in regulating mammalian egg development through large-scale, dynamic zinc fluxes. To expand on these findings, we utilize the model system *Caenorhabditis elegans* to further uncover zinc’s role in egg viability maintenance.

Many regulatory factors involved in the reproductive tract formation in *C.elegans* are conserved in higher organisms. Therefore, *C.elegans* are an excellent model to study zinc. We aim to uncover if zinc fluxes are conserved and characterize how zinc affects egg viability. We hypothesize that zinc fluxes are conserved and that proper zinc levels maintain oocyte viability. We sequenced zinc utilizing the chemical TPEN in the worm’s growth environment. Results demonstrated that zinc sufficient (ZI) hermaphrodites yielded smaller broods and contained fewer unfertilized oocytes compared to controls, whether self-mated or not. By time-lapse confocal imaging, we discovered that zygotes exposed to low TPEN concentrations (10 uM) displayed improper polar body extrusion, hyperploidy, lagging chromosomes and improper cytoplasmic cleavage. Combined, these results suggest that zinc insufficiency impacts unfertilized eggs within the reproductive tract and not sperm from hermaphrodites or males. Moreover, zygotes remain sensitive after fertilization to changes in cytoplasmic zinc concentrations.

Because we determined that zinc insufficiency impacts the maturing egg, we next performed X-Ray Fluorescence Microscopy (XFM) at Argonne National Laboratory to determine total zinc content during oocyte maturation. Total zinc is all of the zinc in the cell, bound and unbound. We discovered that total zinc content sharply increases after fertilization up to the zygote stage, and decreases after meiosis. This indicates that large-scale zinc fluxes in *C.elegans* occur similarly as mammals. We similarly examined labile zinc dynamics during oocyte maturation. Labile zinc is zinc that is accessible to our probes. Utilizing the zinc sensor ZincBy-1, we determined that after fertilization, labile zinc steadily increases in the cytoplasm up to Anaphase II. Then, labile zinc content declines steadily and remains at low levels through 2-cell. Labile zinc from the cytoplasm relocates to the extra embryonic matrix (EEM), and is visible beginning at pronuclear migration.

Future experiments will allow us to understand the mechanism behind zinc fluctuations within maturing oocytes. Work with *C.elegans* can provide insight for the conserved mechanisms for zinc action during fertilization and proper egg formation. We will then be closer to understanding human infertility issues we face today.

**271C** Understanding sister chromosome movements between sperm meiotic division. N.R. Munoz, vanessa cota, Juiching Wu, Diana Chu  san francisco state university, san francisco.

Mechanisms that segregate chromosomes during male meiosis are poorly characterized. Male meiosis differs from oocyte meiosis in *C. elegans* in several ways. In sperm, microtubules are organized by centrosomes while oocytes are acenstromal. Microtubules in sperm do not disassemble as centrosomes split and migrate during anaphase I as they do in oocyte meiosis. Microtubules attach to complexes of kinetochores, that include KNL-1, which attaches to chromosomes. KNL-1 localizes around...
chromosomes in a cup shape and is retained throughout meiosis in sperm, unlike in oocytes where KNL-1 is removed during anaphase. These differences suggest that sperm and oocytes have distinct mechanisms for shifting the orientation of sister chromatids from segregating from the same pole to opposite poles during anaphase 1. It is unknown how microtubule-kinetochore attachment and detachment are regulated to allow sister chromatids to bi-orient to opposite poles when both kinetochores and microtubules do not disassemble. Our goal is to understand how microtubule-kinetochore attachment and detachment are regulated to bi-orient sisters during male meiosis. The retention of KNL-1 during meiosis allows us to study its localization to characterize this chromosomal biorientation. What are KNL-1’s movements when sister chromatids change division plane axes during bi-orientation of spermatogenesis? I hypothesize microtubules use the kinetochore to detach and re-attach to chromosomes to ensure bi-orientation and division plane rotation.

Live imaging sperm expressing knl-1::GFP; tbg-1::GFP allows visualization of kinetochore localization and chromosome movement, in reference to the centrosomes, during meiosis. We have been able to characterize the movement of chromosomes as sisters bi-orient, and have observed distinct stages of the process relative to centrosome migration during anaphase 1. It appears that KNL-1 changes localization from a cup shape, absent on one side, to surrounding the chromosomes as sisters change orientation. This suggests as the DNA changes division plane axes, microtubules organized by the centrosomes are actively using kinetochores to change attachments to chromosomes so that they can be bi-oriented correctly. Our data is allowing us insights into mechanisms that allow microtubules to act on chromosomes to segregate them correctly.

272A Investigating the interaction of DLC-1 and GLD-1 in regulation of gene expression. E. Osterli, M. Ellenbecker, E. Voronina Division of Biological Sciences, University of Montana, Missoula, MT.

The regulation of RNA-binding protein (RBP) activity in cells is a central question in gene expression studies. One important RBP in C. elegans germline is GLD-1; this particular protein prompts the germ cell switch from mitotic proliferation to differentiation thus acting as a tumor suppressor. Previous research in our lab identified a small protein, DLC-1, as a cofactor of an RBP FBF-2. We hypothesized that DLC-1 may also promote the RNA regulatory function of GLD-1 because DLC-1 interacts with many cellular proteins. We tested our hypothesis on several strains of C. elegans that express fluorescent reporter proteins under control of GLD-1. These reporters are normally repressed by GLD-1 during differentiation. When we knock down DLC-1, we can see how it affects the reporter's repression. Fluorescence microscopy revealed that dlc-1 knockdown resulted in derepression of a subset of the reporters during differentiation. Our hypothesis that DLC-1 promotes GLD-1 functions is supported by the derepression of the reporters when DLC-1 is knocked down because loss of DLC-1 results in loss of GLD-1 function. Identifying this relationship between DLC-1 and GLD-1 is important for understanding stem cell balance. When the stem cell balance between mitotic proliferation and differentiation is altered, it can result in serious consequences such as unchecked cell proliferation, tumor formations, infertility, and cancer. Understanding the mechanism(s) by which DLC-1 promotes GLD-1 would be extremely relevant to advancing our understanding of certain human diseases like cancer.

273B Male-specific proteins SMZ-1 and SMZ-2 are required for normal sperm generation in C. elegans. Hsiao-Fang Peng1, Ya-Chu Hsu2, Chang-Shi Chen2, Jui-ching Wu1 1) National Taiwan University, Taipei, TW; 2) National Cheng Kung University, Tainan, TW.

In sexually reproducing animals, sperm are generated in highly-specialized male germline. Therefore, male-specific regulators should be participated in functional sperm production. SMZ-1 and SMZ-2, two highly identical PDZ domain proteins, were found abundant in spermatogenic chromatin through proteomic study in C. elegans. While PDZ domain-containing proteins are known for acting as scaffolds for cell signal transduction, little is known about the roles of PDZ domain-containing proteins in sperm generation.

To investigate the roles of SMZ-1 and SMZ-2 in fertility, we first obtained two smz-2 deletion mutants using CRISPR/Cas9 techniques, and generated smz-1;smz-2 double mutant worms. We compared total brood size and hatch rate of single as well as double mutant hermaphrodites and found that worms bearing either smz-1 or smz-2 single deletion can normally generate offspring. On the other hand, smz-1; smz-2 hermaphrodites do not have any countable progeny. The results show that SMZ-1 and SMZ-2 are functionally redundant in fertility.

Examination of hermaphrodite uterus revealed smz-1; smz-2 uterus filled with unfertilized oocytes. This suggests failure of fertilization, which can result from defects in sperm or oocyte function. To distinguish where the defects take place, we examined SMZ-1 and SMZ-2 protein expression in worms generating either sperm or oocytes. Immunoblotting analyses showed that SMZ-1 and SMZ-2 are highly expressed in sperm but not in oocytes or somatic cells. In agreement to this, the infertility in smz-1; smz-2 double mutant hermaphrodites can be rescued by mating with wild type males. These results indicate SMZ-1 and SMZ-2 only function in sperm.

In order to define the origin defects of smz-1; smz-2 in sperm generation, we examined fixed smz-1; smz-2 male gonad. We found smz-1; smz-2 male germline failed to generate mature sperm comparing with wild type, suggesting there are meiotic division defects. Time-lapse recording of smz-1; smz-2 spermatocytes showed that chromosomes failed to progress into metaphase I. Taken together, SMZ-1/2 are male specific fertility factors required for initiation of meiotic divisions in spermatocytes. We are currently investigating the cellular factors that are regulated by SMZ-1/2.

274C The genetic interaction of wrn-1 with mre-11 or him-6 in embryonic development. B.T.T. Pham, Byungchan Ahn Biological Science, University of Ulsan, Nam-gu, Ulsan, KR.

Werner’s syndrome (WS) is an autosomal recessive disorder in humans characterized by the premature development of age-associated pathologies. WRN, the gene defective in WS, plays a key role in protecting the genome. The cell derived from WS patients show genomic instabilities such as sister chromatid exchange and telomere shortening. Caenorhabditis elegans (C. elegans) with a WRN-1 ortholog also exhibits a shorter life span. Our results showed wrn-1 interacts with mre-11 or him-6 and...
affects during embryonic and larval development. When inhibiting the expression of *mre-11* or *him-6* in *wrn-1* (gk99) by RNA interference (RNAi) resulted in a significant increase of embryonic lethality and the retarded larval development compared with control worms of N2 (RNAi) worms. In addition, *wrn-1* (gk99):*him-6* (RNAi) or *wrn-1* (gk99):*mre-11* (RNAi) worms displayed abnormal premeiotic nuclei and multiple chromosomal abnormalities in oocyte nuclei at the diakinesis stage. Furthermore, observation of embryo cell division showed the slower development. These results suggest that MRE-11 and HIM-6 may interact with WRN-1, which is involved in embryonic development.


Chromatin remodelers such as the nucleosome remodeling and deacetylase (NuRD) complex are required for numerous cellular and genetic processes throughout development. Recently, we discovered a novel role for the NuRD complex during meiosis, wherein loss of any of its members results in the accumulation of apoptotic nuclei. Additionally, we found that disruption of a subset of NuRD components including LET-418, a homolog of Mi2, results in accumulation of persisting recombination intermediates. These findings implicate a putative role for NuRD in the repair of meiotic double-stranded breaks (DSBs). Here, we investigate the genetic, cellular, and molecular role of the NuRD chromatin remodeling complex and its relationship with meiotic checkpoint signaling in the *C. elegans* germ line. The conserved checkpoint kinase CHK-1 is responsible for coordination of the DNA damage response (DDR) that leads to either cell cycle arrest, DNA repair, or apoptosis. It was therefore hypothesized that *let-418* loss-of-function mutants would have elevated levels of phosphorylated CHK-1 (pCHK-1) protein as a result of defective DSB repair. Indeed, we found that *let-418* hypomorphs possessed significantly increased levels of pCHK-1 protein compared to wild-type controls. Additionally, pCHK-1 foci were detected in *let-418* germ lines, which were not observed in wild-type or *let-418; chk-1(RNAi)* controls. In the absence of LET-418, we also observed elevated mRNA expression of the pro-apoptotic gene *egl-1*, a target of the *C. elegans* pi5 homolog CEP-1. These data are corroborated by genetic evidence demonstrating that both *chk-1(RNAi)* and *cep-1* mutants suppress apoptosis in *let-418* as well as other NuRD mutants. Currently, we are investigating the relationship between the NuRD complex, checkpoints, and their role in the execution of error-free DSB repair during meiosis. Intriguingly, our preliminary findings suggest that LET-418 blocks activation of the error-prone repair pathways such as non-homologous end joining. Taken together, these results support a model wherein the NuRD complex ensures error-free gamete formation by attenuating meiotic checkpoints and promoting usage of error-free repair pathways during meiosis.

276B  **Histone dynamics during oocyte meiosis in *C. elegans.* S. Rosu, O. Cohen-Fix LMCB, NIH-NIDDK, Bethesda, MD.

During meiosis, which is essential for gamete formation, homologous chromosomes must pair, undergo recombination, and segregate from each other. Many questions remain about chromatin dynamics during meiosis in oocyte development. To gain new insights into this process, I am using live imaging in *C. elegans* to explore chromatin dynamics during various stages of meiotic prophase. I have constructed strains containing histone H2B fused to Dendra2, a fluorescent protein that irreversibly photoconverts from green to red fluorescence. This allows me to follow the fate of a selected pool of histone H2B in live animals over time. Unexpectedly, I have found widespread histone H2B exchange in the late stages (diplotene to diakinesis) of oocyte meiotic prophase. This is surprising, as at this stage no replication is occurring and transcription is thought to be shut down. Thus the mechanism and role of histone exchange at this stage are unknown. I have confirmed this observation in three different ways: 1) by the complete recovery of mCherry::H2B fluorescence after photobleaching in a strain carrying a mCherry::H2B transgene (indicating new unbleached histones are loaded onto chromatin), 2) by the disappearance of converted Dendra2::H2B in a strain overexpressing the Dendra2::H2B transgene in the germline (indicating that converted histones are unloaded, and new unconverted histones are loaded onto chromatin), and finally, 3) by the redistribution of converted H2B::Dendra2 from a sub-region of chromatin to the entire chromatin in a strain where the Dendra2 tag was inserted at an endogenous H2B locus by CRISPR. This suggests that H2B histones are widely unloaded from chromatin at the diplotene to diakinesis stage in meiosis, and either reloaded when the nucleoplasmic pool is limited (as in the endogenously tagged strain), or degraded when the nucleoplasmic pool is large (as in the over-expressing transgenic strain). This widespread exchange is specific to the diplotene/diakinesis stage of meiosis, as I have not observed this phenomenon in terminally differentiated somatic cells or in germ cells at earlier stages of meiotic prophase in the timeframe assayed. In future work, I plan to screen candidate histone chaperones and chromatin remodelers to find factors involved in this meiotic histone exchange. I will also examine the consequence of blocking histone exchange, which may lead to defects in meiosis or embryo development.

277C  **SMRC-1 maintains genome integrity in *C. elegans.* L. Russell1, B. Yang2, X. Xu2, M. Sullenberger2, E. Maine2, J. Yanowitz1 1) Magee-Womens Research Institute, UPMC, Pittsburgh, PA; 2) Department of Biology, Syracuse University, Syracuse, NY.

In order to maintain genomic integrity, DNA must be replicated without damaging the genetic code. This can be especially difficult under stressful conditions where additional barriers to replication may arise. Additionally, challenging repetitive sequences, such as those in telomeric DNA, provide endogenous barriers to replication. In humans, SMARCAL1 helps maintain genomic integrity by restarting stalled replication forks. This is a critical function, and defects in SMARCAL1 have been implicated in the rare disease Schimke Immuno-osseous Dysplasia. We used CRISPR to generate knockout alleles of the *C. elegans* SMARCAL1 ortholog, smrc-1. We found that these mutants show a decreased brood size and laid fewer eggs than wild type and also show increased germline apoptosis, phenotypes that are dependent on the activity of the DNA damage checkpoint. To further investigate the role of SMRC-1 in DNA damage repair,
we subjected smrc-1 mutants to hydroxyurea (HU) and camptothecin (CPT), which can inhibit replication fork progression, as well as ionizing radiation (IR) which causes double stranded breaks. We found that smrc-1 mutants are sensitive to hydroxyurea (HU) and tagged SMRC-1 proteins relocalize from the nucleoplasm to chromatin-associated foci following HU exposure. Consistent with a role for SMRC-1 at stalled replication forks, smrc-1 mutants enhance the genomic instability phenotype of dog-1, showing increased mutations in a region that forms G-quadruplexes. smrc-1 mutants also exhibit a mortal germline (MRT) phenotype that we attribute to a buildup of mutations over generations. Interestingly, absence of SMRC-1 function enhances the MRT phenotype in the telomere mutant, tt-1, implicating SMRC-1 as a major determinant of telomere integrity. SMRC-1 protein co-immunoprecipitates with the H3K9 methyltransferase, MET-2 and double mutants show enhanced sterility. These studies introduce the exciting possibility that replication fork repair is coupled with the protection of repetitive sequences by methylation.


278A Exploring the role of CLASP<sup>CLS-2</sup> in oocyte meiotic spindle assembly and bipolarity. A. Schlientz, B. Bowerman Institute of Molecular Biology, University of Oregon, Eugene, OR.

Oocyte meiotic spindles assemble in the absence centrosomes, which provide microtubule nucleation and establish bipolarity during mitotic and sperm meiotic spindle assembly. This phenomenon begs the question, what factors are required for establishing/maintaining oocyte meiotic spindle bipolarity given the absence of centrosomes? To enhance our understanding of spindle assembly in the absence of centrosomes, we are investigating the role of the C. elegans CLASP family member CLS-2 in oocyte meiotic spindle assembly. Studies of CLASP family members indicate that these proteins promote microtubule stability by preventing microtubule depolymerization, but how they contribute to oocyte meiotic spindle assembly remains poorly understood.

To better examine the role of CLASP<sup>CLS-2</sup> in oocyte meiotic spindle assembly, we have used CRISPR/Cas9 to generate putative CLS-2 null-alleles. These new alleles show both the previously reported mitotic defects via Nomarski DIC microscopy, as well as occasional instances of multiple maternal pronuclei – a phenotype associated with defects in oocyte meiotic spindle assembly that has not been previously reported after cls-2(RNAi). Using spinning disk confocal microscopy, we have also observed the previously reported oocyte meiotic chromosome segregation defects. Loss of CLS-2 in oocytes results in the formation of highly aberrant spindles lacking distinct bipolarity, which fail to segregate chromosomal masses during both meiosis I and II. Intriguingly, the membrane ingression normally observed during polar body extrusion appears to be absent in our CLS-2 mutants, and polar body extrusion may be completely absent. This is in contrast to several other oocyte spindle assembly defective mutants, in which membrane ingression occurs even if polar body extrusion is abnormal to varying degrees. These preliminary results indicate that CLS-2 may play important roles in both spindle assembly and polar body extrusion. We are characterizing both processes in more detail to better define the requirements for CLS-2 for during oocyte meiotic spindle assembly.


279B Chromosome partitioning in meiosis mutants. K. A. Rivera Gomez1, G. Fabig2, E. Clarke1, A. M. Villeneuve3, T. Muller Reichert2, M. Schvarzeinstein1 1) Biology, City University of New York, CUNY. Brooklyn College, Brooklyn, NY, USA; 2) Dresden University of Technology. Dresden, Germany; 3) Stanford University. Stanford, CA, USA.

Crossovers (COs) are integral in enabling the ordered partitioning of the duplicated genome during the two meiotic divisions. Studies have focused on understanding the meiotic prophase I steps leading to formation of COs connecting each maternal homologous chromosome to its paternal counterpart. However it is understood how different perturbations in prophase I translate into specific chromosome segregation defects. Observations of the product of meiotic mutant divisions by Severson et al. suggest that mutants lacking COs segregate homologous chromosomes in the first division in one of two different patterns, depending on whether mutant chromosomes have the cohesin component REC-8 required for sister chromatid co-orientation in meiosis I. Our live imaging analysis of these meiotic mutants revealed the pattern of chromosomes segregation in the two meiotic divisions.

Meiotic mutants that lack the meiosis cohesin component REC-8 segregate sister chromatids away from each other in the first division, as they would in the second division in wild type meiosis. These mutants fail to partition chromatids in the second division resulting in the formation of diploid gametes. These findings are the basis of a scheme designed to derive viable and stable tetraploids from any C. elegans strain in order to query the roles of genome size on cell division, development, and evolution.

Meiotic mutants with REC-8 include him-3, syp-1, syp-2 and spo-11 that are defective at different steps in CO formation. These mutants keep sister chromatids together as the wild type yet fail to partition in the first meiotic division. Their centrosomes, however, continue to progress through the cell cycle giving rise to spermatocytes with transient tetrapolar spindles. The chromatids eventually segregate to each of the four spindle poles yielding aneuploid sperm. Interestingly, analysis of altered karyotype and special meiotic mutant spermatocytes suggests that a single bi-oriented homologous chromosome pair is sufficient to suppress the formation of the transient tetrapolar spindles. We will report on the mechanism by which a single homologous chromosome pair might prevent formation of tetrapolar spindles.

Together these studies will lead to a better understanding of fundamental mechanisms promoting accurate chromosome inheritance in normal and pathological meiosis.
Meiotic progression requires the initiation of programmed, genome-wide double-stranded breaks (DSBs). Unrepaired DSBs result in defective gametes, which manifest as spontaneous abortions, infertility and chromosomal disorders. Accordingly, faithful repair of DSBs is accomplished by a series of conserved mechanisms which must take place within the constraints of a specialized chromatin architecture. Here, we demonstrate a role for the nucleosome remodeling and deacetylase (NuRD) complex in DSB repair, wherein the conserved Mi2 homologs Chromodomain helicase DNA binding protein (CHD-3) and its paralog LET-418 promote normal meiotic progression by attenuating meiotic checkpoints and ensuring faithful repair of DSBs through homologous recombination (HR). In loss-of-function let-418 mutants, animals possess persisting recombination intermediates and elevated germline apoptosis. We found that apoptosis in these mutants is checkpoint-dependent and coincident with the presence of phosphorylated CHK-1 protein as well as elevated expression of the pro-apoptotic gene egl-1. Additionally, we uncovered a previously unreported role for CHD-3, whose loss alone results in the activation of meiotic checkpoints, a significantly reduced brood size, and mild defects in the repair of DSBs, all of which are exacerbated when combined with let-418 hypomorphs. Interestingly, we also discovered that when non-homologous end joining (NHEJ) is compromised, let-418 germ lines are highly disorganized and possess numerous persisting recombination intermediates. We are currently generating several strains which will enable us to further understand the molecular nature of these defects. Taken together, these data support a model wherein LET-418/CHD-3 maintain genomic stability through reinforcement of a chromatin landscape suitable for HR-driven repair mechanisms.

Cytoplasmic and mitochondrial superoxide inhibit germ-line cell proliferation and reduce functional sperm in Caenorhabditis elegans. G. Wang¹, A. Krauchunas², S. Dharia³, A. Singson², M. Driscoll¹ ¹) Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ; ²) Department of Genetics, Rutgers University, Piscataway, NJ.

Reproduction is essential to species survival. We systematically investigated the essential role of superoxide dismutases (SODs) for the efficient reproduction in C. elegans. SODs are a group of enzymes that specifically scavenge the hyperactive free radical superoxide. Five C. elegans genes encode SOD: sod-1 (constitutive) and sod-5 (inducible) encode the cytoplasmic SODs; sod-2 (constitutive) and sod-3 (inducible) encode the mitochondrial SODs; and sod-4 encodes the extracellular SOD. Deletion of the mitochondrial sod gene in either fly or mouse causes neonatal lethality, but C. elegans has a regular lifespan even in the absence of all five sod genes [1]. However, in the quintuple sod knockout, the progeny number drops to ~40 per-generation, as compared to ~300 in wild type. Progeny numbers are ~120 in the sod-1,5 (cytoplasmic null) mutant or ~90 in sod-2,3 (mitochondrial null) mutants, but there is no change in reproduction in the sod-4 (extracellular) mutant. The number of C. elegans self progeny is dictated by the number of functional sperm, so we first examined the sperm number with whole worm DAPI-staining in sod mutants. It turns out that the sod quintuple mutant can generate ~150 sperm per-generation, which is much larger than its progeny number. After further investigation, we find that the in vitro sperm activation rate of the sod quintuple mutant is about 30% lower than wild type. Thus, the fertility defect in the sod quintuple mutant may be partially due to the failure of sperm activation. We also measured the rate of oocyte production (progeny/day) by counting the progeny of sod mutants mated with wild type males. We found the mated sod-1,2,3,4,5 produced ~80 oocytes on the peak day, compared to ~170 of the wild type. Consistent with defective or slowed oocyte production, DAPI staining of young adult worms showed that the sod quintuple mutant had a low number of nuclei in the gonad. We also found there were no unfertilized eggs and no significant increase of dead embryos from the mated sod-1,2,3,4,5 hermaphrodites. Thus, the quality of oocytes appears close to normal in the absence of all superoxide management, but the quantity of oocytes is largely decreased in sod quintuple mutant. We found the absence of either mitochondrial SOD (sod-2,3) or cytosolic SOD (sod-1,5) also largely reduced the maximal daily oocyte production to ~80. Thus, either a cytosolic or mitochondrial superoxide increase can impact germ-line cells, with a slightly less severe outcome compared to the impact of missing all SODs.

References:

A compartmentalized signaling network mediates crossover assurance and crossover interference in meiosis. Liangyu Zhang¹,², Simone Köhler¹,², Regina Bohn¹,², Abby Dernburg¹,²,³,⁴ ¹) Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA 94720-3220, USA; ²) Howard Hughes Medical Institute, 4000 Jones Bridge Road, Chevy Chase, MD 20815, USA; ³) Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA; ⁴) California Institute for Quantitative Biosciences, Berkeley, CA 94720, USA.

Meiotic crossover control is a central mystery of sexual reproduction. During meiosis, crossover between homologous chromosomes is tightly regulated to ensure proper segregation. Each chromosome pair typically undergoes at least one crossover (crossover assurance) but these exchanges are also strictly limited in number and widely spaced along chromosomes (crossover interference). This has implied the existence of chromosome-wide signals that regulate crossovers, but their molecular basis remains mysterious. Early this year, we reported that the synaptonemal complex, a polymer that assembles paired chromosomes. We recently characterized a family of four RING finger E3 ligases in C. elegans. We found that these proteins act as two heterodimers within the synaptonemal complex, creating a self-extinguishing circuit that controls crossover designation and maturation. These proteins also act at the top of a hierarchical chromosome remodeling process that enables
crossovers to direct stepwise segregation. Work in the other organisms indicates that analogous mechanisms mediate crossover control in most eukaryotes.

Cell Biology - Imaging Methods

283C Hydrogel embedding for long-term, high-resolution imaging of \textit{C. elegans}. K.R. Burnett, N. Otoo, L. Aurilio, D.R. Albrecht Department of Biomedical Engineering, Worcester Polytechnic Institute, 100 Institute Road, Worcester, MA, 01609-2280, USA.

Imaging \textit{C. elegans} at high spatial resolution requires effective immobilization of the organism. Current immobilization methods include the use of agarose pads with paralyzing reagents or nanobeads, or microfluidic devices. Here we present a simple and inexpensive alternative method for rapid worm immobilization that provides a safe, long-term environment for all developmental stages within a photocrosslinked PEG-based hydrogel. We have characterized crosslinking dynamics and immobilization quality for various polymer and photoinitiator concentrations, illumination sources, geometric volumes, buffer compositions, and crosslinking parameters. We determined chemical, thermal, and osmotic conditions for optimal high resolution single cell imaging. Animals were immobilized in seconds without the need for paralytics, and young adults were found to be recoverable even after 24 hours. Further, encapsulated worms are not compressed between impermeable glass coverslips, enabling exposure to liquid stimuli and imaging using light-sheet microscopy. We demonstrate this capability by recording 3D volumetric images of GCaMP responses in sensory neurons expressing the Chrimson red-light-activated ion channel using the diSPIM light-sheet microscope. Recordings were made in the same animal for up to 12 hours. We envision this technique useful for long-term (several hour) imaging of neural activity across state changes and for other applications requiring \textit{C. elegans} immobilization such as microinjection or laser ablation.

284A \textit{In-vivo} high resolution topographic imaging and 3D force-mapping of \textit{C. elegans} using atomic force microscopy. C.L. Essmann¹, V.M. Pawar¹, M.A. Srinivasan¹² ¹) University College London, UCL, London, GB; 2) Massachusetts Institute of Technology, MIT, Cambridge, MA.

Atomic force microscopy (AFM) is a powerful method for topographic imaging of biological surfaces with nanometer resolution yet unexploited for the use in \textit{C. elegans}. AFM offers significant advantages over scanning electron microscopy (SEM) including the acquisition of quantitative 3D-images and biomechanical information. More importantly, for \textit{in-vivo} biological imaging, AFM does not require sample dehydration or labeling. We use this technique to show for the first time high-resolution topographical images and biomechanical maps of the cuticle of wild type \textit{C. elegans} (N2) under physiological conditions. We visualize annuli and furrows, analyze their 3D spatial distribution on young adult worms and compare these results with several collagen (dpy) mutants. Moreover, we are able to describe differences in cuticle stiffness between wild type worms and collagen mutants. Using AFM we were able to visualize previously unseen cuticle structures of the so-called “non-annuli” mutants \textit{dpy-7} and \textit{dpy-10} demonstrating the potential and merit of this imaging technique new to \textit{C. elegans} research. Combining AFM with fluorescence imaging allows us to overlay topographic elements with fluorescently labeled structures within deeper cuticle layers such as mechanoreceptor clusters or collagenos. \textit{C. elegans} is used extensively for drug screening and to study pathogen adherence in innate immunity; both applications highly depend on the integrity of the nematode’s cuticle. Mutations affecting both drug permeability (e.g. \textit{srf-3}, \textit{bus-8}, \textit{bus-17}) and pathogen adherence (e.g \textit{srf-2}, \textit{srf-3}, \textit{srf-5}, \textit{bus-8}, \textit{bus-17}) have been proposed to relate to changes in the cuticle structure, but never visually examined in high resolution. Here AFM could serve as a new and valid tool to analyze these differences. 1. Essmann, C. L. \textit{et al.} Nanomedicine Nanotechnology, \textit{Biol. Med.} 13, 183–189 (2017).

285B Long-Term High-Resolution Imaging of Developing \textit{C. elegans} Larvae with Microfluidics. W. Keil¹², L. M. Kutscher¹, E. D. Siggia², Shai Shaham¹ ¹) Center for Physics and Biology, The Rockefeller University, New York City, NY; 2) Laboratory of Developmental Genetics, The Rockefeller University, New York City, NY.

Decades of research on \textit{C. elegans} development have shed light on a large variety of developmental phenomena; from cell-fate decisions and transdifferentiation to cell migration and cell death. However, long-term high-resolution \textit{in-vivo} imaging of these processes has been accessible only in embryos, because larvae must move and feed to develop, and existing immobilization techniques perturb development and compromise animal viability. To overcome this problem, we have developed a microfluidic setup to simultaneously follow development of ten \textit{C. elegans} larvae at high spatiotemporal resolution from hatching to adulthood (~3 days). Animals grown in microchambers are periodically immobilized by compression to allow high-quality imaging of even weak fluorescence signals. We show that this technology can be used to follow cell-fate decisions, cell death programs, and transdifferentiation events with Nomarski and multichannel fluorescence microscopy. We also obtain cell-cycle timing statistics during the formation of the \textit{C. elegans} vulva in a large number of animals, exposing the limits of fidelity and robustness of \textit{C. elegans} development. Finally, we develop algorithms for automated image registration to generate time-lapse movies. For the first time, these movies enable us to visualize and quantify highly-diverse processes such as neural arborization or cell divisions during gonadogenesis in a feeding, moving, and growing animal. Our technique opens the door to quantitative analysis of time-dependent phenomena governing cellular behavior during \textit{C. elegans} larval development.

286C Imaging mass spectrometry for \textit{C. elegans}. Yoshide \textit{Kimura}¹, Tomomi Kaneko¹, Satoka Aoyagi² ¹) Department of Liberal Arts and Human Development, Kanagawa University of Human Services, Yokosuka, Kanagawa, JP; 2) Department of Material and Life Science, Skeikei University, Musashino, Tokyo, JP.

Imaging mass spectrometry (IMS) is a two-dimensional mass spectrometry to visualize the spatial distribution of biomolecules,
which does not need either separation or purification of target molecules. The free-living soil nematode *Caenorhabditis elegans* is a common model organism, extensively used in life science research. Though, various investigations have been performed for metabolomic profiling of worms, the information of a single worm has been lost by the conventional mass spectrometry (MS) techniques. Thus, the development of a label-free, non-targeted MS technique for molecular mapping in *C. elegans* has been required. We have previously performed MALDI imaging of *C. elegans*. However, the resolution was not enough to analyze cellular or subcellular level of biomolecule distribution. Thus, we next tried the application of TOF-SIMS (Time-of-Flight Secondary Mass Spectrometry) system for *C. elegans*, which enables us to obtain subcellular distribution of metabolites. By comparison of several sample preparation methods, the frozen sections of *C. elegans* fixed by paraformaldehyde (PFA) were suitable for TOF-SIMS analysis. By sputtering of Ar gas cluster ion beam (Ar-GCIB), the sensitivity to fatty acids (e.g. stearic acid (SA), oleic acid (OA), and eicosapentaenoic acid (EPA)) was significantly enhanced, and high-resolution images of biomolecules were acquired. Further modification to prepare *C. elegans* samples for TOF-SIMS imaging is in progress. This is promising to obtain the cellular and subcellular distributions of the various biomolecules easily and efficiently.

### 287A Development of a cell cycle state sensor for *C. elegans*

**A.Q. Kohrman**

Biochemistry and Cell Biology, Stony Brook University; 2) Graduate Program in Genetics, Stony Brook University.

During organismal development, differential regulation of the cell cycle is critical to many cell biological processes, including differentiation and morphogenesis. While the complete cell division lineage of *C. elegans* is known, how the control of cell cycle is linked to fate specification and morphogenesis remains poorly understood, due to our inability to directly visualize cell cycle state. In order to visualize cell cycle state live, we have further developed a CDK2 biosensor for use in *C. elegans*. Our biosensor uses the dynamic nuclear/cytoplasmic localization of a portion of Human DNA Helicase B (DBH) linked to GFP to assess cell cycle state. The dynamic localization is the result of phosphorylation of the biosensor by CDKs. We have modified this sensor to allow for algorithmic assessment of cell cycle state. Similar to reported results from cell culture, we are using this biosensor to quantify lineage specific differences between cycling cells, quiescence and differentiation, provide new biological insights into the control and timing of the cell cycle in specification of uterine cells and the morphogenesis of the *C. elegans* vulva.

### 288B Time and stress constraints of immobilization techniques

**J. Manjarrez**, R. Mailler

Computer Science, University of Tulsa, Tulsa, OK.

It is a daunting task to perform any surgical or physical procedures on a moving worm. Over the years, worm researchers have devised numerous methods to immobilize *C. elegans* for optimal imaging, laser ablation, DNA injections and electrophysiology procedures. These immobilization methods fall into three broad categories: the use of chemical compounds, environmental stimuli, and physical restriction. Many researchers are unfamiliar with the ways that treatment protocols may be altered to prevent experimental difficulties and these techniques are not always fully reversible or free from after-effects of their use. In addition, problems arise when commonly used immobilizing agents are used without considering potential stress-related after-effects attributed to their use in downstream experimentation. But fortunately, most techniques maintain a great deal of flexibility and with the proper constraints at least one technique will provide suitable restraint along with a minimal amount of residual stress.

The focus of this study was quick immobilization for mounting and recovery for future experimentation. The chemical immobilizing agents 1P2P, levamisole and NaN₃ were combined with the physical agent polystyrene beads and cold shock to immobilize worms under determined sub-lethal conditions. The determined exposure and recovery times for each technique were recorded leading to an optimal condition for each method. The optimized immobilization conditions were further studied to determine the stresses attributed to their use. Transcriptional and fusion stress reporters were utilized and fluorescence microscopy was used to assess GFP induction or subcellular localization after treatment. Stress reporters included in this study were *skn-1, daf-16, hsp-4, hif-1, hsp-16, 2, and tmem-135*.

We have presented a combination of concentrations (or durations) for each immobilization technique that provides a range of immobilization, exposure and recovery times for use in experimental optimization. The commonly used 10mM NaN₃ was shown to be ineffective in a humidified environment. Whereas, the data from the stress reporters indicates that many of the commonly used immobilizing agents show no deleterious effects under controlled conditions while others show significant stress-related effects. In the case of *hsp-4*, there was no increase in GFP induction for any condition, however; *daf-16* had a significant after-effect when 1P2P was used at 0.5%. However, utilizing the range of agents tested above it is possible to adapt the experimental protocol to minimize the stress through a determined set of parameters. This is only possible when time and stress constraints have been assessed. Therefore, immobilization techniques come down to carefully controlled conditions and the characteristics related to the techniques whether it be optics, stability or recovery for various experimental uses.

### 289C New Approaches for Bioinorganic Imaging

**G. McColl**

Florey Institute of Neuroscience, Parkville, Victoria, AU.

Bioinorganic chemistry is essential for understanding cell signaling, early development and neuronal function, as well as disease pathogenesis and toxicology. Metal ion cofactors are required for numerous fundamental enzymatic and cellular processes. Synchrotron-based X-ray fluorescence microscopy approaches can provide accurate quantification and spatial resolution of endogenous elements in *Caenorhabditis elegans*. We have been applying different approaches to image essential elements in *C. elegans* at sub-cellular resolution. Our goal is to use combinations of chemical imaging technologies to determine total metal concentration, chemical state and ultimately, the protein to which an element is associated. These approaches must preserve the endogenous state of the examined system, to ensure valid interpretation of concentrations.
and gradients. However, X-ray irradiation induces modifications that accumulate with incident dose. Consequently, the utility of X-ray approaches is intrinsically limited by the radiation damage it causes and the degree to which this damage alters the target features of interest. We have identified the dose threshold, under which the integrity of the specimen and its elemental distribution is preserved following three chemical-free specimen preparations: lyophilisation, cryofixation and live-hydrated. We have determined dose limits for each preparation pertaining to the micron-scale spatial distribution of specific elemental analytes.

In addition, we have developed an X-ray absorption near edge structure spectroscopy method using fluorescence detection for visualizing in vivo coordination environments of metals. This approach can be used to spatially depict metal-protein associations in a native, hydrated state.

In combination, the use of distinct yet complementary chemical imaging techniques provides unique insight into essential and trace elements at the subcellular level.

290A The Relative significance of different fluorophores to Molecular Genetics research. Andrew Papp, Pegah Savehshemshaki Tritech Research, Los Angeles, CA.

Background

Various fluorophores are used to label molecules of interest to determine the presence, location, and timing of gene expression, and gene-gene interactions, as well as neuronal activity. This labeling can be accomplished through antibody binding, the creation of transgenic organisms with fluorescent fusion proteins or reporter genes, or ion-responsive fluorescent proteins. To find out the relative prevalence of various fluorophores in molecular genetics research is important in that it can help researchers choose fluorescent markers most useful to others and to buy equipment most likely to be useful; the data can also be useful to equipment designers so as to focus efforts on lowering the cost of detecting the most common fluorophores. To this end, an online survey ranking the relative use of various fluorophores was posted to the C. elegans and Drosophila bionet groups in November 2016.

Discussion

The data show that green fluorescence is used most often. This could be due to its ease of detection with equipment already present in many labs to detect FITC antibodies, generally high signal levels, and the fact that Green Fluorescent Protein was the first gene with a fluorescent protein product generally available to the scientific community after the publication of "Green fluorescent protein as a marker for gene expression" by Chalfie et al., 1994 in Science. 1994 Feb 11;263(5148):802-5.

Reddish fluorophores can be divided into two groups based on their excitation and emission spectra. One group is excited by greenish light and fluoresces orangish-red light (such as dsRed and TRITC) while the second group is excited by yellowish light and fluoresces deep red (such as mCherry and Texas Red). Our results show that, while not as commonly used as green fluorescence, deep red fluorescence is used more often than orange-red fluorescence. This may be because, in double fluorescence labeling experiments, it is easier to create two distinct fluorescence channels using the redder fluorescence. Unlike the dsRed's orange-red fluorescence, mCherry's deep red is not excited significantly by the blue light used to excite green fluorescence.

Our existing data will be presented in graphical form, and a live continuation of the survey will be conducted at our poster at the C. elegans Meeting Poster Session, with final results being published in the Worm Breeder's Gazette. We will also compile and publish a list of favorite fluorescence marker strains.

291B Automated Wormscan: a low cost, high throughput, scanner-based system for scoring worm mortality. T. Puckering1,2, J. Thompson1, S. Sathyamurthy1, S. Sukumar2, T. Shapira1, P. Ebert1,2 1) School of Biological Sciences, University of Queensland, St Lucia, QLD, 4072, Australia; 2) Plant Biosecurity Cooperative Research Centre, Canberra, ACT, 2617, Australia.

There has been a recent surge of interest in computer-aided rapid data acquisition to increase the potential throughput and reduce the labour costs of large scale Caenorhabditis elegans studies. We present Automated WormScan, a low-cost, high-throughput automated system using commercial photo scanners, which is extremely easy to implement and use, capable of scoring tens of thousands of organisms per hour with minimal operator input, and is scalable. The method does not rely on software training for image recognition, but uses the generation of difference images from sequential scans to identify moving objects. This approach results in robust identification of worms with little computational demand. We demonstrate the utility of the system by conducting toxicity, growth and fecundity assays, which demonstrate the consistency of our automated system, the quality of the data relative to manual scoring methods and congruity with previously published results.

Cell Biology - Intracellular Trafficking and Organelles

292C The transition zone may function as a lipid gate to establish a ciliary signaling compartment. M. Barbelanne, C Li, M. Leroux Simon Fraser University, Burnaby, British Columbia, CA.

Cilia are cellular appendages present on the surface of most cells that perform essential roles in sensory physiology and development, by modulating different signal transduction pathways. Their dysfunction is increasingly recognized as a cause of ciliopathies, a group of genetic disorders affecting many parts of the body. Cilia consist of a microtubules-based axoneme surrounded by a ciliary membrane continuous with that of the cell body. We are studying a specialized subcompartment of the cilium, called the transition zone (TZ). The role of the TZ is to create a ‘barrier’ that controls the selective entry and exit of ciliary
molecular mechanism. suggesting increased skin permeability. These data are being confirmed by testing other neurotransmitter agonists. We are

rab-6.2 across phyla. We assayed rupture in pure water as a measure of cuticle integrity and show that mutant worms missing the

rab-6.2 skin and bones involves hypothesize that there might a defect in the skins of Bejjani Biology, Davidson college, Davidson, NC.

Buechner 1) Department of Natural and Applied Sciences, University of Dubuque, Dubuque, IA; 2) Department of Molecular Biosciences, University of Kansas, Lawrence, KS.

endosome marker, RME-1, suggesting EXC-1's cellular role to be occurring between these two steps. To determine if EXC-1

found to occur prior to the development of a cyst. Along with the buildup of early endosomes there was a loss of the recycling

endosomes around the cyst, as indicated by an EEA-1 fluorescent subcellular marker. This buildup of early endosomes was

focused on the cellular mechanism of EXC-1 in the excretory canal. In a loss-of-function mutation, there is a buildup of early

excretory canal and there is cyst-like structures located along the projections of the amphid sheath cells. Previous studies have

of the amphid sheath and excretory canal.

gene, a homolog of mammalian Immunity-Related GTPases (IRG), has been shown to play a role in the structural maintenance

and provides an ideal model for studying small, single-celled tubes. The amphid sheath encloses the cilia of 12 amphid neurons

is a fundamental process in most organisms. In our lab, we focus on two epithelial, tubular structures that are necessary for

both the excretory canal and amphid sheath. In

rab-6.2 (ok2254) rupture easily when handled. This led us to

hypothesize that there might a defect in the skins of rab-6.2 deletion animals. Significantly, a human genetic disease that affects

skin and bones involves rab-6.2 interacting proteins. This suggests that the role for rab-6.2 in skin integrity may be conserved

across phyla. We assayed rupture in pure water as a measure of cuticle integrity and show that mutant worms missing the rab-

6.2 gene and outcrossed three times rupture at a significantly greater frequency in water than wild-type worms. These data

suggest that rab-6.2 is necessary for proper skin integrity. Preliminary studies show that a PCR product containing 6 Kb of the

rab-6.2 locus rescues the water rupture phenotype. Further, we show that rab-6.2 animals are hypersensitive to levamisole

suggesting increased skin permeability. These data are being confirmed by testing other neurotransmitter agonists. We are

currently asking whether rab-6.2 functions cell autonomously in the hypodermis or in the seam cells and working to describe the

molecular mechanism.

295C Immunity-Related GTPase (IRG) homolog, exc-1, in necessary for the structure of small biological tubes. Kelly Grussendorf1, Kathryn Silberstein1, Taline Holman1, Tsveta Valcheva1, Madeline Miller1, Christopher Trezza2, Matthew Buechner2 1) Department of Natural and Applied Sciences, University of Dubuque, Dubuque, IA; 2) Department of Molecular Biosciences, University of Kansas, Lawrence, KS.

Biological tubes are ubiquitous structures that carry out vital roles. The formation and maintenance of these tubule structures is a fundamental process in most organisms. In our lab, we focus on two epithelial, tubular structures that are necessary for function, the excretory canal and the amphid sheath. The excretory canal is essential for proper osmoregulation in C. elegans and provides an ideal model for studying small, single-celled tubes. The amphid sheath encloses the cilia of 12 amphid neurons and serves as a great structure to study the development of glial cells, where the tubule structure is also prevalent. The exc-1 gene, a homolog of mammalian Immunity-Related GTPases (IRG), has been shown to play a role in the structural maintenance of both the excretory canal and amphid sheath. In exc-1 loss-of-function mutant there is a development of fluid-filled cysts in the excretory canal and there is cyst-like structures located along the projections of the amphid sheath cells. Previous studies have focused on the cellular mechanism of EXC-1 in the excretory canal. In a loss-of-function mutation, there is a buildup of early endosomes around the cyst, as indicated by an EEA-1 fluorescent subcellular marker. This buildup of early endosomes was found to occur prior to the development of a cyst. Along with the buildup of early endosomes there was a loss of the recycling endosome marker, RME-1, suggesting EXC-1’s cellular role to be occurring between these two steps. To determine if EXC-1 may play a role in vesicular trafficking in the amphid sheath, as well, we have continued studies to construct molecular markers for these cells. We are also working to identify other genes that may play a role in the structural development and maintenance of the amphid sheath and excretory canal.
The recruitment and activation of a Rab GTPase in lysosome-related organelle biogenesis.  

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The mechanism by which Rab GTPases are targeted to and activated at specific organelles remains a poorly understood process. It is known that Rab specific guanine nucleotide exchange factors (GEFs) play an important role in this process. Here we present studies that investigate a novel mechanism of Rab GEF regulation, whereby subunit exchange modifies its Rab specificity. In addition, there are examples of GEF independent Rab targeting, suggesting that other factors have this activity. In our work, we identify an ABC transporter that acts in Rab organelle recruitment.

C. elegans gut granules are lysosome-related organelles (LROs) found only within intestinal cells whose biogenesis requires the activity of conserved complexes that act in LRO formation in mammals. It is known that the CCZ-1/SAND-1 complex acts as a GEF to activate RAB-7 and promote early endosome to late endosome maturation and RAB-5 to RAB-7 conversion. Our studies suggest that CCZ-1, but not SAND-1, functions with GLO-3 as a GEF for the Rabs related GLO-1 which functions in LRO biogenesis. We show that ccz-1(-) and glo-3(-), but not sand-1(-), mutants exhibit defects in gut granule biogenesis and the cytoplasmic accumulation of GLO-1. ccz-1(-) and sand-1(-) mutants display defects in conventional endosome biogenesis that are not exhibited by glo-1(-) and glo-3(-) mutants. A fast guanine nucleotide exchange GLO-1 mutant restores gut granules, which contain GLO-1, in ccz-1(-) and glo-3(-) mutants, but not other mutants defective in gut granule formation. Moreover, while ccz-1(-) displays protein trafficking defects distinct from glo-1(-) and glo-3(-), glo-1(-); sand-1(-) and glo-3(-); sand-1(-) double mutants resemble ccz-1(-). These results are consistent with CCZ-1 acting with SAND-1 as a GEF for RAB-7 in endosome maturation and with GLO-3 as a GEF for GLO-1 in gut granule biogenesis.

We screened our collection of gut granule biogenesis mutants for cytoplasmic accumulation of GLO-1 and identified a phenotype similar to ccz-1(-) and glo-3(-) in wht-2(-) mutants. WHT-2 is an ABCG family membrane transporter, which we find is localized to the gut granule membrane. wht-2(-) mutants contain partially formed gut granules, that lack GLO-1. Interestingly, WHT-2 functions in the polarized distribution of gut granules, but not other organelles, in embryonic intestinal cells. We find that the fast nucleotide exchange GLO-1 mutant does not restore the gut granule association of GLO-1 or gut granule formation in wht-2(-) mutants. However, WHT-2 is required for the ability of GLO-1 fast nucleotide exchange mutants to bypass the requirement of GLO-3 but not CCZ-1 in gut granule biogenesis. Together, these data point to a GEF independent role of WHT-2 in GLO-1 organelle recruitment and activation.

Autophagosome biogenesis at the synapse.  
Sarah Hill¹, Daniel Colón-Ramos¹²  ¹) Program in Cellular Neuroscience, Neurodegeneration and Repair, Departments of Cell Biology and Neuroscience, Yale University School of Medicine, New Haven, CT; 2) Instituto de Neurobiología, Recinto de Ciencias Médicas, Universidad de Puerto Rico, San Juan, Puerto Rico.

Autophagy removes bulk cytoplasm or organelles from cells by forming double-membrane autophagosomes that fuse to lysosomes for degradation. Regulation of autophagosome formation and trafficking is especially important in neurons, which have long polarized processes distant from the cell body. We and others have observed that autophagosomes form at presynaptic sites and then undergo retrograde transport towards the cell body, which contains the lysosomes (Stavoe and Hill et al., 2016). However, the function of autophagy and regulation of autophagosome transport at the synapse is not completely understood. We used neurons from the C. elegans thermotaxis circuit as a model to study autophagy at the synapse. This system allows us to visualize autophagosome biogenesis in vivo, control temperature-dependent synaptic firing, and simultaneously monitor potential autophagic regulators and targets at single-cell resolution. Using this system, we found that loss of synaptic activity results in decreased autophagosomes at the synapse and that JIP3/UNC-16, an adaptor protein known to regulate early endosome and lysosome transport, also plays an important role in regulating autophagosome transport in C. elegans neurons.

Glycolytic proteins reversibly form punctate structures with liquid-like characteristics in response to hypoxia.  
S. Jang¹, D. R. Albrecht², D. A. Colón-Ramos¹ ²) Department of Cell Biology, Yale University, New Haven, CT; 2) Department of Biomedical Engineering, Worcester Polytechnic Institute, Worcester, MA; 3) Instituto de Neurobiología, Universidad de Puerto Rico, San Juan, Puerto Rico.

We previously observed that glycolytic proteins, which are metabolic enzymes found in the cytosol, assemble to form punctate structures near the synapse in response to energy stress. However, how cells organize such metabolic structure is not understood. Combining microfluidics with imaging, we characterized the assembly of glycolytic proteins in C. elegans neurons and observed that metabolic enzymes of glycolysis reversibly condense out and dissolve back into the cytoplasm in response to energy stress induced by hypoxia. We also identified that this glycolytic compartment exhibits liquid-like characteristics in that: 1) glycolytic proteins form punctate or spherical structures; 2) glycolytic proteins can internally rearrange within its punctate structure; 3) those structures can fuse with one another. Our study suggests that the glycolytic compartment might undergo liquid-liquid phase separation in their assembly to facilitate a metabolic reaction in response to environmental changes in the cell.

Role of metazoan dynein in transition zone formation, ciliary maintenance, and processive intraflagellar transport.  
Victor L. Jensen, Swetha Mohan, Tiffany A. Timbers, Jerry Cai, Michel R. Leroux  Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, CA.

Cilia are ubiquitous microtubule-based organelles found at surface of metazoan cells. Non-motile (sensory/primary) cilia act to receive signals from the environment, and motile cilia either move cells or the surrounding fluid. Cilia are built and maintained using a cargo-trafficking intraflagellar transport (IFT) machinery powered by kinesin (anterograde) and dynein (retrograde)
molecular motors. How IFT-dynein contributes mechanistically to ciliary processes in metazoans remains poorly studied, in part because null mutants exhibit a severe terminal phenotype with IFT protein accumulations in the bulbous tips of highly truncated cilia. We carried out a screen for retrograde IFT transport defects in C. elegans, and identified the first temperature-sensitive IFT mutant in a metazoan. Using this strain, we show that the IFT-dynein (CHE-3) heavy chain is essential for cilium maintenance and correct formation of the transition zone (TZ) ciliary gate. Cilia resorb upon shift to restrictive temperature (inactive IFT-dynein), and are restored upon return to permissive temperature (active IFT-dynein). Importantly, this resorption and regrowth is observed for primary cilia in terminally differentiated, sensory neurons. We next investigated how IFT-dynein enables processive IFT, in contrast to the bidirectional tug-of-war mechanism of transport used by kinesin and dynein elsewhere in the cell. We find that the IFT-dynein heavy, intermediate and two light chains are trafficked by the IFT-subcomplex B, unlike the light intermediate chain, which is transported independent of the canonical IFT complex. This differential transport provides an effective mechanism by which the retrograde transport machinery is maintained in an inactive state during anterograde transport. Together, our findings reveal that IFT-dynein is required for ciliary maintenance and TZ formation, and regulates the processivity of anterograde IFT by spatial separation and thus inactivation of its components.

300B The role of mitochondria in alcohol-induced muscle damage. Kelly Oh, Timothy Cheung, Hongyun Kim Cell Biol & Anatomy, Rosalind Franklin Univ, North Chicago, IL.

Chronic alcohol consumption causes alcoholic muscle diseases, such as cardiomyopathy or myopathy. Along with the unique role of mitochondria in apoptotic cell death, the alcohol-induced impairment in mitochondrial function at least partially explains muscle weakness and the atrophy-like symptoms in alcoholic muscle disease. Recent studies in mammalian muscles have demonstrated that alcohol influences mitochondrial fusion and fission dynamics, which in turn modulates mitochondrial bioenergetics, autophagy and other signaling pathways. However, it is also recognized that chronic alcohol intake causes an array of adverse changes in cellular events and signaling pathways, including reduced mTOR (mechanistic target of rapamycin) signaling, cellular stress responses and disrupted fatty acid metabolism. We found that the C. elegans muscle exhibited changes in mitochondrial tubular networks upon ethanol exposure. In addition, we observed that chronic alcohol exposure reduced muscle size compared to controls, implicating the involvement of the mTOR signaling pathway that controls cell size and mitochondria biogenesis. Finally, we found that ethanol exposure induces genes that help to cope with stress response. We are in the process of defining the relationship between alcohol-induced mitochondrial dynamics and other cellular signaling pathways. Given the conservation of genes that mediate these events, genetic dissection of the causal relationship in C. elegans will help to understand ethanol-induced muscle diseases.

301C A genetic analysis of the dense core vesicle biology in C. elegans neurons. P. Laurent1,2, Q. Ch’ng3, C.C. Chen2, M Jospin1, M De Bono** 1) ULB institute for neuroscience, Université Libre de Bruxelles, Brussels, BE; 2) MRC Laboratory of Molecular Biology, Cambridge, United Kingdom, ; 3) MRC Centre for Developmental Neurobiology, King’s College London, London, United Kingdom; 4) Institut NeuroMyoGène, Université Lyon 2, Villeurbanne, France.

Although both neurotransmitters and neuropeptides are released in response to calcium entry, they are not packed into the same organelles. The Dense Core Vesicles (DCVs) typically mediate the secretion of neuropeptides. Analyses in mammalian (neuro)endocrine cells link DCVs to multiple pathophysiologies. However, the cell biology of the DCV is poorly understood, particularly in neurons: e.g. how their biogenesis, trafficking and secretion is coupled to neuronal activity.

We generated a new model to study the DCV biology in the well-characterised neuron PQR whose activity can easily be manipulated by the external [O2]1. Fluorescently tagged pro-neuropeptides have proven reliable reporters of DCV in other models. INS-1-VENUS expressed in PQR accumulated in its cell body and in puncta along the length of the PQR axon. Two other tagged-neuropeptides behaved similarly to INS-1 and all colocalised largely with IDA-1, a specific membrane marker for DCV.

Using this assay, we reevaluate the role of multiple genes proposed to mediate neuropeptide secretion in C. elegans and other models. Specific phenotypic signatures based on DCV distribution in the neuron and neuropeptide secretion are revealed for mutations known to alter DCV maturation, anterograde and retrograde trafficking and exocytosis. We performed a candidate gene screen for new mutants potentially affecting DCV biology, and classified each mutant in those phenotypic categories. We explored the new genes with biogenesis mutants-like phenotypic signatures. Together with previously published work (2,3,4,5) 3 new genes identified during this screen suggest new models for DCV biogenesis in neurons.


Polar bodies containing maternal DNA are extruded to form a haploid oocyte, but their fate is poorly understood. We study C. elegans polar bodies to understand how cells deal with released vesicles containing potentially harmful cargos, such as extra chromosomes.

Using time-lapse imaging of fluorescent reporter strains and a panel of mutants, we found that the second polar body is
internalized in a stereotyped manner at the 2- or 4-cell stage. Prior to internalization, actin and the phagocytic receptor CED-1/MEGF11 are enriched around the polar body, consistent with the formation of a phagocytic cup. CED-1, CED-2/Crkl, and other CED-10/Rac-dependent engulfment pathway proteins are required for polar body internalization, indicating that the polar body is internalized via receptor-mediated phagocytosis similar to cell corpse engulfment.

Receptor-mediated phagocytosis demonstrates that there is signaling between the polar body and embryonic cells. This raises the possibility that the polar body has a function after extrusion. We examined the pattern of inheritance of the polar body and did not observe a link between polar body inheritance and cell fate. Thus, the polar body is unlikely to be a cell fate determinant. After polar body phagocytosis, the double membrane phagosome is decorated with RAB-5, RAB-7, and the autophagy-associated Atg8/LC3 protein. Polar body degradation is delayed when Atg8/LC3 lipidation is disrupted by atg-7 knockdown, but not when the macroautophagy-specific PI3K subunit EPG-8 is deleted, suggesting that the polar body is degraded via LC3-associated phagocytosis. Before degradation, the polar body phagosome fragments, a rare example of double membrane budding. Thus, our observations of polar body trafficking establish a new model system for studying the mechanisms of receptor-mediated phagocytosis, double membrane budding, and degradation via LC3-associated phagocytosis.

303B Using the early C. elegans embryo to investigate nuclear size control. M. Mauro, S. Bahmanyar Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT.

The nucleus is comprised of an inner and outer nuclear envelope and acts as a functional permeability barrier that separates cytoplasmic and nuclear components. Underneath the inner nuclear envelope resides the nuclear lamina, which is comprised of inner nuclear envelope proteins and lamin, the main structural component of the nuclear envelope. Nuclear size varies dramatically during organismal development and in different tissue types. While it is known that nuclear size correlates with cell size rather than other factors such as ploidy, the exact molecular mechanisms that govern nuclear size in vivo remain unclear. Prior work investigating nuclear size control has focused primarily on in vitro models, making it difficult to tease apart the role of factors such as the cell cycle. Through this work, it was revealed that nuclear import, specifically of lamin, regulates nuclear expansion and size. Here, we utilize the expansion of the paternal pronucleus directly following fertilization of the C. elegans embryo to investigate the regulation of nuclear expansion in vivo. To examine the dynamics of nuclear expansion, we developed a sensitive semi-automated time-lapse fluorescence based imaging assay that tracks the diameter of the paternal pronucleus over time. Using this assay, we show that the rate of nuclear expansion is independent of cell cycle length, and is in part regulated by lamin. However, cytoplasmic GFP:a-tubulin is not excluded from the nucleoplasm during the majority of nuclear expansion suggesting that the NE permeability barrier is not intact during this time. To monitor the assembly of a functional permeability barrier during sperm pronuclear expansion, we imaged embryos expressing fluorescent markers for the NE and nuclear pores in situ. We found that the sperm NE undergoes breakdown and reassembly upon fertilization and between oocyte meiosis I and II. While a NE rim is present when nuclear expansion initiates it only becomes functional after the majority of nuclear expansion has completed. Thus, nuclear import may not be the primary mechanism of sperm pronuclear expansion. Future work will determine factors that control sperm pronuclear expansion rates in vivo.

304C Characterization of Lysosomal Dynamics and Function during C. elegans Larval Development. R. Miao, Xin Li, Xiaochen Wang 1,2 1) National Institute of Biological Sciences, Beijing, CN; 2) Institute of Biophysics, Chinese Academy of Sciences, Beijing, CN.

C. elegans develops through four consecutive larval stages (L1-L4) separated by molts. During each molt, worms synthesize and secrete a new exoskeleton called cuticle underneath the existing one, followed by their separation (apolysis) and the shedding of the old exoskeleton (ecdysis). Lethargus, an inactive sleep-like state characterized by a gradual decrease in general activity and feeding, occurs at the end of each larval stage coinciding with separation of the old exoskeleton from the hypodermis.1, 2 We found that lysosomes, which are labeled by either the lysosomal enzyme NUC-1 or the lysosomal membrane protein LAAT-1, appear as small puncta or thin tubules during larval development. Interestingly, worms at the lethargus stage contain extensive tubular lysosomes in the hypodermis, whereas globular lysosomes are predominant when animals exit lethargus to enter the ec dysis stage. Moreover, EGF overexpression, which induces behavioral quiescence, leads to abundant tubular lysosome formation, whereas physical disturbance of the locomotive quiescence partially disrupts tubular lysosomes formed during lethargus. This indicates a close correlation between tubular lysosome formation and lethargus quiescence. We found that morphology of other intracellular organelles including ER, Golgi, mitochondria and endosomes remain unaltered during lethargus, suggesting that lysosomes are specifically altered at this stage. In addition to morphological changes, we found that lysosome biogenesis and dynamics appear to be up-regulated during lethargus. We are currently investigating how lysosome dynamics and functions are regulated at this specific developmental stage and how they may contribute to the molting process.


305A Exploring the effects of the V-ATPase inhibitor luteolin on development, germline apoptosis, and FB-MO acidification in C. elegans. J. Millard, K. Dexter, M. Henderson 1) Lincoln Memorial University, Harrogate, TN; 2) DeBusk College of Osteopathic Medicine-Lincoln Memorial University, Harrogate, TN.

The Vacuolar-type Adenosine Triphosphatase (V-ATPase) is a large multi-subunit enzyme complex found within intracellular and plasma membranes of animals, fungi, and plants. Contemporary research efforts have elucidated the role of V-ATPases in neoplastic maladies. Increased V-ATPase expression has been shown to support neoplastic metabolism and migration. The extensive involvement of V-ATPases in pathological conditions qualifies it as a suitable target for pharmaceutical intervention.
Luteolin is a flavone found in a variety of plants, and is frequently consumed as a dietary supplement. Luteolin has been demonstrated to inhibit V-ATPase activity in mammalian cell culture. During spermatogenesis in *C. elegans*, the fibrous body-membranous organelles (FB-MOs) act as secretory vesicles responsible for the delivery of proteins to the surface of the sperm prior to fertilization. V-ATPases function to acidify the FB-MOs, contributing to their maturation to spermatozoa. Successful spermatogenesis is contingent on the fidelity of the V-ATPase in this acidification process. This change in pH can be detected by the use of LysoSensor Blue (Molecular Probes) staining. Disruption or inhibition of the V-ATPase complex is reliably reported by the LysoSensor staining assay. This allows for screening of potential V-ATPase inhibiting compounds such as luteolin. The toxicity of luteolin to *C. elegans* was demonstrated by growth and development assays. Examination of the effects of luteolin on germline apoptosis were performed using the *ced-1: gfp* reporter strain and acridine orange staining. Successful inhibition of FB-MO acidification supports the role of the V-ATPase in spermiogenesis as well as the potential therapeutic role of luteolin. These methods also provide an efficient medium for investigation of other V-ATPase inhibitory compounds.

**306B  Bypass Suppressors of *mrp-5*: Identifying Novel Components of Heme Homeostasis.** Shyrie Mitchell1, Iqbal Hamza1, Michael Krause2, Harold Smith2 1) Biological Sciences, University of Maryland College Park, College Park, MD; 2) The National Institute of Diabetes and Digestive and Kidney Diseases, NIDDK, Bethesda, MD.

Heme is a ubiquitously expressed biomolecule conserved in all eukaryotic systems. This cofactor is vital due to its essentiality in hemoproteins such as cytochromes, hemoglobin, and myoglobin. While it can be intuitively understood that heme is necessary for life because of the role hemoproteins play in the production of ATP, free heme is cytotoxic. Thus, cells must have the capability to transport, traffic, and chaperone heme. *C. elegans* are an excellent animal model to elucidate heme trafficking pathways because this roundworm is unable to synthesize its own heme but instead acquires it from the environment. By exploiting this heme auxotrophy, we built the first molecular framework for heme trafficking in eukaryotes. *C. elegans* acquires dietary heme via HRG-1-related transmembrane heme permeases into the worm intestine while heme is exported from the intestine to extraintestinal tissues by ABCC5/MRP-5. Loss of MRP-5 results in an embryonic-lethal phenotype because heme is trapped within the intestines but extraintestinal cells are heme deprived, a phenotype that can be suppressed by large amounts of heme supplementation. To uncover additional components of heme transport by MRP-5, a forward genetic screen was performed on *mrp-5 (ok2067)* deletion mutants to identify bypass suppressors of *mrp-5*-dependent lethality. Screening of 160,000 haploid genomes yielded thirty-two *mrp-5(ok2067)* suppressors and deep-sequencing variant analyses revealed that three of the suppressors were subunits of adapter protein complex 3 proteins involved in vesicular cargo sorting. We now propose to characterize the remaining suppressor mutants and identify their genetic lesions to construct a detailed cell biological pathway for organismal heme export.

**307C  Screening the Million Mutation collection for defects in gut granule biogenesis.** C. Morris, J. Delahaye, M. Dewey, L. Voss, G. Hermann Lewis & Clark College, Portland, OR.

*C. elegans* intestinal cells are characterized by the presence of gut granules, lysosome-related organelles (LROs) that contain autofluorescent and birefringent material. Gut granule biogenesis requires the activity of evolutionarily conserved genes that function in directing and transporting proteins to LROs. There are likely many undiscovered genes necessary for the generation of LROs. To identify these genes, we have screened 1336 Million Mutation Project (MMP) strains and found 137 strains with altered gut granule formation, size, and/or positioning.

We identified 78 MMP strains with altered gut granule biogenesis. Seven strains have nonsense mutations likely to disrupt the function of 5 genes known to function in gut granule formation. 23 strains have missense mutations in 10 genes that act in gut granule formation. The 48 remaining strains lack mutations in known gut granule biogenesis genes. In 21 of these strains, we have characterized multiple alleles and used rescue experiments to identify mutations in *glo-9, glo-10, lyst-1*, and *wht-2* as causing the defects in gut granule formation. These four genes represent new factors promoting gut granule biogenesis. We are continuing experiments to identify the genes causing altered gut granule formation in the 27 remaining strains.

90 of the MMP strains have increased or decreased gut granule size. Four of these strains have nonsense mutations likely to disrupt function of 2 genes known to play a role in gut granule morphology. 13 strains have missense mutations in 9 genes known to play a role in both gut granule morphology and biogenesis. In 11 strains we have used multiple alleles to identify mutations in *glo-9* and *glo-10* as causing alterations in gut granule morphology. We are continuing our analyses of the remaining 62 strains.

We identified two strains with altered gut granule positioning and have used rescue experiments to show that they result from mutations in *wht-2* and *wht-7*.

Here we present our analyses of *glo-9*, which encodes a regulatory protein that likely impacts Arf GTPase activity. *glo-9* mutants display enlarged gut granules that mislocalize the gut granule proteins LMP-1 and CDF-2::GFP. Introduction of N- or C-terminally GFP tagged forms of *GLO-9* rescue these phenotypes. Introduction of GLO-9 containing a point mutation predicted to disrupt function of 5 genes known to function in gut granule formation. 23 strains have missense mutations in 10 genes that act in gut granule formation. The 48 remaining strains lack mutations in known gut granule biogenesis genes. In 21 of these strains, we have characterized multiple alleles and used rescue experiments to identify mutations in *glo-9, glo-10, lyst-1*, and *wht-2* as causing the defects in gut granule formation. These four genes represent new factors promoting gut granule biogenesis. We are continuing experiments to identify the genes causing altered gut granule formation in the 27 remaining strains.

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The cytosol of the intestinal cells of *C. elegans* was apparently occupied by superficially indistinguishable granular organelles (intestinal granules), which consist of at least three types of organelles: acidified lysosome-related gut granules, lipid droplets, and HEBE (HAF-4/HAF-9-enriched body evanescent with age) granules. Although the former two types of organelles were
studied well, HEBE granules have been largely overlooked. HAF-4 and HAF-9 are homologous to mammalian lysosomal peptide transporter ABCB9 and localize to the membrane of HEBE granules. Population of HEBE granules changes dynamically by aging and food conditions. Although characters we found suggest roles of HEBE granules in nutritional resource allocation in response to physiologic changes during the young adult stage, regulatory mechanisms of the organelle dynamics have yet to be determined.

To reveal the genetic mechanisms underlying the biogenesis of HEBE granules, we performed RNAi screening according to the following scheme: synchronized L1 larvae were fed with RNAi feeding library clones (Open Biosystems) on 12-well plates. Worms at adult day 1, when HEBE granules are most prominent in the control condition, were directly observed using digital microscopes to efficiently evaluate the presence of intestinal granules. Positive clones were then subjected to the second screening by differential interference contrast microscopy.

We previously reported the progress of the screening with preliminary analyses of genes for pentose phosphate pathway (PPP) and vacuolar proton transporter (V-ATPase) subunits (Shiraishi et al., and Nishikori et al., 19th and 20th International C. elegans Meeting). In this meeting, we will present results of the whole screening, in which worms fed with 11,263 out of 11,479 RNAi clones reached adulthood and 142 positive clones were obtained. The gene ontology analysis revealed enrichment of genes associated with metabolic processes including PPP and vacuolar proton transport.

In view of proton transport, we found a Na+/H+ exchanger NHX-2 and its regulator PBO-1 among the positive clones. Since V-ATPase and NHX-2 are assumed to play a role in nutritional uptake via acidification of the intestinal lumen in C. elegans, we examined involvement of H+/oligopeptide symporter PEPT-1 in the biogenesis of HEBE granules using pept-1 mutant. These results suggest that oligopeptide uptake is responsible for the biogenesis of HEBE granules.

**309B  CAB-1 is required at two dense core vesicle trafficking checkpoints.** T. Page1,2, P. Allaire2, M. Schwartz2, E. Jorgensen1,2,3  1) Department of Bioengineering, University of Utah, Salt Lake City, UT; 2) Department of Biology, University of Utah, Salt Lake City, UT; 3) Howard Hughes Medical Institute, Chevy Chase, MD.

The secretion of peptide hormones is an evolutionarily conserved process that regulates diverse physiological processes and behaviors. In neurons and endocrine cells these hormones are packaged in dense core vesicles (DCVs), which bud from the Golgi and are trafficked to the plasma membrane for release. We have found that the conserved transmembrane protein CAB-1 is required for proper DCV trafficking in the nematode C. elegans. Specifically, in this poster we demonstrate a requirement for the two conserved cytosolic domains of CAB-1, CR1 and CR2, in two distinct DCV trafficking checkpoints. Previous work has demonstrated that CAB-1 binds AEX-3, an ortholog of the human MADD protein, which is a GEF for RAB-3 and RAB-27. Our experiments show that cab-1, aex-3, and rab-27 mutants all fail to release DCV cargos and that CAB-1 is required for the localization of AEX-3/RAB-27 to DCVs at the basolateral membrane. We show that in cab-1 mutants the DCV marker AEX-5, a pro-hormone converter, is trapped in a peri-Golgi compartment overlapping with RAB-7/lysosomes. However, in aex-3 and rab-27 mutants CAB-1 and AEX-5 mislocalize to the apical membrane. Furthermore, deletion of CR1 from CAB-1 phenocopies aex-3 and rab-27 mutants while deletion of CR2 phenocopies a cab-1 mutant. This data demonstrates a dual function for CAB-1 in DCV trafficking: 1) CAB-1 via CR2 prevents DCV fusion with lysosomes, and 2) CAB-1 via CR1 recruits AEX-3/RAB-27 to properly drive DCVs to the basolateral membrane as opposed to the apical.

**310C  CDKL-1, a Cyclin-Dependent Kinase-Like protein implicated in neurological disorders, regulates cilium length.** K. Park1, J. Gonzalves2,3, C. Li1, L. Pelletier2,3, M. Leroux1  1) Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, CA; 2) Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, CA; 3) Molecular Genetics, University of Toronto, Toronto, ON, CA.

Primary cilia are non-motile, microtubule-based hair-like organelles emanating from the surfaces of most metazoan cells. They are essential for sensory physiology and mediate cellular signaling, which is important for development. Cilium length is tightly regulated for optimal function within each given cell type, and thus impairment of this regulation results in various human disorders. Protein kinases are involved not only in many signaling pathways and cellular processes, but also in the regulation of cilium formation, length, and function. Specifically, several kinases including Cyclin-Dependent Kinase-Like 5 (CDKL5) are implicated in ciliary length control. In humans, the CDKL family includes four additional members (CDKL1/CDKL2/CDKL3/CDKL4) which are closely related to Caenorhabditis elegans CDKL-1 (the sole C. elegans CDKL member). Yet, it is unclear whether CDKL-1-4 have roles in cilia. In this study, we found that CDKL-1 localizes to the base (transition zone) of neuronal cilia, and is not associated with the function of the ‘membrane diffusion barrier’ established at the transition zone (TZ). The TZ separates cilia from the plasma membrane and helps to maintain the unique protein content within the cilium. However, CDKL-1 controls the cilium length in C. elegans, and its kinase activity is essential for both its localization and function. Interestingly, human CDKL5 mutations cause neurological disorders such as Rett syndrome and epilepsy, and most mutations are present in the conserved kinase domain found in all CDKL family members. We discovered human CDKL5 localizes at the cilium in RPE-1 cells and impairs ciliogenesis when overexpressed. Therefore, we introduced three corresponding CDKL5 pathogenic missense mutations into C. elegans?CDKL-1 to assess their effects on localization and function. All CDKL-1 variants harboring the mutations cause ciliary length defects, and two of them fail to localize to the TZ, suggesting ciliary mislocalization and dysfunction as pathomechanisms for the CDKL5 mutations in human disease. Altogether, our results reveal that CDKL-1 regulates ciliary length at the TZ, and suggest that ciliary dysfunction may lead to neurological phenotypes in humans.

**311A  Miro-1 and localization of mitochondria in the intestine.** Vidhi Patel, Yanqing Shen, Hui Lan Chua, Han Yin Cheng, Takao Inoue. Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, SG.

We are studying the mechanism of mitochondrial localization controlled by Miro, an atypical small GTPase containing two
GTPase domains, separated by a linker containing Ca\(^{2+}\) binding EF hand motifs. Miro proteins are localized to mitochondrial outer membrane by a transmembrane region near the C-terminus. In C. elegans intestinal cells, miro-1 is required for the concentration of mitochondria near the lumen, at the apical surface. This concentration also appears to require components of the dynein motor protein complex, suggesting that Miro and dynein may work together in these cells to transport mitochondria. A small-scale pilot EMS screen found that it is possible to generate novel mutations affecting apical concentration of mitochondria. We are using this and other approaches to identify proteins that interact with Miro to regulate mitochondrial localization.

312B Lamin resists dynein forces to promote recovery from nuclear envelope ruptures in vivo. L. Penfield\(^1\), B. Wyssolmerski\(^1\), R. Farhadifar\(^2\), M. Martinez\(^1\), R. Biggs\(^3\), M. Mauro\(^1\), C. Broberg\(^1\), D. Needleman\(^2\), S. Bahmanyar\(^1\) 1) Department of Molecular and Developmental Biology, Yale University, New Haven, CT; 2) School of Engineering and Applied Sciences, Department of Molecular and Cellular Biology, FAS Center for Systems Biology, Harvard University, Cambridge, MA; 3) Ludwig Institute for Cancer Research, Department of Cellular & Molecular Medicine, University of California San Diego, La Jolla, CA.

The nuclear envelope (NE) provides a barrier between the nucleoplasm and the cytoplasm to protect genome integrity and spatially regulate cellular processes. Recent studies revealed that transient losses in NE compartmentalization, called NE rupture, occur in cancer cells. NE rupture rates increase upon inhibition of lamin, a structural component of the NE. However, NE rupture mechanisms are yet to be identified in a physiologically relevant context. Here, we use the one cell C. elegans embryo to show that lamin prevents NE rupture in vivo, and that this function for lamin depends on a conserved residue that is associated with disease in human lamin. NE rupture sites in lamin perturbed nuclei transiently exclude nuclear pore complex components and accumulate the inner nuclear membrane protein, LEM-2, an adaptor for NE repair machinery. We were able to recapitulate NE repair in wild type cells by laser induced puncture of the NE and found that NE recovery rates depend on NE puncture size. Dynein forces that position pronuceli exacerbate NE ruptures and lamin resists these forces to restrict loss of the NE permeability barrier during NE rupture. We propose a model in which lamin resists dynem-generated tension on ruptured nuclear membranes to prevent enraging of NE holes and thereby promote recovery from NE rupture. This is the first study to dissect mechanisms of NE rupture in vivo and monitor NE protein dynamics during NE rupture and recovery.

313C Pathways that regulate ciliary stability, structure, and transport in response to microtubule post-translational modifications. R. O'Hagan\(^1\), Y.H. Ramadan\(^1\), A. Gu\(^1\), E.A. De Stasio\(^2\), H. Smith\(^3\), A. Golden\(^2\), M. Bart\(^1\) 1) Department of Genetics, Rutgers University, Piscataway, NJ 08854; 2) Department of Biology, Lawrence University, Appleton, WI 54911; 3) National Institutes of Health, Bethesda, MD 20892.

Microtubules form diverse structures, despite being composed of alpha and beta tubulins that vary only in their C-terminal tails. Post-translational modifications (PTMs), most of which can be added to the tubulin C-terminal tail, are thought to increase tubulin diversity, creating a “Tubulin Code” that regulates microtubule-based motor transport, as well as microtubule stability and structure (Verhey and Gaertig 2007). Polyglutamylation is a reversible tubulin modification regulated by tubulin tyrosine ligase-like (TTLL) proteins that initiate and elongate glutamate side-chains on tubulin C-terminal tails, and cytosolic carboxypeptidases (CCPs) that cleave glutamate residues, reducing or removing polyglutamylation. Although molecules that write or erase the Tubulin Code, such as TTLL glutamylases and CCP deglutamylases, have been identified, we lack an understanding of how the Tubulin Code is written, read and interpreted by cells. Cilia provide an ideal model to study PTMs. The axoneme is composed of highly post-translationally modified microtubules that display a diversity of structures and perform a variety of functions. In C. elegans sensory cilia, ccpp-1 and ttll-11 regulate microtubule deglutamylation and glutamylation (O’Hagan et al 2011; Kimura et al. 2010; Chawla et al 2016). To discover effectors of the tubulin code, which may read or interpret tubulin PTMs, we are using genetic and biochemical methods. From a forward genetic screen, we isolated mutants that suppressed the progressive degeneration of cilia characteristic of ccpp-1 mutants. We are currently mapping and cloning these genes. We are also using biochemical approaches to uncover both tubulin and non-tubulin substrates of ccpp-1 and ttll-11. Molecular motors are also influenced by glutamylation, and therefore must read and interpret the Tubulin Code. To understand cell-specific effects of glutamylation on ciliary transport, we are also testing how loss of glutamate and deglutamylase enzymes alters ciliary velocity and distribution of molecular motors in vivo. Our findings on ccpp-1 and ttll-mediated pathways should further our understanding of the basic mechanisms that modify and stabilize cilia, and how defects in these pathways might contribute to human conditions such as male infertility, polycystic kidney disease, impaired vision, and abnormal neurodevelopment.

314A Determining how TGF-ß is trafficked and secreted in C. elegans. G. Ravindranathan, L. Faure, T. Gumieni. Texas WOman's University, Denton, TX.

Transforming growth factor-beta (TGF-ß) comprises a conserved family of secreted cell signaling proteins responsible for regulating numerous cellular processes. Disruption of TGF-ß signaling results in developmental defects and cancer in mammals. In the nematode Caenorhabditis elegans, one TGF-ß superfamily member is DBL-1. To identify potential targets that affect DBL-1 signaling, an RNAi screen was performed that identified candidates that may be involved in neuronal trafficking and secretion of DBL-1 from the sending cell, a process that is not well understood for any TGF-ß signaling protein. Specific aims of this research project are to 1) determine if the candidate genes and dbl-1 interact genetically and 2) Determine if their proteins colocalize. Genetic and confocal analyses of transgenic strains expressing functional, fluorescently tagged DBL-1 and candidate regulators provide insight into how cells regulate the message they send. Candidates include the small GTPases rap-1 and unc-108, caveolin cav-1, and dynamin dyn-1. Genetic interaction and co-localization studies with synaptic vesicle acetylcholine transporter unc-17, suggests this complex also affects DBL-1 within the secreting neurons. While RNAi of the retrograde
trafficking and autophagy gene *bec-1* reduces GFP-tagged DBL-1, tagged, functional BEC-1 does not co-localize with DBL-1, suggesting an indirect involvement in DBL-1 trafficking.

315B Investigating molecules that genetically interact with *tba-6*. A. Rizvi, M. Silva, M. Barr  Department of Genetics and Human Genetics Institute of New Jersey, Rutgers University, Piscataway, NJ 08854, USA.

In humans, ciliary dysfunction causes a wide range of ciliopathies. Human clinical ciliary pathologies include polycystic kidney disease, retinal degeneration, hydrocephalus, polydactyly, male infertility, and situs inversus. How cilia are specialized in form and function remains widely elusive.

Sensory cilia are critical in transducing extracellular signals. The ciliary axoneme, the core structure of the cilium, is composed of cylindrically arranged nine outer microtubule doublets. In *C. elegans*, cilia specialized to sense and transduce external stimuli are found at the dendritic ends of sensory neurons. Based on microtubule ultrastructure, the axoneme is typically divided into three distinct regions: the transition zone, doublet region, and singlet region. The transition zone is composed of nine microtubule doublets connected to the membrane by “Y” links. The doublet region contains nine microtubule doublets that lack “Y” links. In the singlet region, doublets containing A-tubules continue as singlets, while B-tubules abruptly stop. In wild-type cephalic male (CEM) cilia, we found that 9+0 doublet microtubules splay to form 18 A- and B-tubule based singlets that remain joined at proximal and distal ends. We are identifying mechanisms that generate this unique ciliary structure in CEM neurons.

a- and β-tubulins form a-β-tubulin heterodimers, which serve as the basic structural unit of microtubules. Tubulin heterodimers assemble into linear protofilaments. Each doublet microtubule consists of A- and B-tubules. The A-tubule is a complete microtubule with 13 protofilaments, while the incomplete B-tubule contains 10 protofilaments. The *C. elegans* genome encodes nine a-tubulin and six β-tubulin isoforms. *tba-6* encodes an a-tubulin that is specifically expressed in the 27 ciliated sensory neurons that release extraciliary vesicles. We discovered that, in *tba-6* CEM cilia, doublets do not splay to 18 singlets and instead resemble amphil channel cilia with a distal singlet region. CEM ciliary transport and functions are impaired in *tba-6* mutants (Silva et al Current Biology 2017).

To determine the β-tubulin partner of TBA-6, we examined tubulin C-terminal sequences and identified TBB-6 as a potential candidate. Preliminary results indicate that *tbb-6* regulates TRP channel PKD-2 ciliary localization, consistent with a role in CEM cilia. To investigate epistatic relationships between the a-tubulin TBA-6 and β-tubulin TBB-6, we are analyzing CEM structure and function in *tbb-6* single and *tba-6*; *tbb-6* double mutants. Our study of *tbb-6* will further contribute to the scientific knowledge of how mutations in tubulin genes lead to neurodegenerative diseases called tubulopathies.

316C *C. elegans* as a model to study extracellular vesicle biology. F. Rizvi1, J. Akella1, K. Nguyen2, D. Hall2, M. Barr1 1) Department of Genetics, Rutgers University, Piscataway, NJ, U.S.A; 2) Center for *C. elegans* Anatomy, Albert Einstein College of Medicine, Bronx, NY, U.S.A.

Cells release extracellular vesicles (EVs) that serve as nano-sized packages allowing for exchange of protein and genetic content. Cilia are hair-like projections that play important roles in development and signaling. The cilium both releases and binds to EVs. EVs play a role in cell signaling in health and pathologies, and may carry beneficial or toxic cargo. An understanding of the biogenesis, release, uptake, and signaling of ciliary EVs is lacking. Using *C. elegans* as a model, we aim to identify the molecules and mechanisms involved in EV biology.

A subset of the ciliated neurons of *C. elegans* release EVs containing cargo that include the polycystins LOV-1 and PKD-2 and a myristoylated protein CIL-7 (Wang et al. Current Biology 2014; Maguire et al. MBoC 2015). Transcriptional profiling of the EV releasing neurons (EVNs) revealed candidates that could play a role in EV biogenesis and/or release (Wang et al. Current Biology 2015). *rab-28* is expressed in all ciliated neurons of *C. elegans* including the EVNs. *rab-28* encodes a small RAB GTPase. *RAB-28* is important for amphil ciliary ultrastructure, amphil glial sheath cell volumes, and amphil-mediated sensory behaviors (Jensen. et al. PLoS Genetics 2016). Here, we explore the role of *RAB-28* in EVNs.

We investigated the role of *RAB-28* in EV biology. We examined shedding and release of GFP-tagged EV cargoes using live imaging of *rab-28(tm2636)* mutants. *rab-28* mutants display altered localization of ciliary EV cargoes PKD-2 and CIL-7. Defects in the release of EV cargo from the tips of EVNs and/or alterations in the ciliary localization of GFP-tagged EV cargo in the EVNs may indicate defects in ciliary trafficking or defects in EV biogenesis and/or release.

We are examining ciliary ultrastructure and EVs in *rab-28* mutant males using transmission electron microscopy (TEM). The EV releasing CEM cilia have a unique ciliary ultrastructure and are housed within the cephalic sensillum (Silva et al. Current Biology 2017). The male cephalic sensillum is comprised of CEM and CEP neurons, and glial support and socket cells, the latter create a lumenal space that contains EVs. We are determining whether *rab-28* regulates CEM ciliogenesis, the integrity of the cephalic sensillum, or EV biogenesis. Our work could shed light on the contribution of ciliary ultrastructure and cilia-glia interactions to EV biology.

317A Abnormal ER morphology revealed through ultrastructural analysis of *C. elegans* axt-2 mutants. Emily S. Semaya1, Megan M. Gnazzo2, Ahna R. Skop2, David H. Hall1 1) Albert Einstein College of Medicine, Bronx, NY; 2) University of Wisconsin-Madison, Madison, WI.

ATX-2, a conserved RNA binding protein, is the *C. elegans* ortholog of the human RNA binding protein Ataxin-2. Loss of
Ataxin-2 leads to the late-onset neurodegenerative disease spinocerebellar ataxia type-2 (SCA2) and is associated with an increased risk for amyotrophic lateral sclerosis (ALS). To define the cellular role of ATX-2, we sought to determine the ultrastructure of ATX-2-depleted embryos and adults. ATX-2 is necessary for embryonic patterning in C. elegans (Kiehl et al., 2000) and recent work from the Skop and Song labs has shown cell division defects in atx-2 mutants (Stubenvoll et al., 2016; Gnazzo et al., 2016). To examine the cellular defects in the early embryo, we characterized atx-2 ts mutant animals (WM210 (atx-2(ne4297));(Gnazzo et al., 2016)) at high resolution. Transmission electron microscopy (TEM) of ultra-thin sections revealed that ATX-2-depleted animals exhibit fertilization and egg-laying defects. Both unfertilized oocytes and defective embryos are present in the uterus of ATX-2 depleted animals. Mutant embryos also exhibit defects in nuclear division, in which daughter cells are multinucleated. Ataxin-2 localizes to the endoplasmic reticulum (ER) in humans (van de Loo et al., 2009), yet the dynamics of this association during cell division are unclear. Preliminary data from the Skop lab suggests a role for ATX-2 in maintaining proper ER morphology, yet the ultrastructure was unknown. Using TEM of ultra-thin sections, we found that ATX-2 depleted embryos have fragmented rough ER, suggesting that ATX-2 is necessary for maintaining proper rough ER morphology during mitosis. Given the human phenotypes, ultrastructural studies of ER morphology in neurons and glia of ATX-2-depleted adult animals is currently underway. This research is supported by an NSF grant MCB-1158003 (Skop Lab) and an NIH grant OD019043 (Hall Lab).

318B Mutation in cuticle collagen genes affects lysosome morphology and integrity in C. elegans. L. Shi1,2, Y. Wu1,2, Y. Li1,2, T. Hao2, X. Wang1,2 1) National Institute of Biological Sciences, Beijing, CN; 2) Institute of Biophysics, Chinese Academy of Sciences, Beijing, CN.

Lysosomes are membrane-bound organelles containing hydrolytic enzymes that can break down virtually all kinds of biomolecules derived from phagocytosis, endocytosis or autophagy. In addition to acting as the center of cellular degradation, lysosomes are involved in secretion, plasma membrane repair, cell signaling and energy metabolism. How lysosomes are regulated to fulfill these diverse functions remains unclear. To explore this, we employ C. elegans as a model system to investigate regulation and physiological functions of lysosomes.

In C. elegans, we observed that lysosomes appear as small puncta or thin tubules, and display a highly dynamic pattern during development and aging. To understand how lysosome function and dynamics are regulated, we performed genetic screens to search for mutants with altered lysosome morphology. From a screen that covered 20,000 haploid genomes, we isolated 35 mutants that contain extensive tubular lysosomes in hypodermis. Among them, 3 alleles (qx435, qx440, qx442) displayed similar tubular lysosome morphology from day 1 of adulthood. We found that qx435, qx440 and qx442 were 3 dominant alleles that affect cuticle collagen genes col-140, col-8 and col-178 respectively, causing mutations at the N-terminal processing site of the collagens. Moreover, knockdown of the pro-collagen N-terminal processing enzyme BLI-4 induced similar lysosome phenotypes as in qx435, qx440 and qx442 worms. These data suggest that the altered lysosome morphology may be caused by defective processing of pro-collagen and/or incorporation of the partially processed pro-collagen into the cuticle. Loss of SCAV-3, the lysosome membrane protein homologous to human LIMP-2, affects lysosome integrity, causing accumulation of damaged lysosomes (Li et al., 2016). Intriguingly, we found that accumulation of damaged lysosomes in scav-3(qx193) was efficiently suppressed in col-178(qx442) mutants or when BLI-4 was inactivated, suggesting involvement of pro-collagen processing in modulating lysosome membrane integrity. We are currently investigating how lysosome morphology and membrane integrity may be modulated by cuticle collagens.

319C Touch neurons can toss out mitochondria. Joelle Smart, Ilija Melentijevic, Girish Harinath, Marton Toth, Ryan Guasp, Meghan Arnold, Monica Driscoll Rutgers, Piscataway, NJ.

Mitochondria provide energy, execute key steps of metabolism, control calcium, and modulate cellular decisions for life/death. Given these critical functions in cell, tissue, and organism health, it is not surprising that mitochondrial functionality plays an essential role in neuronal maintenance in everyday biology, aging, and late-onset neurodegenerative disease. Mitochondrial quality control is thought to be primarily executed through cell-internal elimination via mitophagy and lysosome degradation. However, the Driscoll lab has discovered, and recently published (Melentijevic, 2017 Nature 542:367) that mitochondria can be thrown out of neurons in large membrane bound vesicles we call exophers. Mitochondria in exophers are present in the uterus of ATX-2 depleted animals. Mutant embryos also exhibit defects in nuclear division, in which daughter cells are multinucleated. Touch neurons can toss out mitochondria for degradation by neighboring astrocytes (Davis, PNAS 11:9633), suggesting that throwing away defective mitochondria may be a mechanism for neuronal quality control. Indeed, some mammalian neurons can throw out their mitochondria for degradation by neighboring astrocytes (Davis, PNAS 11:9633), suggesting relevance across phyla. In C. elegans, beautiful work on degradation of sperm mitochondria upon fertilization have been published (Sato, Science 334:1141).

We will present data on our initial efforts to characterize mito-exopher production and the factors that prompt neurons to extrude mitochondria. Our hope is that our findings will be relevant to understanding neuronal maintenance and neuronal degeneration, especially as associated with perturbed mitochondrial quality as may occur in Parkinson’s disease and many other human disorders.

320A Characterization of lysosome morphology, dynamics and function during C. elegans aging. Y. Sun1,2, W. Shao1, D. Zhao1,2, X. Wang1,2 1) National Institute of Biological Sciences, Beijing, China; 2) Institute of Biophysics, Chinese Academy of Sciences, Beijing, China.

Lysosomes, the primary catabolic compartment of eukaryotic cells, contain a variety of hydrolytic enzymes that are active at acidic pH and collectively degrade macromolecules derived from endocytosis, phagocytosis or autophagy. Defects in lysosomes...
cause inherited storage diseases, indicating importance of lysosomes in cellular homeostasis. In *C. elegans* hypodermis, we observed that lysosomes appear as both small puncta and thin tubules and the lysosome pattern changes when worms age. At day 1 of adulthood, lysosomes appear as vesicular structures, while tubular lysosomes are visible at day 3 and become increasingly evident, leading to formation of tubular network in aged adults. Moreover, we found that lysosomes moved quickly in larvae but become static in aged adults. We developed assays to examine various aspects of lysosomes including morphology, motility, dynamics, acidification and biogenesis in both wild type and mutants that affect genes of multiple longevity-regulatory pathways. These studies will help us to understand how lysosome dynamics and functions are regulated and contribute to adult aging.

321B Structural monitoring of neuronal receptive endings by glia. K.C. Varandas, S. Shaham Laboratory of Developmental Genetics, The Rockefeller University, New York, NY.

Cell structure is critical for cell function. Cells in the nervous system, neurons and glia, have particularly elaborate and extreme structures, and structural defects in both cell types are associated with disease. Furthermore, experience-dependent structural remodeling of neuronal receptive endings (NREs) is believed to correlate with learning and memory. Glia are known to monitor the structures of their interacting neurons. For example, neuronal injury results in protective glial responses and axon diameter defines the extent of glial myelination. Nonetheless, relatively little is known about how glia monitor and respond to physiological changes or pathological defects in NRE structure. *C. elegans* with defects in amphid sensory neurons exhibit changes in their ensheathing glia (AMsh), including accumulation of glial secretory vesicles and changes in protein expression and localization. We developed two assays for detecting the glial response to defects in NRE structure based on changes in the expression and localization of AMsh glia secreted proteins. We plan to use these assays to determine the cellular basis of glial structural monitoring of NREs, to characterize the glial response to defects in NRE structure and its functional consequences, and to identify molecules involved in glial monitoring of NRE structure. These studies may not only shed light on how glia monitor neuron integrity, but may also illuminate how cilia mutations in humans cause diverse developmental defects called ciliopathies. Additionally, as sensory NREs and synapses are functionally and molecularly related, these studies may uncover how neurons and glia interact at the synapse.

322C "TAG-63 is a neurofilament-like protein that facilitates the fast axonal transport machinery in *C. elegans". Ding Wang, Prerana Bhan, Chih-Wei Chen, Meng-Chieh Wang Dept. of Life Science, National Tsing Hua University, Hsinchu, TW.

**Purpose:** Various neurological diseases baring defects in neurofilaments (NF’s) are known including Parkinson’s disease, Amyotrophic Lateral Sclerosis, Charcot-Marie-Tooth disease etc. Though a critical role of NF’s has been ascertained little is known about how these diseases develop on the molecular level, critical to facilitate future drug design. Nematodes have been increasingly used to dissect these underlying molecular mechanisms. However, whether an NF homolog exists in the nematode still remains unclear. Therefore, the major goal of this study was to identify and characterise a putative NF homolog in *C. elegans*. Using bioinformatics tools, we identified TAG-63 with numerous sequence homologies to NEFH, three coiled coils as well as various phosphorylation sites.

**Methods:** We then employed a broad range of techniques like Western blotting, Immunohistochemistry, Worm imaging, motility analysis etc. to delineate the role of tag-63 in *C. elegans*. 

**Result:** To identify NF homologs, we employed WormBase and received hits from *C. elegans* gene tag-63. Using KEGG database, we received hits for NEFH orthologs. Using KEGG, we also note a cluster of NEFH orthologs in a rooted phylogenetic tree emerging from a common TAG-63 ancestor. Though we cannot detect KSP repeats in TAG-63, we identified nine potential phosphorylation sites using Scansite tool and coiled coil prediction tool identified three potential coiled coils in TAG-63. Using worm lysates from N2 wild-type animals, we clearly identified a band around 68kDa in Western blots by a monoclonal mouse anti-NEFH antibody, but this band cannot be detected in lysates from *C. elegans* knock- out. Using KEGG, we also note a cluster of NEFH orthologs in a rooted phylogenetic tree emerging from a common TAG-63 ancestor. Though we cannot detect KSP repeats in TAG-63, we identified nine potential phosphorylation sites using Scansite tool and coiled coil prediction tool identified three potential coiled coils in TAG-63. Using worm lysates from N2 wild-type animals, we clearly identified a band around 68kDa in Western blots by a monoclonal mouse anti-NEFH antibody, but this band cannot be detected in lysates from *C. elegans* knock-out worms leading to increased accumulations of motors along axons. In summary, we identified and characterised an NEFH ortholog in *C. elegans*, and demonstrate that lack of this protein limits axonal transport efficiencies suggesting that this model organism may be used for studying neuropil-based neurological diseases.

323A Vesicle fusion meets autophagy. C.M. Wnukowski, E.G. Bend, N.D. Okerlund, R.J. Hobson, E.M. Jorgensen Biology, University of Utah, Salt Lake City, UT.

Synaptic vesicles fuse to the plasma membrane to release neurotransmitters and are quickly rebuilt from the reinternalized membrane and proteins. These component membranes and proteins accrue damage as they experience repeated use.

Autophagy, a pathway by which damaged cellular material is degraded, has been observed at synapses, but it is unknown how damaged vesicles may be directed to the autophagy pathway. In addition to its vesicle fusion role, SNAP-25 may function in targeting synaptic vesicles for degradation. *snap-25* null *C. elegans* have an accumulation of synaptic vesicles at synapses. Overexpression of SNAP-29, a SNAP-25 paralog, reduces vesicle numbers to normal in *snap-25* mutants. These phenotypes are consistent with simple redundant roles for SNAP-25 and SNAP-29 in vesicle fusion. However, other observations are not consistent with this interpretation. First, SNAP-29 is known to function in autophagy. Second, vesicle
fusion rates in snap-25 snap-29 double mutants are not more impaired than in SNAP-25 single knockdown mutants. This suggests SNAP-29 does not function redundantly to SNAP-25’s role in the SNARE complex. Instead, we propose SNAP-29 rescues snap-25 phenotypes by upregulating the autophagy pathway, suggesting a possible noncanonical role for SNAP-25 in triggering autophagy-driven degradation of synaptic vesicles. We are currently testing overexpression of other autophagy promoting proteins in snap-25 mutants to further argue against redundancy in the SNARE complex, and to identify other components of the autophagic pathway.

324B  TORC2 regulates the maturation of endosome via SGK-1 in the intestine of C. elegans. Y. Yan1, Wenjing Qi1, Ralf Baumeister1,2 1) Bioinformatics and Molecular Genetics (Faculty of Biology), University of Freiburg, Freiburg, Germany; 2) ZMBZ (Faculty of Medicine), University of Freiburg, Freiburg, Germany.

In human cells, the serum- and glucocorticoid-inducible kinase (hSGK) has been implicated in the regulation of a wide variety of ion channels that participate in the regulation of transport, hormone release, neuroexcitability, inflammation, cell proliferation, and apoptosis. We have previously shown that SGK-1, the only Caenorhabditis elegans homolog of hSGK, acts in parallel to the AKT kinases to mediate DAF-2 signaling to regulate metabolism, growth, development, and longevity. Besides, SGK-1 is the direct downstream target of the TORC2 kinase Rictor/RICT-1 that is involved in the regulation of fat metabolism, feeding, growth and lifespan in worms. In addition, SGK-1 is also the downstream component of the cold-sensitive TRPA-1 calcium channel and calcium-sensitive PKC-2 in a signaling pathway that detects temperature reduction. Thus, SGK-1 integrates several pathways in the regulation of metabolism, development, longevity, and stress response in C. elegans. Besides its role in the crosstalk between insulin signaling and TORC2 pathway, hSGK3 was recently reported to localize to endosomes and render cancer cells resistant to prolonged treatment by PI3K or Akt inhibitors. It counteracts inhibition of PI3K/Akt signaling by activating TORC1 in a hVps34 dependent manner. Like hSGK3, structural predictions indicate that C. elegans SGK-1 also contains a Phox homology domain that is thought to preferentially bind to phosphatidylinositol-3-phosphate, therefore enabling its endosomal localization in a VPS-34 dependent manner. We now show that SGK-1 may also be a regulator of endosome/lysosome system in polarized epithelial cells of C. elegans. Misexpression of sgk-1 results in a vacuole like structure (VLS) phenotype in intestinal cells that we have used to systematically screen for modifier genes by RNAi. We have identified several candidates supporting a role of SGK-1 in the regulation of the endocytic pathway. Moreover, sgk-1 mutant was reported to have endocytic sorting defects of cargo proteins, which further supports its function in endomembrane trafficking. Epistatic analysis showed that SGK-1 function in the TORC2 pathway mediates endosomal functions, while insulin/DAF-2 and TRPA-1 pathways do not play functional roles. This suggests that serum-and-glucocorticoid-inducible kinases act in a conserved VPS34-TORC2 pathway involved in endocytic trafficking and signaling transduction.

325C  Molecular mechanisms of ATG-9 localization in neurons. S. Yang1, S. Hill1, D. Colón-Ramos1,2 1) Program in Cellular Neuroscience, Neurodegeneration and Repair, Departments of Cell Biology and Neuroscience, Yale University School of Medicine, New Haven, CT; 2) Instituto de Neurobiología, Recinto de Ciencias Médicas, Universidad de Puerto Rico, San Juan, Puerto Rico.

Autophagy is an evolutionarily conserved cellular degradative process in which cytosolic components are engulfed by double membrane structures known as autophagosomes. ATG-9 is a transmembrane protein, which is thought to be important for delivering lipids from membrane compartments for autophagosome formation. Previous work from our lab demonstrated that ATG-9 localizes to presynaptic regions and is associated with autophagosome biogenesis at the synapse (Stavoe and Hill et al., 2016). How ATG-9 localizes to presynaptic regions and traffics between membranes to regulate autophagy is not well understood. We have established an in vivo system to visualize ATG-9 in a pair of Caenorhabditis elegans interneurons called AIY. In wild type animals, ATG-9 diffusely distributes at presynaptic regions. We performed unbiased forward genetic screens and candidate screens to identify molecules required for ATG-9 localization in AIY. We identified two novel alleles which display highly penetrant, abnormal distribution of ATG-9 at presynaptic regions. We now plan to use these alleles to determine the molecular mechanism governing ATG-9 localization at the synapse to better understand the role of autophagy in neurons.

326A  Understanding the secretion mechanism of VAPB/ALS8 MSP. H. Zein-Sabatto, M. Miller  University of Alabama at Birmingham, Birmingham, AL.

Instead of traveling via the conventional Golgi-ER secretory pathway, some eukaryotic proteins are unconventionally secreted. The major sperm protein (MSP) domain of VAPB/ALS8 has been shown to be cleaved from the rest of the protein and secreted. The goal of this project is to understand how VAPB MSP (vMSP) is proteolytically processed, secreted, and regulated. Using C. elegans, our lab discovered that the nervous system, germ line, and intestine are cellular sites of vMSP secretion. C. elegans null mutants for vpr-1, the Vapb homolog, are sterile and have striated muscle mitochondrial abnormalities. VAPB is a ubiquitously expressed type II membrane protein found anchored into the endoplasmic reticulum with the N-terminal MSP domain extending into the cytosol. The secreted MSP domain does not harbor a signal peptide characteristic of conventionally secreted proteins. Based on these studies, we hypothesize that vMSP is secreted in a regulated fashion via an unconventional mechanism.

In order to test our hypothesis, we developed an RNAi screening method using transgenic C. elegans to identify genes required for MSP secretion. vpr-1(tm1411) null mutants expressing VPR-1 only in the intestine (pGES-1::vpr-1) are fertile and can be grown as transgenic homozygotes. We predicted that RNAi of genes essential for VPR-1 MSP secretion would cause sterility. ykt-6, a v-SNARE, was a candidate gene resulting from the 420 genes screened. Further genetic studies will be used to determine the relationship between ykt-6 and vMSP. I am also conducting a structure/function analysis to identify regulatory regions within VPR-1 necessary for MSP secretion. Currently, I am using genome-editing techniques to tag the termini of endogenous vpr-1 and develop a direct read-out of secretion. Finally, we have been characterizing multiple mouse Vapb
mutants with skeletal muscle mitochondrial defects, in collaboration with Jin Chen’s lab at Vanderbilt. I plan to use western blots of isolated mouse tissues to help define the mammalian cell types that proteolytically process VAPB to liberate MSP.

327B  The intrafagellar transportation of sensory cilia is impaired in aged C. elegans.  Y. Zhang, Y. Shen Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, CN.

Sensory functions decline with age. As in many other organisms, sensory functions depend on cilia of sensory neurons in C. elegans. Most major tissues of C. elegans, including body wall muscle, intestine and reproductive system, undergo dramatic changes with ageing. The ageing nervous system undergoes more subtle changes such as dendritic restructuring and synaptic deterioration. However, the changes of cilia in sensory neurons with age remain poorly understood. Sensory cilia are only found at the dendritic endings of sensory neurons in C. elegans, including amphid and phasmid cilia. Cilia functions depend on intraflagellar transport (IFT), which delivers cilia proteins into and within sensory cilia. In order to examine the IFT trafficking in C. elegans in vivo, we tagged endogenous IFT proteins with GFP using CRISPR/Cas9. Using live imaging microscopy to monitor the velocity and frequency of IFT components in young and aged worms, we show that ageing impaired many IFT components in velocity and frequency. Understanding the mechanisms underlying the decline of IFT functions in cilia will help identify cellular factors that protect the cilia structure and function during normal ageing and in disease states.

Development - Cell Fate and Patterning

328C  Investigating the relationship between birth order and cell fate in the AC/VU decision, a simple lateral specification paradigm for stochastic cell fate specification.  M. A. Attner1, W. Keil2,3, S. Shaham3, E. D. Siggia4, I. Greenwald1  1) Dept. of Biological Sciences, Columbia University, New York, NY; 2) Center for Physics and Biology, The Rockefeller University, New York, NY; 3) Laboratory of Developmental Genetics, The Rockefeller University, New York, NY.

Notch-mediated lateral specification is utilized during the development of all animals to generate different cell fates from initially equivalent cells. The anchor cell (AC)/ventral uterine precursor (VU) cell fate decision is a simple lateral specification paradigm in which two initially equivalent cells interact via LIN-12/Notch such that one becomes the AC and one becomes the VU (reviewed in Greenwald 2012). Studies using this paradigm have illuminated many fundamental features about how differences in Notch signaling between equivalent cells arise and are amplified during a cell fate decision. During the AC/VU decision, Z1.ppp and Z4.aaa initially express both LIN-12/Notch and its ligand LAG-2; a stochastic small difference in ligand or receptor activity is amplified by feedback mechanisms that restrict transcription of lin-12 to the presumptive VU cell and lag-2 to the presumptive AC cell. The AC/VU decision is random, as 50% of animals have an AC derived from Z1.ppp and 50% have an AC derived from Z4.aaa. However, the first-born of these cells is biased towards the VU fate (Karp and Greenwald, 2003), suggesting that stochasticity in birth order or a process correlated with birth order is at play.

A recent advance in microfluidic technology for imaging C. elegans larvae (Keil et al., 2017) has allowed us, for the first time, to image dozens of somatic gonad lineages and to probe the relationship between lineage patterns, birth-order, and cell fate specification in the AC/VU decision. We fluorescently labeled Z1, Z4, and their descendants, followed Z1 and Z4 lineages at 8-min intervals through their three rounds of division and somatic primordium formation, and observed how the timing of cell divisions in the Z1 and Z4 lineages correlates with the AC/VU decision. We find that Z1.ppp and Z4.aaa birth-order differences generally reflect accumulating birth order differences higher up in each respective lineage. Importantly, we find that at 20°C, when the birth-order difference between Z1.ppp and Z4.aaa is longer than 16 minutes, the first-born cell always becomes the VU (n=19). When the birth-order difference between Z1.ppp and Z4.aaa is less than or equal to 16 minutes, birth-order no longer biases cell fate (n=24). Thus, above a threshold in birth-order time difference, the AC/VU decision becomes predictable. We are currently investigating how birth-order controls the AC/VU decision, testing specifically whether birth-order bias is affected by environmental conditions. We hypothesize that the stochastic pattern of cell divisions in the Z1 and Z4 lineages contributes to robustness in the AC/VU decision.

329A  De-repression of TCF/POP-1 in Wnt-dependent asymmetric cellular divisions alters cell fate determination.  Kimberly N. Bekas, Bryan T. Phillips Genetics Graduate Program, University of Iowa, Iowa City, IA.

Asymmetric cellular divisions (ACDs) are a fundamental component of developmental processes that result in two daughter cells with differential cell fate at birth. C. elegans uses a modified version of the conserved Wnt/beta-catenin signaling pathway to regulate its many ACDs in embryonic and larval development. The DNA binding protein TCF/POP-1 functions in the Wnt/beta-catenin asymmetry pathway to differentially regulate gene expression in the daughter cells resulting from an ACD. The ability of POP-1, to repress or activate gene expression relies on interactions with Groucho family corepressors or the coactivator beta-catenin/SYS-1, respectively. The Groucho corepressors function in fate determination and are expressed in asymmetrically dividing tissues, such as the seam cells, yet a role for these corepressors in ACD has not been demonstrated. For this reason, we investigated the function of Groucho in the seam cell lineage, which divides asymmetrically to produce a pluripotent seam cell and terminally differentiated hypodermal cell. Seam cell fate appears to heavily rely on repression rather than activation since knockdown of the POP-1 increases seam cell number while knockdown SYS-1 does not affect seam cell number. If Groucho were required for POP-1 repression in the unsignaled daughter after ACD, we expect to see duplication of the signaled fate following Groucho depletion, similar to the POP-1 RNAi phenotype. Surprisingly, no defects in seam cell fates following corepressor depletion were seen. However, we sensitized cells via POP-1 RNAi and determined that, at low levels of POP-1 knockdown, additional knockdown of Groucho results in an increase in seam cell number that resembles the full POP-1 knockdown phenotype. This enhancement provides evidence that Groucho functions in terminal differentiation of the dividing
seam cells to confer hypodermal cell fate. Current efforts include testing the role of POP-1 domains that interact with SYS-1 and DNA, using specific pop-1 alleles in combination with Groucho loss-of-function. Preliminary evaluation of a transgenic strain encoding a POP-1 deficient in SYS-1 binding, pop-1(q645) coupled with a sys-1 hypomorph (q544), shows no significant defects in seam cell ACD. These data indicate that differential transcriptional repression between the two daughters provided by Grouchos, rather than activation SYS-1, may be the critical effect of Wnt signaling in the unsignaled daughter.

330B Long-term C. elegans Immobilization enables High Resolution Developmental Studies in vivo. Simon Berger1, Evelyn Lattmann2, Xavier Casadevall i Solvas1, Tinri Aeagarter-Wilmsen2, Michael Hengartner2, Alex Hajnal2, Andrew deMello1 1) Institute of Chemical- and Bioengineering, ETH Zurich, Zurich, CH; 2) Institute of Molecular Life Sciences, University of Zurich, Zurich, CH.

Owing to its small size, fecundity and genetical tractability C. elegans has become one of the most widely used model organisms in biology. One major challenge when working with C. elegans however, is its high mobility, making immobilization necessary for the study of most cellular and subcellular processes. Immobilization is usually achieved by simply placing the worms of interest on an agar pad, limiting motion through the pressure exerted on the animal and, if necessary, through addition of chemical tranquilizers. Unfortunately, this process has proven to be a major limitation when trying to follow developmental processes over extended periods of time, as it rapidly causes slowdown or arrest of many such processes. Here we present a novel set of microfluidic devices able to trap, feed and image C. elegans in a variety of developmental stages (L1 to adult), without any of the adverse effects encountered when using conventional immobilization methods.

Our immobilization platform is based on a simple PDMS microfluidic device mounted on the back of a coverslip. Operation of the device is readily learned and the microfluidic device can easily be integrated with any type of microscope (inverted or upright), all imaging modalities commonly used (brightfield, epifluorescence and confocal microscopy), and any type of objective (high magnification and high numerical aperture). Thus requiring only small changes to existing microscope setups and microscopy protocols prior to adaptation, enabling the study of a great variety of developmental processes, interference free, in vivo. We demonstrate the platforms capabilities in several case studies. First, we studied long-term viability of adult C. elegans on-chip, readily achieving viabilities exceeding 100 hours while immobilized, all while observing normal feeding and egg laying rates. Optimal quality images of the adult gonad were then acquired, showcasing the platforms capabilities of following complex developmental processes over long time periods. Specifically we studied germ cell apoptosis, a process known to arrest on agar pad within 20 minutes, and factors involved in the cell fate decision by tracking 100 cells over the course of 12 hours. For the first time determining an apoptotic rate of 60% in vivo (n = 100). Secondly we demonstrate that crucial developmental process, e.g. anchor cell invasion and distal tip cell migration, occur normally on-chip in all larval stages assessed, and at rates comparable to on plate culture. Compared to development on agar pads, processes occurred 5-7 times faster and more reliable on-chip, with all worms undergoing normal development. This strongly suggests that our immobilization device has minimal negative effects on sensitive developmental processes, thus making it ideally suited for long-term studies of processes so far inaccessible, all while allowing the capture of high resolution images.

Determinants of robust cell fate in C. elegans. F. Coraggio1,2, R. Püschel1,2, P. Meister1 1) Cell Fate and Nuclear Organization, Institute of Cell Biology, University of Bern, Switzerland; 2) Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland.

A promising therapy for degenerative diseases is the replacement of deficient cells by in vitro differentiated progenitors. Differentiation is a crucial step since incompletely differentiated cells could lead to the formation of teratomas. Therefore, understanding which epigenetic determinants and signalling pathways maintain or drive cell fate is of prime importance for regenerative medicine. Using ectopically induced muscle or endoderm transdifferentiation, we test cell plasticity in fully differentiated animals. Different from previous systems, we assess transdifferentiation by the expression of single copy fluorescent tissue-specific markers, which allows us to track cell fate in vivo. We find that cell plasticity is highly restricted in larvae, with a single cell per animal transiently expressing muscle markers. By using genetic mutants for enzymes depositing silent histone marks (histone 3 lysine 9 and 27 methylation), we show that unlike in embryos, H3K9 methylation is dispensable for cell fate maintenance, while H3K27 methylation is essential to restrict plasticity. In the absence of this mark, animals completely arrest development upon ectopic muscle transdifferentiation induction, while a number of additional cells express muscle markers. Using developmental arrest as a read-out, we used this system to screen for plasticity enhancers which knock-down would suppress the arrest. We find that the Notch signalling pathway rescues the arrest, suggesting Notch acts to enhance plasticity in vivo, thereby antagonizing H3K27 methylation. Surprisingly, we discovered that cell plasticity additionally depends on the metabolic state of the animal: starved animals are insensitive to fate challenges, while fed ones arrest development. We currently investigate the mechanisms and determinants leading to larval arrest and the link between metabolism and cell fate plasticity.

Cell cycle-independent role of the pre-replication complex during anchor cell invasion. Ting Deng, Evelyn Lattmann, Alex Hajnal Institute of Molecular life sciences, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.

Anchor-cell (AC) invasion is serving as an excellent model of study fundamental aspects of cell invasion, a process occurring during normal development as well as tumor metastasis. By screening the C. elegans orthologs of genes up-regulated in invasive human melanoma cells, we have identified five pre-replication complex (pre-RC) components, mcm-7, cdc-6, cdt-1, orc-2 and orc-5, as regulators of AC invasion. The pre-RC is required for the licensing of replication origins during the G1 phase. Since the invading AC never replicates its DNA and remains arrested in G1, the similar penetrance of AC-specific and systemic mcm-7 RNAI suggest a cell-autonomous and cell cycle-independent function of MCM-7. An endogenous GFP::MCM-7 reporter
shows a transient nuclear expression pattern in the AC prior to invasion, suggesting a function of the pre-RC in inducing the epithelial to mesenchymal transition of the AC. Accordingly, MCM-7 regulates expression of metalloproteinase zmp-1 in parallel with FOS-1A. To further study the function of the pre-RC during AC invasion, we have inserted FRT sites into the mcm-7 locus, allowing us to perform a conditional mcm-7 knock-out.

We propose that components of the pre-RC have adopted a cell-cycle independent function in the AC to regulate cell invasion.

333B A mitochondrial isocitrate dehydrogenase prevents direct reprogramming of germ cells to neurons in C. elegans. Nida ul Fatima 1,2, Ena Kolundzic 1, Anna Reid 1, Baris Tursun 1 1) The Berlin Institute for Medical Systems Biology (BIMSB), Max Delbrück Centre (MDC) for Molecular Medicine in the Helmholtz Association; 2) National University of Sciences and Technology, Islamabad Pakistan.

Direct reprogramming makes use of transcription factors (TFs) that induce the identity of specific cell types. These TFs often act in a context dependent manner, and are restricted in most cell types by inhibitory mechanisms that maintain cell fates (Graf & Enver, 2009; Zhou & Melton, 2008). In order to identify these barriers, we study the model organism Caenorhabditis elegans (C. elegans) using the zing-finger TF CHE-1 that is required to induce the glutaemateric ASE neuron fate. Upon ectopic expression of CHE-1 and removal of barrier genes by RNAi, induction of the ASE neuronal fate marker gcy-5prom::gfp can be seen in a variety of cell types including germ cells, the epidermis and the intestine.

We identified a candidate barrier gene for reprogramming germ cells into neurons (we call this the GeCo phenotype), which is the NAD+ dependent mitochondrial isocitrate dehydrogenase idha-1. We observed that upon RNAi knockdown of idha-1 and ectopic expression of CHE-1, cells in the germline acquire neuron-like morphology and express a number of neuronal fate markers. Furthermore, upon ectopic expression of the Pitx family of homeodomain-containing TF UNC-30, which specifies the fate of GABAergic motor neurons, germ cells express a reporter for the GABAergic neuron fate upon knockdown of idha-1.

Recent studies on mitochondria in the context of reprogramming and iPSCs, show that mitochondrial dynamics change during the process of differentiation and cells show distinct mitochondrial and metabolic signatures during proliferation and differentiation (Bukowiecki et al. 2014). This suggests that disturbing mitochondrial function may feed back to chromatin thereby altering gene expression and allowing reprogramming. However, this process seems to be more specific since the depletion of most other genes that are required for mitochondrial function do not result in the GeCo phenotype. Interestingly, the idha-1 depletion-mediated reprogramming of germ cells to neurons is partially repressed in animals that lack the hypoxia-induced factor HIF-1. HIF-1 has been implicated in regulating iPSC reprogramming, a process that is triggered by changes in the level of the metabolite alpha-ketoglutarate (DeBerardinis et al., 2008; Xu et al., 2010). Importantly, alpha-ketoglutarate levels are regulated by isocitrate dehydrogenases such as IDHA-1 (Zeng et al., 2014; Zhang et al., 2015). Moreover, alpha-ketoglutarate is known to act as a co-factor of histone demethylases (Tsukada et al., 2006) and we are currently studying the underlying signaling mechanisms as well as the chromatin regulation involved during this cellular conversion.

334C Characterising mutations that increase seam cell number variability. R. Ghose 1, M. Fasseas 1, N. Gritti 2, C. Essmann 3, J. van Zon 1, M. Barkoulas 1 1) Department of Life Sciences, Imperial College London, London, United Kingdom; 2) AMOLF, Amsterdam, The Netherlands; 3) Department of Computer Science, University College London, London, United Kingdom.

Living systems have a remarkable ability to buffer against a multitude of perturbations and continually produce invariant phenotypes, known as robustness. One example of developmental robustness relates to cell fate patterning in C. elegans, which is so consistent that these animals are generally considered eutelic. We focus on the bilaterally present epithelial stem cell populations in C. elegans, the so-called seam cells, to identify factors that influence cell number variance using unbiased forward genetic approaches. In wild type, animals hatch with 10 seam cells on either lateral side and have a very invariant terminal seam cell number of 16 by adulthood. We show here that mutations in bus-19, which encodes a conserved transmembrane protein and srf-3, which encodes a nucleotide sugar transporter, lead to increased variability in seam cell number. Consistent with previous reports, we find that both mutations lead to a more permeable and morphologically defective cuticle. We show that srf-3 expression is highly specific to the daughter cell that maintains the seam cell fate potential following the asymmetric divisions, whereas bus-19 is expressed more broadly in the seam and hypodermis. Using time-lapse imaging of seam cell divisions, we characterise the errors that increase seam cell number variability. These errors occur more often during the L3 and L4 stages and are mainly of two kinds: conversion of asymmetric stem cell divisions to symmetric with both cell daughters either differentiating to hypodermis or retaining the seam cell fate, thus leading to a reduction or increase in terminal seam cell number respectively. We also see a large number of other patterning errors that do not modify the terminal seam cell number. These results identify a new link between seam cell patterning and cuticle integrity.

335A Characterising mutations that increase terminal seam cell number in C. elegans. I Razzaq, L Mestek Boukhbar, S. P. R. Gilbert, M Barkoulas Department of Life Sciences, Imperial College London, London, GB.

Regulating cell proliferation and differentiation is crucial during the development of any multicellular organism in order to generate, and then maintain, the correct number and function of cells and cell types. Impairment of regulatory pathways involved in these processes can lead to disease, the most common example being cell over-proliferation in cancer. The C. elegans epidermis contains stem-like seam cells that have previously been shown to be an excellent model for studying conserved molecular mechanisms controlling the balance between cell proliferation and cell differentiation (refs). However, our understanding of the seam cell gene network remains rather incomplete, thus many aspects of seam cell patterning and fate choice are yet to be fully understood. Through forward genetic screens and mapping by whole-genome sequencing, we have isolated alleles that modify terminal seam cell number. Using smFISH, lineage and genetic analysis, we have characterised two
conserved genes involved in seam cell development: *kin-3*, encoding the alpha subunit of the holoenzyme complex casein kinase II (CKII), and the zinc finger transcription factor *ztf-6*. Both genes are expressed in many tissues including the seam, and the identified mutations convert asymmetric divisions to symmetric, most commonly at the L4 stage. Consistent with previous results, we describe an upstream role of CKII in regulating the Wnt pathway. We show that *ztf-6* also acts through Wnt to restrict aberrant seam cell hyperplasia, with mutant alleles exhibiting abnormal symmetrisation of Wnt pathway targets in daughter cells of asymmetric divisions, leading to both cells adopting the seam (proliferative) cell fate. Intriguingly, the dosage of ZTF-6 appears to be tightly regulated in wild-type animals, as heterozygous loss-of-function alleles exhibit haploinsufficiency phenotypes and *ztf-6* over-expression causes loss of seam cell fate and seam hypoplasia. These results highlight that seam cell screens are not saturated and further our understanding of the regulation of fate choice in the seam cell lineages.

336B Expression of odd-skipped transcription factors in *C. elegans*. E. Del Buono, A. Groth Department of Biology, Eastern Connecticut State University, Willimantic, CT.

The odd-skipped transcription factors are conserved across metazoans, and are involved in early development and patterning. In mammals, they are needed for proper development of the kidney and heart, among other tissues. *C. elegans* has two odd genes, *odd-1* and *odd-2*. The DNA-binding domain of *odd-2* is most similar to both human versions, odd-skipped related 1 and 2 (OSR1 and OSR2). We have performed GFP reporter studies of the odd genes in adult *C. elegans*. We have confirmed strong expression of ODD-1 and ODD-2 in the posterior intestinal nuclei, although fainter expression can be seen in most intestinal nuclei. We have further shown that ODD-1 is localized to the nucleolus in the most posterior nuclei, while ODD-2 colocalizes with DNA and is largely excluded from the nucleolus. Additionally, ODD-2 is expressed in cells posterior to the intestine that appear to be the three rectal gland cells, rectD, rectVL and rectVR. The expression does not colocalize with a DsRed2 promoter reporter for BUS-1, a protein that is expressed in the rectal epithelial cells, K, K’, F and U. The ODD-2 expression is immediately anterior to the BUS-1 rectal epithelial expression, consistent with the position of the rectal gland cells.

337C Gene regulatory networks underlying cell fate specification of a *C. elegans* sensory/inter/motor neuron-type. W. Heo, K. Kim Brain and Cognitive Sciences, DGIST, Daegu, KR.

Neuronal differentiation and specification are orchestrated through a cascade of gene expression via interactions between trans-acting transcription factors and cis-regulatory elements of their target genes. However, the mechanisms of how specific transcription factors determine or specify neuronal cell fate during development are not fully understood. In *C. elegans*, the IL1 sensory/inter/motor neurons consist of six neurons that regulate the rate and pattern of spontaneous foraging movement (Kaplan et al., 1997). To identify molecular and cellular mechanisms by which the IL1 neurons are terminally differentiated, we searched for cis- and trans-acting factors that are necessary and sufficient for specification of the IL1 neurons. First, we performed promoter analysis of the set of genes that are specifically expressed in IL1s, including *flp-3* neuropeptide gene (Kim et al., 2004). We found several cis-regulatory regions of which deletion caused altered *flp-3* expression in the IL1 neurons. More specifically, we identified a motif that is necessary for *flp-3* expression specifically in the IL1U/R neurons. We are currently testing whether this motif exists in promoters of other IL1-expressed genes including *aakg-1* and *eat-4*, and this motif is also sufficient for the expression in IL1U/R. In addition, we performed candidate gene searches and mutagenesis screens to identify trans-acting factor of IL1s, and isolated several mutant animals which show decreased *flp-3* expression in the IL1 neurons. We are currently identifying molecular lesions in these mutants.

338A Cap-dependent protein synthesis is critical for embryonic development in *C. elegans*. Hayden P. Huggins, Andrew J. Friday, Jenna L. Hoffman, Brett D. Keiper Biochemistry and Molecular Biology, East Carolina University, Greenville, NC.

When and where specific proteins are synthesized is critical for determining cell identity and fate. Salient examples of this occur during gametogenesis and early embryogenesis in metazoans. Selective mRNA translational control and targeted protein turnover are cell-determining processes that generate an embryo from a fertilized egg. Yet, we know very little of how protein synthesis and turnover act in tandem in the early embryo to establish proper development. Here, we address translationally regulated mRNAs that may link synthesis and turnover between the oocyte and early blastula stages. Translation initiation comes in two flavors – one requires the mRNA cap binding protein eIF4E (cap-dependent), and one does not (cap-independent). Both begin by recruiting mRNAs to the scaffold protein eIF4G, and then to ribosomes. Some mRNAs translate more efficiently in a cap-dependent state, whereas others are efficiently translated in a cap-independent state. We previously showed that cap-independent translation increases germ cell apoptosis *in vivo*. Here, we show that cap-independent mRNA initiation also caused marked aberrations in embryonic development. Remarkably, *C. elegans* embryos genetically depleted of cap-dependent protein synthesis completed early cleavage events, but arrested prior to elongation. This suggests that embryonic mitoses do not require cap-initiated translation. However, arrested embryos were rounded and showed an increased frequency of germ mRNPs called P granules in somatic blastulae. We are currently characterizing mRNAs/proteins associated with eIF4G and eIF4E complexes, to evaluate overlap with P granules and other germ mRNPs. We assessed the translational status of all mRNAs in nematodes with normal and depleted cap-dependent translation by polysome RNA-Seq. We identified 144 mRNAs that depend on cap-mediated translation, and 55 mRNAs that have enhanced translation when the cap-independent mechanism predominates. Interestingly, several of these genes are implicated in autophagy (*e.g.*, *atg-13*, *epg-2*, and *epg-4*) and polarity establishment (*e.g.*, *occ-3*, *par-2*, and *par-4*). These cap-dependent mRNAs may be responsible for the phenotypes we observe. Our findings suggest that the balance of translation initiation mechanisms determines the spatiotemporal synthesis of lineage proteins and promotes somatic identity during embryogenesis in *C. elegans*. 

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Integration of environmental stress signals into the heterochronic developmental timing pathway via DA-independent regulation of daf-12. O. Ilbay, V. Ambros Molecular Medicine, Umass Medical School, Worcester, MA.

C. elegans development is remarkably robust against environmental stresses. Two major environmental sources of stress, food (type and quantity), and the population density pheromones, alter the developmental trajectory by affecting the overall rate of larval development and/or cell fate choices at the end of the L2 stage (L3 fates vs quiescence). However, despite the striking changes in the developmental trajectory, the temporal patterning of developmental cell divisions and cell fates is robustly maintained by the developmental timing pathway. A key component of the developmental timing pathway that coordinates temporal cell fates with the developmental trajectory is the nuclear hormone receptor DAF-12. In response to an endocrine signal, dafachronic acid (DA), DAF-12 controls both the developmental trajectory (reproductive vs dauer-interrupted) and the expression level of certain let-7 family microRNAs (high vs low). Our recent data suggest that DAF-12 integrates environmental signals into the developmental timing pathway also in a DA and let-7 independent manner. First, the developmental timing defects of the daf-12(rh61) mutant, which has low let-7 levels due to its inability to bind the DA ligand, is suppressed by growth in the presence of dauer-promoting ascarosides, or by the presence of hypomorphic alleles of daf-7 or daf-2, demonstrating a DA-independent interaction between environmental signals and the developmental timing pathway. Second, this ascaroside-mediated suppression of heterochronic phenotypes does not affect the levels of let-7 family microRNAs and does not require any particular let-7 family microRNA, suggesting a let-7-independent change in the developmental timing control in response to ascarosides. Moreover, we observe that a half-dose of the lin-28 gene [lin-28(If)/+] suppresses the developmental timing defects of the daf-12(rh61) mutant. This suppression requires lin-46, which was shown to perform developmental timing functions specific to the dauer-interrupted developmental trajectory. We propose that environmental stress signals are integrated into the developmental timing pathway via down-regulation of lin-28 in a DA and let-7 independent manner.

Revisiting the role of eff-1 in epidermal seam cell patterning. Sneha Latha Koneru1, Ritobrata Ghose1, Fu Xiang Quan1,3, Nicola Gritti2, Jeroen Sebastiaan van Zon2, Michalis Barkoulas1 1) Department of Life Sciences, Imperial College London, London, GB; 2) FOM Institute AMOLF, Science Park 104, Amsterdam, The Netherlands; 3) Department of Applied Mathematics and Theoretical Physics, University of Cambridge, Cambridge, GB.

Developmental patterning in Caenorhabditis elegans proceeds in a stereotyped manner and is considered highly invariant. We use the terminal number of seam cells as an experimental model to understand mechanisms of developmental robustness to stochastic noise. To this end, we have conducted mutagenesis screens to identify mutants showing increased variance in seam cell number without necessarily displaying changes in the mean. One of the mutations we have recovered represents a null mutation in the fusogen gene eff-1, which has been previously implicated in most cell fusion events in C. elegans including the fusion of the anterior cell daughter of the asymmetric seam cell divisions with the surrounding hyp7 syncytiun. To understand the developmental basis of cell number variability in eff-1 mutants, we performed long-term time-lapse imaging of post-embryonic seam cell divisions. We found that despite the fusion defects in eff-1 animals, seam cell division and differentiation patterns were mostly unaffected. This is largely because anterior cell daughters, despite their inability to fuse, differentiate normally by adopting a hypodermal fate, as evidence by the acquisition of hypodermal (dpy-7, elt-3) rather than seam cell fate marker expression (egl-18, nhr-73). We show that acquisition of hypodermal fate in anterior daughters correlated with breaks in adherens junctions, allowing their cytoplasmic continuity with the hyp7 syncytium. Surprisingly, we found that the rare decrease in seam cell number was explained by a loss of seam cell identity in early larval stages due to inappropriate differentiation of both daughter cells to hypodermis. However, these errors were mostly compensated at later developmental stages, due to loss of anterior daughter cell differentiation to hypodermis, which contributes towards an increase in seam cell number. Taken together, these results refine the role of eff-1 in epidermal seam cell patterning.

Variability in BAR-1/β-catenin activation drives a cell-autonomous stochastic cell fate decision by a feed-forward loop. J. Kroll, J. van Zon Quantitative Developmental Biology Group, Institute for Atomic and Molecular Physics (AMOLF), Amsterdam, The Netherlands.

Stochastic cell fate decisions occur frequently during animal development. In many cases, individual cells randomly choose one cell fate out of a limited repertoire of fates, but with the relative frequency of the different possible fates tightly controlled on the population level. It is not understood how signaling networks enable such cell-autonomous stochastic decisions and what network properties control the relative frequency of the resulting cell fates. To address these questions, we studied the stochastic differentiation of the C. elegans P3.p cell as a model system. During the L2 larval stage, P3.p randomly assumes vulva precursor cell (VPC) fate or fuses to the hypodermis. This decision is cell-autonomous and the relative frequency of VPC and hypodermal fate is strongly impacted by mutants for the signaling network underlying the decision. We use a novel time-lapse microscopy technique to measure the single-cell dynamics of BAR-1/β-catenin and LIN-39/Hox, two key regulators that inhibit hypodermal fate, while using cell fusion markers to monitor both the timing and outcome of the P3.p decision. Surprisingly, we found that mutations which strongly impacted the frequency of hypodermal fate had only limited impact on the timing of fusion. In addition, we found that LIN-39 levels in P3.p remained constant in time, but showed significant animal-to-animal variability. However, this variability did not correlate with cell fate outcome. In contrast, we found that BAR-1 levels exhibited a dramatic peak around the time of the P3.p decision. Cells that fused with the hypodermis showed lower levels of BAR-1 prior to fusion than those that assumed VPC fate, indicating that variability in the amplitude of the BAR-1 pulse is the primary driver of the stochastic cell fate decision. Our results suggest a model where an external trigger simultaneously induces activators of fusion and BAR-1, an inhibitor of fusion. In this model, the relative frequency of VPC or hypodermal fate is determined by the balance between the degree of activation relative to inhibition by BAR-1 and LIN-39. Such a mechanism, known as an incoherent feed-forward loop, would provide a new model for precisely-timed and cell-autonomous stochastic cell fate decisions.
Proper Wnt/β-catenin pathway regulation is essential in various organisms to ensure proper embryonic development, homeostasis in adult tissues, and asymmetric cell differentiation (ACD). In \textit{C. elegans}, SYS-1/β-catenin is required for transcriptional activation of Wnt target genes and is regulated by components of a conserved “destruction complex” as seen in other metazoans. Like mammalian β-catenin, the SYS-1 contains an unstructured N-terminal domain (NTD) followed by twelve armadillo repeats (R1-R12). The central armadillo repeats (R5-R8) interact with the β-catenin binding domain of POP-1/TCF in a conserved fashion, but SYS-1 lacks a C-terminal domain, which vertebrate β-catenin uses to recruit several transcriptional co-activators such as MED12 and CBP. Yet worm homologs of these activators are required for proper ACD. Yeast-1-hybrid assays reveal that the NTD and R1-R4 individually exhibit transcriptional activity. Further in vivo experiments done in worms showed that these fragments activate Wnt target genes resulting in additional distal tip cells (DTC), a Wnt signaled fate. The NTD and R1-R4, when linked to YFP, localized primarily to the nucleus. In contrast, the R5-R8 and R5-R12 fragments exhibit a reduced DTC and localize primarily in the cytoplasm. To address how SYS-1 nuclear localization is regulated, a mass spec screen was performed and provided candidate proteins. One such candidate, IMB-3, appears in preliminary data to be associated with SYS-1 import into the nucleus. \textit{imb}-3\textit{(RNAi)} showed a reduction in DTC number in worms, a phenotype consistent with loss of proper Wnt signaling due to decreased levels of nuclear SYS-1. In conclusion, these studies will aid in identifying SYS-1 transactivation and localization mechanism and possible novel mechanism of Wnt target gene regulation.

The \textit{C. elegans} achaete-scute homolog \textit{hlh-4} is a terminal selector of nociceptive neuron identity. \textit{N. MASOUDI}\textsuperscript{1}, L. Ryu\textsuperscript{2}, K. Kyuhyung\textsuperscript{2}, S. Tavaozo\textsuperscript{2}, O. Hobert\textsuperscript{1,4} 1) Department of Biological Sciences, Columbia University, New York, NY; 2) Department of Brain and cognitive Sciences, South Korea; 3) Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY; 4) Howard Hughes Medical Institute.

In the developing nervous system, proneural genes are among the most-early acting factors required to precisely govern the decision of an undifferentiated cell to become a neuron. bHLH transcription factors such as Drosoophila achaete-scute complex (AS-C) genes, their vertebrate orthologues Ascl/Math and \textit{C. elegans} AS-C homolog \textit{hlh-14} have been reported to carry such proneural activity. Thus far, bHLH-s have been shown to be the only regulatory factors to exhibit the proneural activities in only a handful of neurons in \textit{C. elegans}. To assess how broadly proneural genes are involved in \textit{C. elegans} neurogenesis, we undertook an expression pattern analysis. First, we systematically lineaged fosmid-based reporters for eleven of bHLHs. Unexpectedly, we found that one of the \textit{C. elegans} AS-C homolog, \textit{hlh-4}, is expressed and functions in a manner that is fundamentally different from classic proneural bHLH genes: In the developing embryonic, larval and adult nervous system, \textit{hlh-4} is exclusively expressed in a single, nociceptive neuron type, ADL. \textit{hlh-4} expression in ADL is maintained via autoregulation throughout the life of the animal. Moreover, rather than displaying proneural activity, \textit{hlh-4} is required to regulate the expression of ADL’s unusually large repertoire of GPCR genes, as well as all other known features of terminal ADL identity, including its nociceptive function. Further, the expression of these terminal identity features is mediated by a conserved E-box motif. We found that loss of the ADL-expressed LIM homeobox gene \textit{lin-11} also controls expression of multiple ADL markers and is also required for ADL-mediated avoidance behavior. This observation suggests that \textit{hlh-4} may act together with \textit{lin-11} to define ADL identity and function.

Taken together, we have shown an unusual function for a member of the proneural bHLH family, \textit{hlh-4}, to act as a terminal selector of ADL neuronal identity. Considering a previous report from our lab, in which we deciphered a role for \textit{hlh-14}, in neurogenesis of ADL-lineage, we conclude that two members of As-C orthologues in \textit{C. elegans}, \textit{hlh-14} and \textit{hlh-4}, act sequentially for neurogenesis (\textit{hlh-14}) and ensuing specification of ADL terminal identity (\textit{hlh-4}).

\textbf{344A SWI/SNF chromatin remodeling complexes regulate the SGP/hmc cell fate decision. \textit{LD Mathies, AG Davies, JC Bettinger} Dept of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA.}

The two somatic gonadal progenitors (SGPs) of \textit{Caenorhabditis elegans} are multipotent progenitor cells that give rise to all somatic tissues of the reproductive system, including uterus, sheath, spermatheca, distal tip cells and the anchor cell. The SGP sister cells are head mesodermal cells (hmcs); one undergoes programmed cell death and the other migrates anteriorly and terminally differentiates shortly after it is born. We use this cell fate decision, between SGP and hmc, as a simple and genetically tractable model to understand the determinants of multipotency. We have used forward and reverse genetic approaches to identify \textit{hnd-1}/βHand and three subunits of the SWI/SNF chromatin remodeling complex (\textit{swsn-1}, \textit{swsn-4}, and \textit{pbrm-1}) as regulators of the SGP/hmc cell fate decision. Loss-of-function mutants in any of these genes result in three phenotypes: 1) fewer than the normal number of SGPs, 2) SGPs that sometimes express a marker that is characteristic of the hmc, and 3) the anterior gonadal arm is missing more frequently than the posterior gonadal arm. We suggest that all three of these phenotypes result from SGPs that are partially transformed into hmc’s in the mutants. \textit{Hnd-1} is a transcription factor and SWI/SNF chromatin remodeling complexes are important for the proliferation of mammalian multipotent progenitors. The identification of downstream targets of SWI/SNF in the SGP/hmc cell fate decision may therefore uncover conserved molecular mechanisms regulating multipotency in diverse species.
Glii cells comprise half of our nervous system and play critical roles in its function, yet basic understanding of how glia arise during development remains surprisingly limited. For example, what molecules establish glial fate and how do glii become different from each other? To address these questions, we have turned to the well-defined and experimentally tractable nervous system of *C. elegans* as a simple model of glii cell type diversity. We have focused on specification of two different glii cell — the sheath and socket cells of the amphid sense organ. Although these two glii cells are in close proximity, they are morphologically and molecularly distinct: the sheath is larger, more highly secretory, and expresses different molecular markers than the socket. In a forward genetic screen, we identified sheath and socket fate defects caused by a missense mutation (W201G) in the DNA-binding domain of a conserved Forkhead transcription factor, UNC-130. Among mutant animals, ~25% lose expression of sheath-specific markers and ~35% independently exhibit ectopic expression of socket-specific markers in other cells. In contrast, animals bearing a different missense mutation (R218C) in a nearby region of the DNA-binding domain exhibit sheath fate defects yet do not have "extra" socket glia. These results suggest that UNC-130 affects sheath and socket specification by different means, for example, by binding to distinct classes of DNA sequence motifs. To test this hypothesis, we used protein-binding microarrays to measure the affinity of wild-type, W201G, and R218C variants of UNC-130 for all possible 10-mer DNA sequences. Wild-type UNC-130 preferentially bound motifs consistent with canonical Forkhead sites. UNC-130(W201G) exhibited almost no specific sequence binding, while UNC-130(R218C) retained the ability to bind high-affinity motifs but was severely impaired in its binding to some lower-affinity motifs. These results suggest a model in which UNC-130 binds distinct DNA motifs to control amphid sheath and socket glial fate, with both activities being lost in UNC-130(W201G) and only sheath-specific activities being lost in UNC-130(R218C). Importantly, the vertebrate homolog of UNC-130, FoxD3, has also been implicated in specifying glial fates. We found that expression of human FoxD3 completely rescues the glial fate defects of unc-130 mutants, suggesting that the glial specification pathways we uncover in *C. elegans* may be well conserved in humans.

**346C**  The subcellular localization of asymmetrically distributed mRNA transcripts during *Caenorhabditis elegans* early embryogenesis.  **D. Parker, E. Nishimura, M.T. Nishimura**  Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO.

Asymmetric cell divisions are important for cell-fate determination in the early embryo. After the first *C. elegans* embryonic cell division, roughly 200 mRNA transcripts partition asymmetrically between the AB and P1 daughter cells. However, the mechanisms driving their asymmetric inheritance are not known. We have observed distinct subcellular localizations for transcripts enriched in the AB or P1 daughter cells using single molecule Fluorescence In Situ Hybridization (smFISH). For example, *erm-1* transcripts were enriched at the AB cell cortex, *imb-2* transcripts at the nuclear periphery, and *chs-1, nos-2, clu-1, and cpg-2* transcripts in granular patterns in the P lineage. These findings lead to the hypothesis that a transcript’s subcellular localization drives its asymmetric distribution between AB and P1 daughter cells, which in turn impacts cell-fate determination.

smFISH, however, requires embryo fixation thus limiting the ability to observe transcript distributions in real-time. To overcome this limitation of smFISH, current efforts are underway to visualize mRNA localization in live embryos using an MS2 system. We aim to use this system to identify the sequences and machinery required to direct subcellular mRNA localization and to determine its role throughout early embryogenesis.

**347A**  Rap1 reinforces Ras signaling in *C. elegans* vulval patterning.  **N.R. Rasmussen¹, D.J. Dickinson², D.J. Reiner¹**  1) Center for Translational Cancer Research, Texas A&M HSC Institute of Biosciences and Technology, Houston, TX; 2) Department of Biology, University of North Carolina, Chapel Hill, NC.

The small GTPase Ras is the most mutated oncprotein. Its close relative Rap1 shares 100% identity in the core effector-binding region. Yet the role of Rap1 in Ras-dependent signaling remains unclear, particularly relative to their potentially shared effector, Raf. *C. elegans* encodes a single inessential Rap-1 isoform, so we are using vulva cell fate specification to ascertain the role of Rap-1 relative to LET-60/Ras and LIN-45/Raf. LIN-3/EGF induces the six equipotent vulval precursor cells (VPCs), resulting in a distinct and highly reproducible pattern of 3°-3°-2°-1°-2°-3°. 1° fate is induced via the canonical LET-60/Ras-LIN-45/Raf-MEK-2/MEK-MPK-1/ERK MAP kinase cascade, while 2° fate is induced by LIN-12/Notch. We hypothesize that initial cell fate specification is accompanied by partial re-wiring of signaling mechanisms, resulting in increased pattern fidelity via reinforcement of the initial pattern and suppression of contradictory signals. Fluorescently tagged endogenous Rap-1 localized to the plasma membrane of the six VPCs. The endogenous Rap-1(G12V) activating mutation was sufficient to induce ectopic 1° VPCs as well as excretory duct cell duplications, both hallmarks of activated LET-60/Ras. Conversely, rap-1-directed RNAi or deletion reduced excess 1° induction in sensitized backgrounds. We are currently testing whether endogenous Rap-1(G12V) can rescue the absent 1° VPCs and duct cells in the putative Ras null mutant, let-60(dx16). If successful, we will similarly test the ability of endogenous Rap-1(G12V) to rescue the absence of these cells in the putative Raf null mutant, lin-45(dx84). We have tagged endogenous MPK-1/ERK to measure activation-dependent nuclear translocation as a biomarker for in vivo LET-60/Ras and Rap-1 activation. We analyzed a transgenic promoter::GFP fusion of the Rap GEF, PFX-1, a potential upstream activator of Rap-1. Prior to LIN-3/EGF signal, GFP was expressed at low levels in all six VPCs. After LIN-3/EGF signal, GFP expression was increased in presumptive 1° but decreased in presumptive 2°s. Accordingly, we found that vulval-specific pxf-1-directed RNAi reduced excess 1°s in a sensitized genetic background, consistent with PFX-1 functioning to activate Rap-1 in presumptive 1° VPCs. We speculate that Ras>Raf>MEK>ERK signaling in presumptive 1° VPCs increases PFX-1 expression and hence Rap-1 activation, while exclusion of PFX-1 expression in presumptive 2° VPCs reduces Rap-1 activation in these cells. Thus, we hypothesize that the initial LET-60/Ras>LIN-45/Raf signal is reinforced by a positive feedback loop using Rap-1.
Our findings establish a novel regulatory feature of the vulval signaling network.

348B Genetic Control of the Maintenance of the AIA Cell Fate. Josh Saul\textsuperscript{1,2}, Takashi Hirose\textsuperscript{1,2}, Bob Horvitz\textsuperscript{1,2} 1) HHMI; 2) Dept. Biology, MIT, Cambridge, MA 02139.

Cell fate can be considered to involve two steps: establishment, in which an undifferentiated cell commits to and expresses a final differentiated state, and maintenance, in which a differentiated cell preserves the expression of its fate while precluding the expression of other fates. Cell-fate establishment has been well studied and is characterized by changes in gene expression, cell morphology and cellular function. Cell-fate maintenance has received comparatively little attention, and while several examples of defects in cell-fate maintenance exist, the mechanisms by which cell fate is maintained are poorly understood.

We have identified a gene, \textit{ctbp-1}, that appears to be involved in maintaining one or more cell fates in \textit{C. elegans}. \textit{ctbp-1} encodes the only worm member of the C-terminal Binding Protein (CtBP) family of proteins, which in mice and \textit{Drosophila} have been shown to act as transcriptional corepressors that regulate gene expression during embryonic development\textsuperscript{1-3}. \textit{ctbp-1} mutant adult worms show ectopic expression of an M4 neuron-specific reporter in the two embryonically-born AIA neurons, whereas L1 \textit{ctbp-1} animals do not display this abnormality. Furthermore, adult but not L1 \textit{ctbp-1} animals are defective in an AIA-mediated turning behavior. These observations suggest that AIA gene expression and function are normal in L1 \textit{ctbp-1} animals but abnormal in \textit{ctbp-1} adults, consistent with the hypothesis that \textit{ctbp-1} mutants properly establish the AIA cell fate but fail to maintain this fate.

We are further testing this hypothesis and investigating the mechanism by which \textit{ctbp-1} might regulate cell-fate maintenance. We performed an EMS mutagenesis screen for suppressors of the M4-specific reporter misexpression phenotype of \textit{ctbp-1} mutants to identify genetic interacting partners of \textit{ctbp-1} and have identified 21 independent isolates that we are currently mapping. We are also attempting to determine if any cells besides the AIAs are defective in cell-fate maintenance in \textit{ctbp-1} mutants.

We hope to better understand the genetic control of cell-fate maintenance by determining how \textit{ctbp-1} promotes the maintenance of the AIA cell fate. This work might provide insight into diseases, such as cancer, in which perturbation of the maintenance of tumor cell fate might intervene with the oncogenic process.


349C Identification of a novel Ral signal transduction cascade in \textit{C. elegans} 2° vulval fate patterning. H. Shin\textsuperscript{1}, R. Kaplan\textsuperscript{1}, T. Duong\textsuperscript{1}, D. Reiner\textsuperscript{1,2} 1) Institute of Biosciences and Technology, College of Medicine, Texas A&M University, Houston, TX, USA; 2) Department of Pharmacology and Lineberger Cancer Center, University of North Carolina, Chapel Hill, NC, USA.

Vulval precursor cells (VPCs) are patterned through graded action of EGF from the anchor cell (AC) in conjunction with LIN-12/Notch-mediated lateral signaling. Together these signals control the highly reproducible 3°-3°-2°-1°-2°-3° pattern that develops to form the vulva. A key protein in this process is LET-60/Ras, which in mammals is the most mutated oncprotein. LET-23/EGFR promotes LET-60/Ras activation to induce 1° fate via the canonical Raf-MEK-ERK MAP kinase cascade. We previously demonstrated that LET-60/Ras also switches effectors to activate its effector RalGEF-Ral to induce 2° fate in support of Notch. In general, 1°- and 2°-promoting signals are strongly antagonistic. Thus, in equipotent cells Ras switches effectors between Raf and RalGEF-Ral to promote mutually antagonistic 1° and 2° cell fates, respectively. However, the Ral signaling cascade is poorly understood in all systems. We therefore screened candidate Ral binding partners from the literature. Two subunits of the Exocyst complex have been previously shown to bind Ral in other systems. We found that loss of Exo84 but not Sec5 caused the same phenotype as loss of Ral, suggesting that Ral signals through Exo84 to promote 2° fate. To move further downstream we analyzed a little known family of MAP4K kinases. In \textit{Drosophila} Sec5 and Msn/MAP4K, the ortholog of \textit{C. elegans} MIG-15, physically interact and promote JNK signaling, but function in opposition to Ral signaling. Consistent with the \textit{Drosophila} genetics, our preliminary data in vulval development do not support the model that Sec5 and Msn function as a Ral effector. We hypothesize that the paralogous MAP4K subfamily, \textit{C. elegans} GCK-2 and \textit{Drosophila} Hppy, constitutes the real Ral effector. We found loss of GCK-2 phenocopies loss of Exo84, and GCK-2 functions cell autonomously downstream of Ral in the vulva. Using CRISPR/Cas9, we tagged the N-terminus of endogenous GCK-2 with mNeonGreen (mNG). mNG::GCK-2 is localized in the cytoplasm of 1° and 2° cells. The mechanism by which Ral and Exo84 activate GCK-2 remains unclear. We tagged endogenous Ral with mKate2 and observed red signal at plasma membrane and junctions. We also knocked a constitutively activating mutation into endogenous tagged Ral. We further found that Ral-Exo84-GCK-2 signals through a conserved MAP3K/MLK-1-MAP2K/MEK-1-p38/PMK-1 cascade. This cascade may further activate downstream MAPKAP kinase, MAK-2. We generated PMK-1::mNeonGreen to measure the activity of the Ral signaling cascade through activation-dependent cytoplasmic-to-nuclear translocation of p38. Our results support a model where Ral promotes 2° fate in support of Notch via MAP4K activation of a p38 MAP kinase cascade.
Temporal cis-regulatory control of embryonic fate specification.

Accurate adoption of cell fates during development requires transcription of developmentally important genes at the requisite time and abundance. In C. elegans, the number of cells, their division timing and lineage history is uniform, with only minor variations between animals. The need for temporal coordination is especially important in developmental contexts such as the C. elegans embryo, where rapid cell division occurs simultaneously with complex patterns of cell fate decisions, and where temporal misregulation could significantly alter the final pattern of cell fates. The E lineage provides an excellent test case for the study of temporal mechanisms because it is specified early, and clonally produces all intestinal cells. This requires a group of GATA transcription factors, which are expressed at different times in the E lineage. These include end-1, end-3 (transcribed beginning in E), elt-7 (transcribed beginning in E2) and elt-2 (transcribed beginning in E4, and maintained through the life of the animal by autoregulation). These factors share a common DNA binding motif, but the difference in timing of transcription of the E-specific genes during development raises the question of how this temporal specificity is achieved. We mined RNA-seq data from the Yanai lab to identify hundreds of genes specifically expressed either early (prior to E4) or late (after E8) in isolated E cell progeny. In the pharynx, temporal control is achieved by motif strength; early expressed genes have higher affinity for the pharynx-specific transcription factor PHA-4, allowing them to be bound and regulated earlier when the PHA-4 concentration is lower. In contrast, early E lineage genes actually have fewer promoter GATA motifs and less GATA factor binding in yeast one-hybrid data compared with late E lineage genes. This suggests that other factors may regulate early E lineage expression. Consistent with this, we identified several candidate transcription factors (TFs) with motifs enriched upstream of early genes including motifs for the E2F, Atf, Myb and Ets family TFs. All of these are expressed at the appropriate stage and thus are candidate temporal regulators. Intriguingly, a similar analysis of early vs. late genes expressed broadly across lineages identifies a different set of motifs, suggesting that dissimilar mechanisms may control their expression timing. To test the role of these predicted temporal regulators, we are examining the effect of their loss of function on the timely expression of E lineage genes using automated lineage tracing methods. This will be followed up by mutagenizing the motifs in the promoter regions of affected genes, and testing for TF binding to these motifs in vitro by electrophoretic mobility shift assays. Our results will help understand how the integration of lineage and cell division specific transcription codes direct expression timing and developmental robustness.

Investigating the role of the ARF GTPase arf-3 in regulating seam cell development and secretion.

We have recently identified the promoter region of arf-3, a small GTPase implicated in intracellular trafficking, as the tissue-specific driver of the seam cell marker scm::gfp (identified in an enhancer trap screen) commonly used as a fate marker of seam cells in the study of seam cell development. RNAi of arf-3 leads to variable seam cell numbers suggesting a possible failure in the regulation of seam cell divisions. Furthermore, a deletion mutant of arf-3, tm1877, causes embryonic lethality, with escapees dying as larvae with seam abnormalities. The deletion phenotype is difficult to interpret however, because the deletion removes an upstream ncRNA in addition to arf-3, thus we are currently producing a clean knockout strain by Cas9 mediated deletion of the arf-3 coding region. Although the arf-3 enhancer in the context of the scm::gfp marker is expressed exclusively in seam nuclei (due to the presence of a NLS) we found that a translational reporter of arf-3 is cytoplasmic with ARF-3::mCherry localised to distinct puncta within the seam cells. We have found that ARF-3::mCherry colocalises with RAB-7::GFP in the seam, suggesting a role for arf-3 in regulating late endosomes. The seam cells are known to play an important secretory function, giving rise to the cuticle and alae but the cell biology of this is very poorly understood, and we are interested in the potential role of arf-3 in this process.

To identify potential interactors of arf-3 we performed a targeted RNAi screen to look for mislocalisation of the ARF-3::mCherry translational reporter upon the silencing of genes involved in intracellular trafficking and signalling. We found that knockdown of apr-1, an orthologue of human APC (a component of the ß-catenin destruction complex) altered the localisation of ARF-3::mCherry. Simultaneous knockdown of arf-3 and apr-1 exacerbated the seam cell phenotype and also caused other morphological defects not observed in the single knockdowns. Given the involvement of apr-1 in Wnt signalling in C. elegans, this may suggest an interaction between arf-3 and the Wnt pathway. Preliminary evidence suggests that knockdown of arf-3 in the hypomorphic apr-1 allele, bp298, leads to fertility and synthetic lethality with escapees phenocopying the lethal arf-3(tm1877) allele. This suggests that arf-3 may play a role in regulating embryonic as well as seam cell Wnt signalling and supports an important role for intracellular signalling in regulating these processes.

You are what you experience: The impact of environment on cellular identity.

The cells of the organs and tissues of multicellular organisms require to maintain their specialized identity over time. However, under certain circumstances, cells can change their identity, a process known as Transdifferentiation or Td, to the benefits of the animal. The balance between maintenance of cellular identity and cellular plasticity (as the potential for a change in identity at the functional and morphological levels) is a major challenge for tissues inside an organism. Uncontrolled cell fate changes can cause several dysfunctional cellular behaviors such as cancer and degenerative diseases. Unraveling the mechanisms behind cell type conversion will help to develop a safe environment for regenerative medicine. Here, we describe how several external factors can impact on cellular identity and increase its plasticity potential.

We use a natural cell identity conversion in the worm to determine how a cell can change or maintain its identity. C. elegans
rectal to neuronal Y-to-PDA transition is a *bona fide* transdifferentiation event: During L2 larval stage the epithelial identity of the Y rectal cell is erased completely, followed by a very robust and unipotent redifferentiation into a fully functional motoneuron, PDA.

We previously described a subset of essential factors that are crucial for the initiation of Td such as *egl-27/MTA*, *sem-4/SALL*, *ceh-6/OCT* and *sox-2*, whose loss-of-function lead to severe defects in PDA formation. We identified two novel regulators of Td: *lin-15A* and *lin-56* that appear to act as licensers of the process. Their null mutants show a lower penetrance of PDA defects and are highly variable under different environmental conditions. We found starvation and caloric restriction, as well as virulence or different food sources to decrease PDA defects in these mutants, and thus, to increase the potential of cellular plasticity of the Y cell.

We will present our detailed analysis of the impact of the environment on cellular plasticity, and our evidence that a general mechanism may underlie the effect of these various external factors. Environmental impact - an often neglected aspect - might have more general implications to the use of cell identity conversions as a tool in regenerative medicine - and the worm might lead us the way to understand these.

**353A The role of ztf-16 in the maintenance of multipotency after dauer in *C. elegans*.** M. A. Hansen1, A. Matharoo1, T. A. Bernstein1, E. Montoye1, B. K. Lardie1, A. L. Daul2, A. E. Rougvie2, A. F. Alessi3, J. K. Kim3, X. Karp1 1) Biology, Central Michigan University, Mount Pleasant, MI; 2) Genetics, Cell Biology, Development, University of Minnesota, Minneapolis, MN; 3) Biology, Johns Hopkins University, Baltimore MD.

Most adult stem cells are multipotent and found in a state of reversible cell cycle arrest known as quiescence. However, the mechanisms that maintain multipotency during quiescence are relatively unknown. We use *C. elegans* as a model organism to study this process. *C. elegans* larvae develop through one of two different life histories: continuous or dauer. Dauer is a stress-resistant and developmentally arrested stage occurring after the second larval molt in response to adverse environmental conditions. If conditions improve, dauer larvae recover and complete development normally. *C. elegans* contains a set of stem cell-like seam cells that undergo specific division patterns at each larval stage, resulting in self-renewal and generation of hyp7 nuclei. During dauer, seam cells remain multipotent and quiescent, modeling mammalian stem cells. Following recovery from dauer, seam cells complete their development identically to larvae that did not experience dauer. Heterochronic genes ensure correct temporal control of seam cell division and differentiation. At adulthood, seam cells terminally differentiate, which can be visualized by the expression of the adult cell fate marker *col-19::gfp*. Intriguingly, many heterochronic genes that are required during continuous development are dispensable after dauer, suggesting that there is a separate genetic pathway operating during this time. To identify heterochronic genes that function after dauer, we performed a genetic screen for mutants with precocious post-dauer *col-19::gfp* expression. We screened 6000 genomes and isolated five independent alleles with high penetrance phenotypes. Using whole-genome sequencing, we found that two alleles contain mutations in *ztf-16*, which encodes a zinc finger transcription factor. We are currently confirming that *ztf-16* is the causal mutation by rescue experiments and looking for phenocopy with RNAi and established *ztf-16* mutations. Using established alleles plus our newly identified mutations, we found that *ztf-16* is required to prevent precocious *col-19::gfp* expression during both continuous and dauer development. Furthermore, passage through dauer does not correct the *ztf-16* precocious phenotype, making it unique among characterized precocious mutants. Ongoing genetic experiments are investigating the position of *ztf-16* within the heterochronic pathway and determining its relevant expression pattern. Characterization of *ztf-16* will shed light on mechanisms that maintain multipotency throughout quiescence.

**354B Endocrine signaling modulates *Caenorhabditis elegans* reproductive plasticity resulting from early life starvation.** Maria Ow, Alexandra Nichitean, Saraj Hall Department of Biology, Syracuse University, Syracuse, NY 13244.

Adult phenotypic plasticity in animals can result from programmed changes in gene expression due to environmental conditions experienced early in development. However, the mechanisms that modulate phenotypic plasticity are largely unknown. In *C. elegans*, stress during a critical period after hatching can result in larvae entering the developmentally arrested, stress resistant dauer stage. Larva exit dauer to resume continuous development only if environmental conditions improve. Our previous work has shown that adults that passed through dauer due to high thermoneutral conditions (PDPha) exhibit significant changes in gene expression profiles and an increased brood size compared to animals that experienced continuous growth (controls).

Here, we show that postdauer larvae that experienced starvation (PDShs) early in development retain a cellular memory of their early life history distinct from PDPha animals, and instead exhibit a significant decrease in their brood size compared to controls. Given that *C. elegans* hermaphrodites are sperm-limited, we hypothesized that altering the onset of germline proliferation in control and PDShs larva could result in the observed reproductive plasticity. We found that while the numbers of mitotic rows are similar between the two populations, the number of meiotic rows is significantly decreased in PDShs larva compared to controls, consistent with a delayed onset of germline proliferation due to starvation-induced passage through dauer. To explore potential signaling mechanisms regulating germline proliferation, we performed brood size assays for control and PDShs adults carrying mutations in genes of interest. Our results indicate that autonomous, but not systemic, endogenous RNAi is required for the brood size phenotype. Moreover, we find that endocrine signaling via nuclear hormone receptors DAF-12 and NHR-49 is required for the reduced brood size in PDShs adults. Taken together, our results indicate that autonomous RNAi and endocrine signaling are required for regulation of germline proliferation in PDShs animals, and suggest a model where endocrine signaling orchestrates signals between somatic and reproductive tissues to re-program an animal following the experience of starvation.
355C Identify and characterize novel factors that inhibit direct reprogramming to body wall muscular cell types in adult C. elegans. Yuliang Qu1,2, Andreas Ofenbauer1,2, Baris Tursun1,2 1) Berlin Institute for Medical Systems Biology, BIMSB; 2) Max-Delbrück-Center for Molecular Medicine, MDC, Berlin, DE.

Induced direct reprogramming has become a promising way to produce the desired cell resource in disease modeling, drug discovery, and regenerative medicine. However, achieving the process experimentally is difficult in most cases, because cell identities are usually safeguarded by defined maintenance mechanisms that act as barriers for direct reprogramming. Using C. elegans as an excellent model organism, we are systematically applying high-throughput genetic as well as molecular and biochemical methods to elucidate the mechanisms that inhibit direct conversion of body wall muscular cells from other differentiated types in vivo, regardless of their lineages of origin. To this end, we engineered transgenic strains for reverse genetic screening, where the myogenic regulatory factor in C. elegans, HLH-1, is ectopically expressed in both epidermis cells and intestinal types of adult worms. As expected, HLH-1 mis-expression alone failed to reprogram epidermal cells and/or intestinal cells into muscle-like cells. We hypothesized that the removal of refractory factors in combination with expressing c-ofactors of HLH-1 would be able to achieve the conversions. Given the important roles of epigenetic factors in regulating cell plasticity, we are currently exploiting the transgenic worms that express HLH-1 in the epidermis and intestine to screen the RNA interference (RNAi) library against chromatin regulating genes. The use of an automated large-particle sorter (BioSorter system) in conjunction with the LPsampler (both Union Biometrica) allows high-throughput RNAi screening from solid media and will be exploited for P0 and F1 RNAi screens. We hope the ongoing work will identify some more novel factors involved in cell fate specialization, cell identity maintenance or direct reprogramming in vivo.

356A Differentiated coelomocytes may be amenable to direct reprogramming into an array of cell fates in C. elegans. M. Quiniou1,2, A. Reid1, B. Tursun1 1) Max-Delbrück Center (MDC) for Molecular Medicine in the Helmholtz Association, Berlin, DE; 2) University of Exeter, Exeter, UK.

Although cell fate decisions are generally considered to be fixed, reprogramming studies are revealing that differentiated cells can be converted into other cell fates (transdifferentiation) by forced expression of specific transcription factors (TF). For instance, brief expression of the GATA-like TF ELT-7 in C. elegans is sufficient to reprogram committed, post-mitotic pharyngeal cells into differentiated intestinal cells (Riddle et al., Development, 2013). Similarly, ectopic expression of the C2H2-type zinc finger-containing TF CHE-1, responsible for specifying the ASE neuronal fate, is capable of converting fully differentiated cells. Recent work in the Tursun laboratory suggests that induction of CHE-1 leads to reprogramming of the mesoderm cells called coelomocytes into neuron-like cells, with striking morphological and transcriptional changes (Reid et al., unpublished data). We set out to assess the plasticity of coelomocytes by testing whether these cells can reprogram into an array of cellular identities including GABAergic neurons, seam, hypodermal, muscle and intestinal cells. Preliminary results suggest that coelomocytes are amenable to cell fate conversion. For example, ectopic expression of the basic helix-loop-helix (bHLH) TF HLH-1 may reprogram coelomocytes into muscle-like cells. A portion of coelomocytes appear to lose their ovoid shape, elongate and adopt a typical muscle-like morphology. These cells faintly express a marker for the endogenous coelomocyte fate suggesting a partial loss of the original cellular identity at the observed point during conversion. Coelomocytes and muscle cells arise from the mesoderm and are closely related in the cell lineage. We therefore tested if coelomocytes can convert into the more distant hypodermal and intestinal fates. Initial results suggest that coelomocytes expressing ectopic ELT-1, a TF involved in specifying the hypodermal fate, may lose their capacity for endocytosis. Furthermore, ectopic expression of ELT-7, a TF involved in specifying the intestinal fate, may lead to expression of the elt-2 intestinal fate reporter in the coelomocytes. However, these cells do not appear to undergo endoreduplication, as observed for the endogenous intestinal nuclei, suggesting a partial conversion of cellular identity. By understanding the extent of coelomocyte conversion and how functional the reprogrammed cells are, we hope to gain valuable insight into the mechanisms by which these cells convert and why coelomocytes display such remarkable cellular plasticity.

357B usp-48 is a negative regulator of plasticity during development. D.P. Rahe1, O. Hobert1,2 1) Biological Sciences, Columbia University, New York, NY; 2) Howard Hughes Medical Institute.

Both classic and more modern developmental biological experiments have shown that differentiation includes both the adoption of key cell fate markers as well as the restriction of developmental potential. The restriction of plasticity can be assayed by transcription factor (TF) overexpression experiments: the overexpression of a TF at an early stage of development often can induce the expression of its target genes, whereas overexpression in later stages has minimal to no effect, indicating a reduction in plasticity over developmental time. In order to understand this phenomenon, we generated mutants that lack the ability to restrict fate. In genetic screens we have isolated usp-48, a conserved, ubiquitous nuclear deubiquitinase, as a key regulator of cell fate in the hypodermis. In such mutants, the hypodermis adopts its cell fate normally, but no longer inhibits the ability of an overexpressed TF to induce target gene expression. This function is cell-autonomous and depends on the deubiquitinating activity of USP-48. Studies with a temperature-sensitive mutant reveal that loss of USP-48 at any stage results in the ability of an overexpressed TF to induce its target genes, indicating a continuous requirement for its function. Interestingly, in addition to TF overexpression now being able to induce target genes, the hypodermis loses several cell fate characteristics in usp-48 mutants upon TF overexpression. Using an improved INTACT protocol, we are generating and analyzing RNAseq profiles of wt and usp-48 hypodermal cells in both normal and TF overexpression conditions in order to reveal the extent and specificity of the changes in the usp-48 mutants upon TF overexpression. We have also begun to explore the mechanism of action of USP-48, which is likely affecting the nature of the chromatin landscape in the hypodermis during development. We have also isolated several new mutants with a similar phenotype as usp-48. Experiments to determine their role in relation to usp-48 are ongoing.
In vivo reprogramming of coelomocytes into ASE neurons in C. elegans. A. Reid¹, M. Quiniou¹,², A. Ofenbauer³, B. Tursun³ 1) Berlin Institute for Medical Systems Biology (BIMSB), Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Berlin, DE; 2) University of Exeter, Exeter, UK.

Understanding how cells maintain and safeguard their fate in vivo is a crucial step towards the efficient and direct reprogramming of differentiated cells into other cell types. Reprogramming studies in C. elegans have characterised several transdifferentiation events with, for example, the naturally occurring conversion of the epithelial Y cell into a PDA neuron (Jarriault et al., PNAS, 2008) and the adoption of the intestinal fate by pharyngeal cells following ectopic ELT-7 expression (Riddle et al., Development, 2013). In addition, Tursun et al. showed that ectopic expression of the C2H2-type zinc finger-containing transcription factor (TF) CHE-1, which specifies the fate of the gustatory ASE neurons, together with the knockdown of the reprogramming barrier lin-53 (homolog of human CAFC1-p48 (RBBP4/7)), leads to conversion of germ cells into neuron-like cells (Tursun et al., Science, 2011). We are currently developing novel and complementary systems to study induced transdifferentiation in C. elegans, driving CHE-1 expression from tissue-specific promoters. For one of these systems we utilize the six ovid, mesodermal cells called coelomocytes. Ectopic expression of CHE-1 in differentiated coelomocytes results in a striking change in cell morphology and identity. Reprogrammed coelomocytes express a reporter specific to the differentiated right ASE neuron in addition to a pan-neuronal marker. In addition, immunostaining showed that UNC-10 (homolog of vertebrate Rim1), a synaptic protein, was expressed in ASE marker-positive coelomocytes. Importantly, a portion of coelomocytes show gross morphological changes including shrinkage of the cell body and growth of long neuron-like projections. Our data suggests that coelomocytes may also be amenable to reprogramming into diverse cell types including GABAergic neurons and intestinal cells. Using our novel system we aim to gain valuable insight into why coelomocytes are amenable to TF-mediated reprogramming and the mechanisms by which these cells accomplish it.

Transdifferentiation is prevented by TLK-1 kinase that represses the level of histone variant H3.3. Y. Shibata, K. Nishiwaki Dept. of Bioscience, Kwansei Gakuin University, Sanda, Hyogo, JP.

In general, differentiation is the irreversible step that loose the potential to produce certain cell-types, although artificial transdifferentiation or induction of IPS cells overcomes the loss of developmental potential. We previously reported that histone H2A variant HTZ-1/H2A.z and acetylated histone H4 binding protein BET-1 prevent transdifferentiation in multiple cell lineages in normal development. Here, we report that, in addition to H2A variant, the balance between two histone H3 variants is also important for the maintenance of differentiation states. We screened mutants with extra-DTCs phenotype that is caused by abnormal transdifferentiation of somatic gonadal cells, as shown in bet-1 mutants. We isolated tlk-1 mutants that showed severer phenotype in adulthood than in larvae. It is consistent with abnormal transdifferentiation during the development. In addition to somatic gonadal lineage, tlk-1 is required in multiple cell lineages, including the Q and V5.pa lineages, indicating that TLK-1 functions as one of the fundamental factors that maintains differentiation states.

tlk-1 encodes a homolog of Tousled like kinase that phosphorylate histone chaperone ASF. It is known that ASF is upstream of histone chaperone CAFC1 that mediates replication-coupled nucleosome assembly by deposition of histone H3.1. We found that CAFC1 deficient mutant, chaf-1, is phenocopy of tlk-1 mutants in the somatic gonadal, Q, and V5.pa lineage. Interestingly, the levels of histone H3 variant, H3.3, were higher in the nuclei of tlk-1 and chaf-1 mutants compared to the wild type, suggesting that H3.3 is deposited instead of H3.1. Since H3.3 correlates with open chromatin, tlk-1 appears to maintain gene silencing through balancing between histones H3.1 and H3.3. Phenotype of tlk-1 mutant indicated that DNA-binding transcription factors that induce specific cell type are regulated by TLK-1. For example, extra-DTCs in tlk-1 mutants showed multiple DTC-specific characters. Since tlk-1 mutants have extra-AMVs, we examined mec-3 expression and found that ectopic mec-3 expression occurred. All together, TLK-1 maintains differentiation state through silencing of genes that encodes DNA-binding transcription factors through regulation of histone H3 deposition. Since CAFC1 prevents transdifferentiation and induction of IPS in mammals, our results raise the possibility that the transdifferentiation inhibitory mechanism involving TLK-1 and CAFC1 is evolutionarily conserved.

Transcriptome and functional analysis of ELT-7-directed reprogramming and transorganogenesis of the C. elegans gonad and pharynx. E. Spickard, J. Rothman MCDB, UC Santa Barbara, Santa Barbara, CA.

Organogenesis is a tightly controlled process that ensures a robust and stable developmental output through regulatory mechanisms that buffer against changes in the fates of cells once they have differentiated. We have found that post-mitotic, fully differentiated cells of the C. elegans pharynx, and cells undergoing organogenesis of the somatic gonad, can be redirected to develop into an intestine-like organ by forced expression of the ELT-7 GATA-type transcription factor. This reprogramming, which does not require inhibition of other factors or the generation of dedifferentiated intermediates, occurs within 20 hours of ELT-7 induction. The reprogrammed somatic gonad, which has undergone what we have called “transorganogenesis,” is virtually indistinguishable at the ultrastructural level from that of the endogenous intestine. To investigate the mechanisms of this cell fate switching, we analyzed the transcriptional profiles of worms during early transorganogenesis by mRNA-seq. Statistical comparison of worm transcriptomes at three hours after hs-elt-7 induction, versus a hs-gfp control, identified over 5,000 strongly differentially expressed transcripts. Tissue enrichment analysis of differentially expressed genes (DEGs) revealed specificity for somatic gonad-related genes based on anatomy ontology terms, particularly among genes that are downregulated by ELT-7. These results suggest that, while the hs-elt-7 construct is expressed globally, the somatic gonad is specifically susceptible to alterations in gene expression patterns. Transcription factor binding site (TFBS) predictions for DEGs that are upregulated by ELT-7 reveal enrichment for GATA binding sites, indicating that a significant fraction of early transcriptional changes may be attributable to the direct activity of ELT-7 and/or other GATA factors. TFBS

Developmental transitions can be observed as changes at the level of cellular and molecular composition, morphology, and behavior. The complexity of the nervous system with its highly connected network of diverse cell types requires the gradual maturation of post-mitotic neurons as they migrate, extend dendrites and axons, and form novel connections throughout much of the organism’s lifetime. This intricately timed developmental sequence imply fundamental molecular mechanisms to coordinate the maturation of the nervous system, however, there is a lack of comprehensive understanding of both the molecular composition of the nervous from juvenile to adult life stages and of the mechanisms regulating this maturation. Here, taking advantage of the large libraries of existing fluorescent transcriptional reporters that exist within the C. elegans community, we have identified candidate from several functionally important gene families (GPCRs, innexins, synaptic molecules) in the nervous system that demonstrate developmentally-regulated expression patterns. Concurrently, we are taking a genome-wide approach (isolation of nuclei from the entire nervous system as well as individual neuron pairs) by profiling the transcriptome of the nervous system across developmental stages. Furthermore, we have characterized the behavioral plasticity of C. elegans by tracking its locomotor behavior across developmental stages. In addition to further characterizing the role of the heterochronic pathway in the developmental regulation of post-mitotic nervous system maturation, we are conducting cis-regulatory element dissectional analysis and EMS mutagenesis on several candidate genes (inix-19, lin-4) that are not regulated by the heterochronic pathway to reveal novel regulators of neuronal developmental plasticity. Together, we aim to elucidate upstream mechanisms coordinating post-mitotic nervous system development and maturation.

362A Deciphering the role of Notch signaling in Y-to-PDA transdifferentiation in vivo in C. elegans. L. Vibert, Daniele Thomas, Fischer Nadine, Jarriault Sophie Developmental Biology and Stem Cells, IGBMC, Illkirch CEDEX, FR.

Cell differentiation is a key process in Developmental Biology as it is essential during organism development; in addition, dysregulation of stable cell differentiation can lead to cancer. Manipulation of the cell differentiation process has seen great progress the last ten years leading to the demonstration, in vitro, of the capacity of cells to dedifferentiate by forcing the expression of pluripotency transcription factors in differentiated cells. However, whereas the transcription factors used for pluripotent reprogramming have led to the successful reprogramming of all cell types reported, intriguingly, several studies showed that direct reprogramming induced by one or more transcription factors is only fully effective in a restricted number of cell types, i.e is cell context-dependent. Thus, as cells could differ in their abilities to be reprogrammed by a given inducing method, some cells appear more permissive to reprogramming. To understand what makes a cell prone to change its identity, we have used an exceptional in vivo event of single cell identity change during C. elegans development. More precisely, during Y-to-PDA transdifferentiation, the rectal Y cell goes through a step-wise process to erase its rectal identity and acquire another, neuronal, identity (to become the PDA motoneuron). The dramatic changes observed in markers expression suggest that these changes are driven by activation of key regulators which would allow the rapid switch from a cell state to the next one. Previous work in the laboratory has suggested that the Y cell acquires the competence to change its identity after its birth in the embryo, rather than inheriting it during its lineage history. Importantly Y is competent for cell transdifferentiation, but its neighbouring and seemingly identical rectal cells are not. We have found that one key difference between the Y cell and the other rectal cells that do not change their identity is that the sole Y cell expresses the lin-12/Notch receptor. Furthermore, this expression appears to be dynamic and restricted to the embryogenesis stage. Importantly, we found that a pulse of the LIN-12/Notch receptor activity is sufficient to convert another cell, that we have identified as AB.pppppaaa, into a competent Y cell that then Td into an additional PDA neuron. The major aim of the project is to identify the target genes that Notch uniquely activates in the Y cell and that may be involved in setting up the competence to reprogram. This work will also lead to investigate the gene regulatory network that allows a cell to transdifferentiate - but not its neighbours.

Development - Germline Development

363B A tail of two transcriptomes: the landscape of cytoplasmic polyadenylation in C. elegans. Peter Boag1, Paul Harrison2, Adele Barugahare1,2, Andrew Pattison1, Angavai Swaminathan1, Stephanie Monk1, Gregory Davis1, Eva Heinz1, David Powell2, Traube Beilharz1 1) Development and Stem Cells Program, Monash Biomedicine Discovery Institute and Dept. of Biochemistry and Molecular Biology, Monash University, Melbourne, VIC, Australia; 2) Monash Bioinformatics Platform, Monash University, Melbourne, VIC, Australia.

During oogenesis the germline nuclei synthesise at least two distinct mRNA populations; one required for immediate germ cell functions, and another for late oocyte and early zygote development. To study this division, we mapped the pathway of transcriptome separation through changes to mRNA poly(A)-tail length-distributions. By comparing the adenylation-state of adult gld-2(q497) and wild type transcriptomes, we identified more than 1000 targets of GLD-2 mediated cytoplasmic polyadenylation through statistically significant changes in their steady-state polyadenylation. Among the mRNA with the most significant change in poly(A) length-distributions were those encoding RNA binding proteins, such as mex-5, pos-1 and oma-1, known to regulate spatial and temporal patterning of embryonic programs. The 3'UTR of mRNAs that depend on cytoplasmic polyadenylation were overall longer, showed enrichment of non-standard adenylation-sites (eg AAUGAA) and contained more cytosine residues. To identify the initiating deadenylase responsible for silencing mRNA, we depleted the five known deadenylases in the gld-2 mutant by RNAi. Only, the loss of ccf-1 suppressed the short-tailed phenotype of GLD-2 target mRNA, suggesting that in addition to its
general role in RNA turnover, this is the major silencing deadenylase for the oocyte transcriptome. We present a model whereby the nuclei of the adult maternal germline are responsible for the transcription of at least two separate gene-expression programs: the ‘ready-to-wear’ transcriptome that sustains and builds the germ cells and oocytes and the ‘couture’ line, selected, silenced and partitioned, waiting for specific cues for reactivation during oocyte maturation and early embryo development.

### 364C LIN-35 is necessary in both the soma and germline for buffering fertility in *C. elegans.* Brian Mikeworth, Frances Compere, Lisa Petrella Biological Sciences, Marquette University, Milwaukee, WI.

The temperature sensitivity of the germline is conserved across phyla but the molecular pathways that are sensitive to temperature in the germline are unknown. The study of mutants that show a loss of fertility at temperatures lower than wild type organisms may allow us to uncover pathways that are important for buffering fertility at high temperature. We have uncovered a unique temperature sensitive phenotype in the primordial germ cells of *lin-35* mutants; *lin-35 L1s* raised at 26°C fail to form canonical perinuclear punctate P granules ~20% of the time. Does LIN-35 therefore play a role in buffering germ line function in response to temperature as it does in somatic tissues? *lin-35* mutants are known to have a reduced brood size even at 20°C but the temperature sensitivity of *lin-35* germine defects have not been explored as *lin-35* mutants raised at 26°C demonstrate ~100% larval arrest. We have developed two transgenic strains that rescue *lin-35* expression in either all somatic cells or solely in intestinal cells which rescues *lin-35* high temperature larval arrest, allowing investigation of the role of *lin-35* in the germline at 26°C. Interestingly, both transgenic lines partially rescue the fertility defects of *lin-35* mutants at 20°C, indicating that part of the loss of fertility in *lin-35* mutants is due to a loss of *lin-35* function in the soma. However, both transgenic lines are almost completely sterile at 26°C, a temperature 1°C below that which causes sterility in wild type. This suggests that *LIN-35* functions within the germline to buffer high temperature stress. The buffering of temperature effects appears to be a conserved feature of *LIN-35* function as many *lin-35* phenotypes, including synMuv and somatic germline gene repression, are temperature sensitive. However, unlike these other phenotypes, we have found that *lin-35* temperature sensitive fertility is not maternal effect and can be rescued by zygotic expression. As loss of fertility in wild type germ lines has been linked to loss of sperm function, we supplied *lin-35* transgenic worms with wild-type sperm. Sterility was not rescued at 26°C in either transgenic background. However, there was ectopic localization of male sperm in the uterus in mutants expressing *lin-35 (+)* only in the intestine, but proper localization of male sperm in the spermatheca in the mutants expressing *lin-35 (+)* pan-somatically. This data suggests that LIN-35 may be necessary in the somatic gonad under high temperature stress conditions. We are currently testing this hypothesis with tissue specific expression. Taken together, we have found unique roles for LIN-35 in the both the soma and germline that are necessary for fertility in *C. elegans* when faced with high temperature stress.

### 365A Visualizing nuclear GLP-1/Notch NICD. Sarah L. Crittenden1,2, ChangHwan Lee1,2, Erika Sorensen1,2, Sindhu Battula3, Tina R. Lynch2, Hannah S. Seidel1,2, Cecilia Lei2, Judith Kimble1,2 1) Howard Hughes Medical Institute, University of Wisconsin-Madison, Madison, WI; 2) Department of Biochemistry, University of Wisconsin-Madison, Madison, WI.

Notch is a conserved regulator of stem cells and differentiation. Upon activation by ligand, the Notch receptor is cleaved, releasing its intracellular domain (NICD) for assembly of a nuclear complex and transcriptional activation of downstream targets. Our work focuses on Notch signaling in the *C. elegans* germline stem cell niche, where well-defined signaling and receiving cells facilitate quantitative analyses. We recently reported that the transcriptional response to GLP-1/Notch activation occurs in a steep gradient across the germline stem cell pool (Lee et al., 2016). An outstanding question is whether this transcriptional gradient reflects a corresponding gradient in GLP-1/Notch activation. We are visualizing nuclear GLP-1/Notch NICD as a readout of GLP-1/Notch activation. Our reagents include MOS-SCI transgenes encoding a tagged GLP-1 receptor that rescues a *glp-1* null allele as well as CRISPR-inserted tags at the endogenous locus. To date, we have detected signal with Myc, V5, OLLAS and Halo tags. The signal is low, however, and we are working on methods to bring the signal into a quantifiable range without changing receptor abundance. Our progress will be reported at the meeting.

### 366B Dynein Light Chain 1 Participates in Post-Transcriptional Regulation of Germline mRNAs. N. Day, M. Ellenbecker, E. Voronina Division of Biological Sciences, University of Montana, Missoula, MT.

Post-transcriptional regulation in the *C. elegans* germline is critical for the maintenance of stem cells. RNA binding proteins (RBPs) are involved in maintaining the balance between differentiation and proliferation in these stem cells. Our lab has previously identified dynein light chain 1 (DLC-1) as an important cofactor for the function and localization of the RBP FBF-2, independent of the dynein motor (Wang et al., 2016). DLC-1 is known to have multiple interacting partners, therefore we hypothesize that DLC-1 interacts with and regulates many RNA binding proteins. The interaction network of DLC-1 with germline RBPs is not well understood and necessitates further study. Here we investigate the role of DLC-1 in promoting RBP function and study its role in a post-transcriptional regulatory network. We generated a FLAG-tagged DLC-1 transgene that rescues the loss of function *dcl-1* mutant and used this worm to co-immunoprecipitate DLC-1-associated RBPs and their mRNA targets. High throughput sequencing was used to identify what mRNAs associate with DLC-1 in vivo. Using the R package RIPSeeker (Li et al., 2013), we have identified mRNAs that are enriched in the FLAG::DCL-1 pulldown. Our list contains mRNAs that are at least three-fold enriched in the pulldown compared to the input library (Zhao et al., 2010). DLC-1-associated mRNAs are significantly enriched in oogenic transcripts but are depleted in spermatogenic transcripts (Ortiz et al., 2014). Some mRNAs identified in our pulldown were also found in association with specific RBPs reported by other groups, suggesting a potential co-regulatory role of DLC-1. We find that approximately 30% of FBF-2 targets (Prasad et al., 2016) associate with DLC-1, indicating that our RIP-seq approach captures the known interaction and will be useful for studying unknown DLC-1/RBP interactions. We also find that other reported RBP targets vary from 25 to 41% overlap with the DLC-1 associated mRNAs. We expect that overlap of 30% or greater suggests that RBPs may interact with and rely on DLC-1 for function. We are using reporter assays to study how DLC-1 binding influences the expression of a subset of these enriched mRNAs in the germline. Using g:profiler, our
gene ontology search of mRNAs enriched in the FLAG::DLC-1 pulldown indicates that DLC-1 may regulate many mRNAs that are responsible for a wide range of functions including: cell fate specification and RNA metabolism. These findings suggest that DLC-1 is an important component of translational regulation of germline mRNAs.

367C Knockout of *C. elegans* Ariadne/ARI family members leads to partial sterility and masculinization of the germline.  K.R. Di Bona, D.S. Fay  The University of Wyoming, Department of Molecular Biology, Laramie, WY.

The addition of ubiquitin (Ub) to a protein substrate (ubiquitination) is a critical regulatory post-translational modification, which can lead to degradation via the proteasome, translocation, or alteration of the activity of the targeted protein. *C. elegans* ARI-1 is a highly conserved member of the Ariadne (ARI) RBR E3 Ub-ligase subfamily. Though ari-1 (*C27A12.8*) is expressed in numerous tissues including neurons, muscles, and the germline, strains homozygous for a null mutation in ari-1 (tm2549) are viable and do not lead to obvious defects. The absence of a strong phenotype in ari-1 mutants is likely due to genetic redundancy, as *C. elegans* possess two additional ari family members adjacent to ari-1 (*C27A12.6 and C27A12.7*).

Recently, our lab has utilized CRISPR technology to generate a null triple mutant in all three ari homologs [ari3X]. Three independent strains were obtained that contain large deletions in the coding regions of C27A12.6, 7, and 8. Initial observations indicate a reduced brood size and ~25-60 % sterility in the ari3X mutants. Sterile ari3X mutants were found to have a normal soma but exhibit excessive sperm production, consistent with a Mog (masculinization of the germline) phenotype. Likewise, a null allele of *ubc-18 (tm5426)*, which encodes a conserved E2 partner of ARI proteins, also exhibits partial sterility and Mog. Furthermore, an independent screen recently reported a synthetic lethal interaction between *ubc-18* and *fbf* family members. Consistent with this, we find that both *ubc-18 (tm5426)* and ari3X mutants are hypersensitive to *fbr(RNAi)*, resulting in a highly penetrant Mog phenotype. In addition, ari3X; *fbr*(RNAi) worms exhibited a multivulval (Muv) phenotype and reduced germline proliferation (Glp), which have previously been observed in strong loss-of-function mutations in the *fbfs*.

FBF-1 and FBF-2 are closely related RNA-binding proteins, which have the ability to bind thousands of different mRNAs and have been shown to regulate targets involved in germline development. Our data indicate that ARI-mediated ubiquitination plays an integral role in germline development and that ARI–UBC-18 functions coordinately with the FBFs to promote germ cell proliferation and oocyte differentiation. Studies are ongoing to determine whether ARI–UBC-18 affects FBF levels or corepresses specific germline targets with the FBFs that are involved in spermatogenesis.

368A Interactions between dynein, dynactin and MEL-28 impact fertility in *C. elegans*.  G. Vida1, A. Tacinelli1, A. Quental1, J. Conklin1, A.C. Agudelo Rivera1, F. Piano1, K.C. Gunsalus2, A.G. Fernandez1,2  1) Biology Department, Fairfield University, Fairfield, CT; 2) Biology Department and Center for Genomics and Systems Biology, NYU, New York, NY.

Most cellular processes require inputs from multiple gene products. We have been studying genetic interactions that include *mel-28*, a conserved and essential gene with key roles in the post-mitotic rebuilding of the nuclear pore. As a classic maternal-effect lethal gene, *mel-28* mutants from heterozygous mothers live to produce normal-sized broods that consist entirely of dead embryos. We did an RNAi screen to identify genes that cause sterility in *mel-28* mutants. Among the genes we found were those encoding components of dynactin, a minus-end directed MT motor, and dynactin, a complex known to help couple dynactin to its cargo. To better characterize phenotypes caused by disrupting these genes simultaneously, we have generated double and triple mutants using a severe hypomorphic *mel-28* allele and temperature sensitive mutants of *dnc-1*, which encodes the heavy chain of dynactin, and *dnc-1*, which encodes the *p150<sup>siai</sup>* subunit of dynactin. We have found that *mel-28*(t1684);*dnc-1 (or483ts)* double mutants show a severely reduced brood size compared with each single mutant. Reciprocal crosses suggest the female gonad is defective in *mel-28; dnc-1* double mutants, and indeed the proximal gonad is disorganized in these animals. The *dnc-1* (or404ts) mutant has a fertilization defect that is rescued in the *dnc-1; mel-28* double mutant, suggesting that *dnc-1* and *mel-28* act antagonistically in fertilization. While *dnc-1; dnc-1* double mutants showed a similar fertilization defect compared to the *dnc-1* single mutant, *dnc-1; mel-28; dnc-1* triple mutants resembled the rescued *mel-28; dnc-1* double mutant. This suggests that the *dnc-1* (or404ts) defect does not influence the loss of fertility in *dnc-1* mutants nor its rescue by disruption of *mel-28*. The triple mutant brood size was significantly larger than the brood size in the *dnc-1; mel-28* double mutant, suggesting that loss of *dnc-1* function mitigates the fertility defects caused by disruption of *mel-28* and *dnc-1*. Taken together this suggests that *dnc-1* and *mel-28* act antagonistically in a fertilization process that is indifferent to defects caused by *dnc-1* (or404ts). In contrast, fertility defects caused by the *mel-28; dnc-1* lesions affect processes that are sensitive to dynactin function.

369B Complexes regulating *C. elegans* eggshell formation and egg activation are scaffolded by a common protein.  Delilna Gonzalez, Helen Lamb, Diana Partida, Zachary Wilson, Sara Olson  Pomona College, Claremont, CA.

Fertilization triggers rapid remodeling of the zygote surface to create an extracellular barrier that protects the developing embryo and prevents polyspermic fertilization. In nematodes, this barrier is a multi-layered eggshell that assembles in a hierarchical manner. The outermost layer of the eggshell is called the vitelline layer, which is present on the oocyte before fertilization. Immediately following fertilization, the vitelline layer lifts off the zygote and initiates remodeling of the extracellular surface. Despite its importance, little is known about the composition of the vitelline layer or how it is remodeled to form the outermost eggshell layer. A previous RNAi screen in *C. elegans* identified PERM-2 and PERM-4 as proteins required for eggshell formation. PERM-2 and PERM-4 localize to the vitelline layer and are co-dependent for proper localization based on RNAi and CRISPR null allele studies. PERM-2 and PERM-4 localization also depends on CBD-1, a protein previously shown to stabilize the EGG/CHS-1/MBK-2 complex involved in the oocyte-to-embryo transition. Interestingly, CBD-1 appears to scaffold the PERM complex and the EGG/CHS/MBK complex independently via distinct domains, suggesting dual roles for CBD-1 in eggshell formation and regulation of egg activation. Depletion of PERM-2/4 compromises the structural integrity of the outermost
vitelline layer of the eggshell, but also disrupts subsequent assembly of the innermost permeability barrier that serves as the major line of defense in protecting the newly fertilized embryo.

370C  **HOP-1-deficient hermaphrodites exhibit a delayed Notch phenotype.**  K. Kim, J. Jiang, B. Brady, D. Leet, R. Solomon, V. Hale, C. Goutte  Department of Biology, Amherst College, Amherst, MA.

The final activating step in the Notch signaling pathway is the proteolytic cleavage that releases the intracellular receptor domain from its transmembrane domain tether. This cleavage is mediated by the gamma-secretase complex, whose four subunits have been shown to be essential for Notch signaling. The presenilin subunit contains the catalytic core of the enzyme complex, and many organisms contain two alternative forms of presenilins. In *C. elegans*, Notch signaling is mediated by the SEL-12 and HOP-1 presenilins. For most known Notch signaling events, SEL-12 and HOP-1 function redundantly, such that Notch activation can be successfully mediated by either presenilin. The SEL-12 presenilin mediates at least one Notch signaling event for which HOP-1 is not available, or is otherwise not effective, because sel-12 mutant hermaphrodites display a penetrant egg-laying defective phenotype that is similar to that of lin-12 Notch hypomorphic mutants. In contrast, a unique role for the HOP-1 presenilin has not yet been demonstrated. We analyzed the null hop-1(ak179) phenotype and demonstrate a reduction in germ line output, as measured by self-brood size, mated brood size, and reproductive span. Normally, GLP-1 Notch signaling in the distal gonad is necessary to support germ cell proliferation during larval development as well as continuously during adulthood. In the absence of any presenilin function, germ cells prematurely enter meiosis without significant proliferation, resulting in a sterile animal. Since both hop-1 and sel-12 single mutants are fertile, a likely explanation for the reduced fecundity of hop-1(ak179) hermaphrodites is that HOP-1 and SEL-12 presenilins contribute additively to the total Notch signaling capacity of the gonad, such that both presenilins are always needed for maximum fecundity. Our investigation into the underlying cause of hop-1(ak179) reduced fecundity revealed a different explanation: First, we found that hermaphrodite oocyte production, but not spermatogenesis, is compromised in hop-1(ak179) hermaphrodites. Second, we found that hop-1(ak179) animals begin adulthood with robust germ cell proliferation, but then fail to sustain proliferation through adulthood. We conclude that hop-1(ak179) animals have reduced fecundity because Notch signaling ceases in the adult gonad, generating a "delayed Notch phenotype." These results reveal an absolute requirement for HOP-1 function in adult gonads, where SEL-12 is likely unavailable. We are currently mapping the timing of the switch to HOP-1-dependence, and its ramifications on germ line maintenance in the adult.

371A  **CYK-4 functions independently of kinesin-6 to promote intercellular bridge closure during oocyte formation in the syncytial *C. elegans* germline.**  R.A.* Green1,2, K.Y.* Lee1,2, E Gutierrez2, J.S. Gomez-Cavasas1,2, A.B. Desai1,2, A Groisman2, K.F. Oegema1,2  1) Ludwig Institute for Cancer Research; 2) University of California, San Diego.

In metazoa, germ cells undergo incomplete cytokinesis to form syncytia with cells connected by intercellular bridges. Centralspindlin, a complex containing two molecules of kinesin-6 and two of the Rho family GTPase-activating protein (GAP) CYK-4, is a conserved bridge component. Here, we analyze centralspindlin function in the *C. elegans* oogenic germline, which contains ~1000 cellular compartments connected by intercellular bridges to a cytoplasmic core. In contrast to cytokinesis, where both centralspindlin components are essential for spindle midzone and contractile ring assembly, CYK-4, but not kinesin-6, is required for a germline cytokinesis-like event where intracellular bridges close to bud oocytes off the syncytium. The CYK-4 C1 domain, predicted to interact with the plasma membrane, and GAP domain interface predicted to interact with RhoA. Hence, knockdown of RhoA, but not Cdc42 or Rac, disrupted germline structure, suggesting that the GAP domain recruits CYK-4 to intercellular bridges by binding RhoA.

372B  **The putative helicase ddx-15 interacts with other spliceosomal proteins, suggesting a dual role in splicing and ribosome biogenesis.**  J.E. Karpel  Biology, Southern Utah Univ, Cedar City, UT.

*ddx-15* is a putative helicase of the "DEAH box" family that is a homologue of the yeast spliceosomal protein, Prp43p. In yeast, Prp43p is required for the release of the lariat intron from the spliceosome and also plays a significant role in ribosome biogenesis. Although Prp43p and *ddx-15* are homologous in amino acid sequence, there seem to be key differences in function between the two proteins. For example, loss of Prp43p in yeast is lethal, while homozygous *ddx-15* knockouts are viable but display a sterile phenotype. In addition there is evidence of a nucleolar defect in germline stem cells. In this study, immunoprecipitation was used to show that *ddx-15* also interacts with other putative spliceosomal proteins in *C. elegans*. This evidence continues the search for the *ddx-15* protein's dual role in splicing and ribosome biogenesis.

373C  **Reproductive and germline aging in young *C. elegans*.**  Z. Kocissova, K. Kornfeld, T. Scheld  Washington University, St. Louis, MO.

Infertility is increasingly a concern for women who wait until middle age to start families. Aging research has focused primarily on somatic aging and organismal lifespan. While *Caenorhabditis elegans* hermaphrodites live 14 to 21 days, reproductive senescence begins long before significant declines in somatic functions. In mated hermaphrodites with excess sperm, the peak of reproduction occurs at days 2-3 of adulthood (20°C). By day 4, reproduction is about half of peak; by day 5 it is about a quarter of peak. Several labs, including ours, have described techniques to analyze the development and peak function of the *C. elegans* reproductive system. Germline stem cells differentiate and mature in a linear assembly line-like pattern as they progress away from the distal tip cell toward the spermatheca and uterus over the course of about two days.

Our goal is to characterize the cellular and molecular changes that underlie age-related functional decline in egg laying in mated hermaphrodites with sufficient sperm. Our rationale is that identifying those changes in the germline which correlate with an observed decline in egg laying will lead us to identify the causes of reproductive decline. We speculate that an early
decline in reproduction is caused by a decrease in the number of progenitor cells, a slower progenitor cell cycle, dormant progenitor cells, slowing of the assembly line of meiotic progression and gametogenesis, and/or other meiotic defects.

At day 5 we observed defects in coordinating meiotic maturation with ovulation. Oocytes in the proximal gonad were frequently endomitotic, affecting nearly one third of animals in one or both gonad arms. This defect is well-known to cause sterility. In longitudinal experiments, the endomitotic phenotype was highly enriched in the subpopulation with the lowest egg-laying. The endomitotic defect alone explains about a fifth of the loss of reproductive ability at day 5.

Even as early as day 3, when the animals’ morphology and reproductive output were at the peak of health, the distal germline experienced significant age-related decline. The progenitor zone was smaller. There were fewer S-phase and M-phase nuclei, yet the S-phase and M-phase index remained unchanged. Fewer nuclei had completed G2 in a four hour or ten hour period. Together, these results point to a proportionally slower mitotic cell cycle. The rate of meiotic entry decreased. Because progression of germ cells through the meiotic prophase part of the “assembly line” takes many hours, these declines at day 3 will contribute to declines in reproductive output at day 5.

The reported changes are due to genuine female reproductive aging rather than sperm depletion, as sperm-depleted animals were excluded. We find alterations in multiple processes at days 3-5 contribute to the decline in progeny production at day 5. The distal changes occur surprisingly early, at a time when animals are still very fertile.

374A Identification of multiple new genes required for C. elegans spermiogenesis. A.R. Krauchunas1, E. Mendez1, D. Chen1, K. Shuleski1, J. Ni2, H. Smith3, S. Gu2, G. Stanfield2, A. Singson1 1) Waksman Institute of Microbiology, Rutgers, The State University of New Jersey, Piscataway, NJ; 2) Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ; 3) Department of Human Genetics, University of Utah, Salt Lake City, UT; 4) National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD.

In order to successfully fertilize an egg, most sperm must go through the processes of post-meiotic differentiation and activation whereby they gain polarity, motility, and fertilization competence. In C. elegans, differentiation and activation take place simultaneously during spermiogenesis, transforming round immobile spermatids into motile, fertilization-competent spermatozoa. Previous work has shown that C. elegans spermiogenesis can be initiated through one of two pathways: the spe-8 pathway or the try-5 pathway. In males, these two pathways are redundant and appear to act in parallel, but in hermaphrodites only the spe-8 pathway is utilized to activate self-sperm. While several genes in each pathway have already been discovered, our limited understanding of how the components work together to effect the cellular changes of spermiogenesis suggests that required genes still remain to be identified.

We are in the process of characterizing three new mutants that are defective in spermiogenesis. We find that spe-43 is a component of the spe-8 pathway and displays a classic "spe-8 class" phenotype. Namely, spe-43 hermaphrodites are self-sterile while spe-43 males show wild-type fertility, and spe-43 spermatids fail to activate in vitro when exposed to Pronase. In contrast, the requirement for spe-48 and as41 seems to be shared between both spermiogenesis pathways since we find that, in addition to the hermaphrodites being self-sterile, males are also sterile in these lines. We have used whole-genome sequencing to identify the spe-43 and spe-48 genes. SPE-43 is predicted to be a single-pass transmembrane protein with a single DX domain and SPE-48 has homology to human ubiquitin-associated protein 1. Sequencing analysis is in progress to identify the genetic nature of as41. Future work will aim to understand the mechanism by which these proteins function within the sperm and determine whether they are signal transducers or effectors during spermiogenesis.

375B The Exocyst complex is required for germline development and embryonic cell divisions in Caenorhabditis elegans. P. Kumari, S. Mylavarapu  Regional Centre for Biotechnology, Faridabad, Haryana, IN.

Polarized vesicular trafficking in eukaryotes is regulated by a conserved protein complex called the Exocyst complex containing eight subunits namely SEC-3, SEC-5, SEC-6, SEC-8, SEC-10, SEC-15, EXO-7 and EXO-8. The Exocyst is proposed to be a molecular-tether that facilitates fusion of Golgi-derived vesicles and recycling endosomes at distinct sites on plasma membrane. It functions in several cellular processes like polarized trafficking, migration, division and autophagy (Win and Guo, 2015). In C. elegans, the Exocyst has been shown to play role in hypodermal cell migration (Frische et. al, 2007), excretory canal lumino genesis (Armenti et al., 2014) and dendritic branching (Taylor et al., 2015). However its role in germline development and embryonic cell divisions has not been demonstrated.

A combination of mutant analysis and partial depletion of Exocyst components by RNAi revealed roles for the Exocyst complex in multiple stages of development in C. elegans. Exocyst components are required for viability and fertility confirming its role in both embryonic and germline development. Exocyst depleted worms have a small germline with a poorly formed lumen. The total number of germline stem cells (GSCs) is significantly reduced and they show low rates of proliferation. The somatic stem cell niche signals GSCs to remain in mitosis via canonical Notch/Delta signaling. We found that the somatic stem cell niche plexus was shortened in the absence of Exocyst components that might reduce the amount of signaling required for GSC proliferation. Further, epistasis analysis of Exocyst with notch/glp-1 indicated that it positively regulates notch/glp-1 function in the germline.

The Exocyst complex is required for proper oocyte development; however spermatogenesis appears to be unaffected. Exocyst depleted worms have very few mature oocytes in the gonad and display a delay in cellularization from the syncytial gonad. Ovulation is also severely affected in these worms. Ovulating oocytes get torn in the process of exiting the gonad and spermatheca resulting in a mass of torn cells in the uterus. The trapped oocytes in the gonadal arm exit meiotic arrest and begin endo-replication. In addition these oocytes show severe intracellular trafficking defects. Further, a few embryos that are...
formed are extremely osmo-sensitive, indicating defects in egg-shell formation. Several of the embryos failed to complete cytokinesis, resulting in multinuclear blastomeres during early embryogenesis. All these results put together indicate that the Exocyst complex is required for germline development and cell division in embryos. In the future, we will focus on elucidating the mechanistic basis of these functions by exploring the protein interactions of Exocyst components in *C. elegans*.

376C  *K01D12.7: a sperm gene identified through its suppression of spe-27 by inducing premature spermatid activation.*  Gaurav Prajapati, Thomas Sokolich, Nicole Lynn, Fermina Aldaco, Steven Keung, Craig LaMunyon  Dept Biological Sci, Cal Poly Pomona, CA.

A critical decision in the life of a male gamete is when to become active. The timing of this seminal event normally co-occurs with ejaculation for males. Failure to activate in a timely manner dooms the sperm to the Darwinian junk heap. In most species, sperm activation occurs after the nuclear material is ultra-condensed and expressionless. Thus, the genesis of motility is orchestrated through preexisting gene products, which in *C. elegans* either prevent or promote activation. An external signal promotes activation by interfacing with surface receptors on the sperm. Interestingly, two parallel activation pathways exist: (i) a TRY-5 signal in male seminal fluid interacting with a SWM-1 receptor, and (ii) an unknown signal in both seminal fluid and in the hermaphrodite reproductive tract interacting with the SPE-8 group receptor complex (SPE-8, -12, -19, -27, and -29). Here, we focus on the SPE-8 group complex, which awaits the activation signal bound to the plasma membrane. Upon encountering the signal, SPE-8 is released to the cell’s interior, where this tyrosine kinase presumably phosphorylates targets. Mutations in any of the spe-8 group genes results in spermatids that cannot activate in unmated hermaphrodites. A suppressor screen of *spe-27(0)fs(32ts)* recovered numerous mutations that restore partial fertility. Our laboratory has identified a number of these *spe-27* suppressor mutations. In most cases, these mutations result in premature activation, bypassing the signaling pathways altogether. We have learned that SPE-6 prevents activation until being down regulated, SPE-4, SPE-46, and SPE-47 all can be mutated to cause premature sperm activation, and that SPE-6 and SPE-27 interact directly during activation. Here, we describe the *K01D12.7(hc201)V* suppressor mutation. Like the other suppressors, hc201 bypasses the need for signaling and causes premature sperm activation. The *K01D12.7* protein is sperm specific and tiny, has 4 sperm specific paralogs, and resembles absolutely nothing outside of its nematode paralogs and homologs. A reporter construct indicates plasma membrane localization but does not rule out localization to other cellular compartments. The role of this gene product in spermatogenesis is still not clear.

377A  *DIV-1/PolA2 Promotes GLP-1/Notch-Mediated Cellular Events in Caenorhabditis elegans.*  DS Yoon1, DS Cha3, MH Lee1,2  1) Internal Medicine, East Carolina University, Greenville, NC; 2) lineberger Comprehensive Cancer Center, UNC-Chapel Hill, NC, USA; 3) woosuk University, Jeonbuk, Republic of Korea.

Notch signaling is a highly conserved cell signaling system in most multicellular organisms and plays a critical role in animal development. In various tumor cells, Notch signaling is elevated and has been considered as an important target in cancer treatments. In *C. elegans*, GLP-1 (one of two Notch receptor) activity is required for cell fate specification in germline and somatic tissues. Here, we have identified div-1 gene (a homolog of the mammalian PolA2) as a positive regulator for GLP-1/Notch-mediated cellular events. *C. elegans* div-1 encodes the B subunit of the DNA polymerase alpha- primase complex and is highly expressed in proliferative germ cells. Functional analyses demonstrated that i) DIV-1 is required for the robust proliferation typical of the germline, ii) loss of DIV-1 enhances and suppresses specific phenotypes that are associated with reduced and elevated GLP-1/Notch activity in germline and somatic tissues, and iii) DIV-1 works together with FBF/PUF RNA-binding proteins, downstream regulators of GLP-1/Notch signaling, to promote germline stem cell (GSC) maintenance and germline proliferation. To maintain GSCs and proliferative cell fate, GLP-1/Notch activity must remain above a threshold for proliferation/differentiation decision. Our results propose that DIV-1 may control the level of a threshold for GLP-1/Notch-mediated germline proliferation.

378B  *Investigating the role of poly(U) polymerases in germline development.*  Y. Li, M. Snyder, E. Maine  Department of Biology, Syracuse University, Syracuse, NY.

Poly(U) polymerases (PUPs) add uridine to the 3’end of RNA, a common modification that correlates with reduced RNA stability in many organisms. Three PUP proteins have been identified in *Caenorhabditis elegans* (Kwak & Wickens, 2007). PUP-1/DE1 (co-suppression defective) is known to target a subset of siRNAs and is expressed in the germline (van Wolfswinkel et al., 2009). PUP-2 activity is known to regulate a subset of miRNAs that act in the soma (Lehrbach et al., 2009; Ha & Kim, 2014). Our genetic studies reveal a redundant role for PUP-1 and PUP-2 in germline survival and chromatin regulation. Under temperature stress, *pup-1/-2(0)* hermaphrodites produce progressively fewer progeny over successive generations and become sterile by the third generation. The majority of those sterile adults contain no germ cells due to germ cell death during mid-larval development. Chromatin regulation, particularly the H3K9me2 modification, is abnormal in meiosis in survivors (generations 1-2) if unpaired chromosomes are present. In addition, *pup-1/-2(0)* sperm are fertilization defective as endomitotic oocytes develop in hermaphrodites and males fail the mating assay.

Our molecular studies indicate that PUP-1 and PUP-2 localize to different cellular compartments. PUP-1 localizes to P granules throughout the mitotic and meiotic germline. PUP-2 is detected as cytoplasmic foci in meiotic germ cells beginning at L4 stage. Thus, both proteins are expressed during the stage when H3K9me2 misregulation is first detected and, in later generations, when the germline dies. The expression patterns are consistent with PUP-1 and PUP-2 acting at different points in the RNA turnover process.

To better understand the role of uridylation in germline development, we abolished the activities of all three PUPs. Preliminary results suggest the loss of PUP-3 function can restore germline survival in the absence of PUP-1 and PUP-2
function. We hypothesize PUP activities modulate the balance of RNA levels. Absence of any single PUP increases the abundance of its targets, which perturbs the stoichiometric relationships of gene products. However, loss of all three PUPs elevates the gross RNA level, which restores the balance of gene products such that germline viability is restored. Taken together, our findings reveal critical functions for PUPs in germline development.

379C Investigating the remodeling of C. elegans primordial germ cells. C. Maniscalco, J. Nance Helen L. and Martin S. Kimmel Center for Biology and Medicine at the Skirball Institute of Biomolecular Medicine, NYU School of Medicine, New York, NY.

Germ cells allow us to transmit our genome to future generations. The embryonic precursors of germ cells, called primordial germ cells (PGCs), are set aside early during development and are subjected to unique regulation, such as transcriptional repression, so that differentiation does not occur. In order to produce gametes, quiescent PGCs must activate and transition into dividing germline stem cells (GSCs). The mechanism of this transition is poorly understood. The two embryonic PGCs in C. elegans extend large cytoplasm-filled lobes into the surrounding endoderm; lobes and their contents are subsequently removed and digested by endodermal cells (Abdu et al., 2016), suggesting that they may function as a means for PGCs to discard unwanted components before they transition to GSCs. In support of this hypothesis, live imaging results show that PGC lobes concentrate certain cellular components, such as oxidant-rich mitochondria, before the lobes and their contents are pinched off and eaten by adjacent endodermal cells. Although lobe formation occurs cell autonomously, the cellular mechanisms of lobe formation are unclear. I observed that non-muscle myosin (NMY-2), anillin, and septin, components of the contractile ring in dividing cells, form a ring at the neck between PGCs and their lobes, suggesting that a contractile ring-like structure constricts the cell body into two halves. I am currently testing this hypothesis using temperature-sensitive mutations in components required for contractile ring function. Preliminary data shows that contractile ring components are necessary for lobe genesis, and without them, contraction between the main body and lobe is unable to form.

380A Using a forward genetic approach to uncover the molecular basis of gamete interactions. X. Mei, A. Krauchunas, K. Flanagan, P. Manich, S. Jain, A. Singson Rutgers University, Piscataway, NJ.

Fertilization occurs when the sperm and egg recognize and bind with each other and fuse to form a zygote. The molecular basis of sperm and egg interactions are largely unknown. In the mammalian systems, Izumo is a sperm surface receptor required for the sperm to fuse with the egg. It was only recently that Juno was identified as the binding partner for Izumo on the egg (Bianchi, 2014). So far, this is the only known receptor pair that is necessary for fertilization. Using forward genetics, our lab and others have been able to identify essential genes for sperm function; those that affect only the ability of sperm to fertilize the egg but not spermatogenesis or spermiogenesis. These genes are defined as the SPE-9 class and include spe-9, spe-13, spe-36, spe-38, spe-41, spe-42, spe-45, and fer-14, with SPE-45 being an IZUMO-like protein (Krauchunas, 2016). On the other hand, not much is known what molecules on the egg surface are responsible for binding with these SPE proteins to mediate fertilization. EGG-1 and EGG-2 are the only known egg-surface molecules that are required for fertilization but so far our preliminary data indicate that they do not bind with SPE-9. This leads to the hypothesis that there are other components on the egg surface that are yet to be identified. Here, using an EMS-based random mutagenesis screen, we set out to find genes that are required for fertilization, with a special interest on egg mutations. We have been able to isolate four independent, temperature-sensitive (ts) sterile mutant lines. By DIC imaging and DAPI staining, they all showed the presence of sperm in the spermathecae, and unfertilized oocytes in the uterus. We are working to outcross and further characterize these lines. We are particularly interested in any mutants that do not recover fertility when mated with males at restrictive temperature. Further mapping and complementation testing will show whether they are mutations in genes with no previously known role in fertility. If yes, we will use whole-genome sequencing to determine the causative mutations and identify new genes. Taking together, our strategy allows us to uncover genes that are important for gamete functions and sperm-egg interactions.

381B AAK-1/AMPK signals for feedback control of germline stem cell proliferation in adult hermaphrodites. P. Narbonne, P.S. Maddox, J-C Labbé 1) Department of Medical Biology, Université du Québec à Trois-Rivières, Trois-Rivières, QC, CA; 2) Department of Biology, University of North Carolina, Chapel Hill, NC, USA; 3) Institut de recherche en immunologie et en cancérologie (IRIC), Université de Montréal, QC, Canada.

Under replete growth conditions, abundant nutrient uptake leads to the systemic activation of insulin/IGF-1 signaling (IIS) and the promotion of germline stem cell (GSC) growth/proliferation at all stages of development or adulthood. Stem cell proliferation rates can further be locally controlled in adult hermaphrodites to meet oocyte needs. This regulation relies on a feedback signal that initiates upon oocyte accumulation and shuts down the production of further oocytes, and GSC proliferation. We had previously shown that spermless animals required DAF-18/PTEN to inhibit oocyte production and GSC proliferation, and that they could do this independently in each gonad arm, despite systemic IIS activation. We now provide evidence that DAF-18 signals through a novel cryptic signaling pathway that requires PAR-4/LKB1, AAK-1/AMPK and PAR-5/14-3-3 to inhibit the signals through a novel cryptic signaling pathway that requires PAR-4/LKB1, AAK-1/AMPK and PAR-5/14-3-3 to inhibit the activity of MPK-1/MPK, antagonize IIS, and inhibit both GSC proliferation and the production of additional oocytes. Interestingly, our results suggest that DAF-18, through PAR-4, can activate AAK-1 in the absence of apparent energy stress. As all components are conserved, similar signaling cascades may regulate stem cell proliferation in other organisms and be widely implicated in tumorigenesis.

382C Decreased mating ability may play a role in C. elegans male low fertility at high temperature. Emily M. Nett, Lisa N. Petrella Biological Sciences, Marquette University, Milwaukee, WI.

The germline, especially the spermatogenic germline, is temperature-sensitive, such that as the temperature increases, germline function and fertility drop rapidly. Our previous work has shown wild type strains of C. elegans males raised at 27°C...
are almost all sterile, while males raised at 20°C but upshifted to 27°C for mating are almost all fertile. Additionally, there are significant differences between wild type strains in the ability of males to regain fertility upon downshifting from 27°C to 20°C (Petrella, 2014). High temperature loss of male fertility could be due to loss of sperm function, reduced sperm number, changes in mating ability, or all three. To test sperm function, we have analyzed the rates of in vitro sperm activation in males that were raised at 27°C. We found that activation rates are reduced by approximately 10-15%. This level of decreased activation is not sufficient to explain the almost complete loss of fertility seen at this temperature. Experiments are currently underway to quantify the number of sperm produced by males at 27°C. However, as the number of sperm does not decrease with temperature in hermaphrodites, we anticipate there may not be a significant difference in males. Finally, male mating interest and execution were tested with males raised at 27°C. All wild type strains show declines in multiple steps of male interest and mating execution during timed assays. However, the decrease in total successful mating events at 27°C differed significantly between strains. Our data indicate that reduced sperm activation is not the main cause of low fertility at 27°C but that decreased mating ability may play an important role. As loss of fertility at high temperature is conserved across phyla, our research may uncover conserved temperature-sensitive germline pathways that account for the germline temperature sensitivity.

383A Premature sperm activation is prevented by SPE-6 in the nematode C. elegans. Gaurav Prajapati1, Thomas Sokolich1, Craig LaMunyon1, Diane Shakes2 1) Dept. of Biological Sciences, Cal Poly Pomona, Pomona, CA; 2) Dept. of Biology, College of William and Mary, Williamsburg,

Spermatogenesis, pauses only at the penultimate stage, the spermatid. The pause ends when an external signal activates the spermatid into a spermatoozoon, activation involves two pathways (i) TRY-5 in seminal fluid interacting with SWM-1 and (ii) an unknown signal both seminal fluid and in the hermaphroditic interacting with the SPE-8 group complex. Mutations in the spe-8 group genes (spe-8, -12, -19, -27, -29) result in spermatids that cannot activate in unmated hermaphrodites. We focus on one likely target of the SPE-8 complex, the SPE-6 serine-threonine kinase. Here, we test the hypothesis that active SPE-6 maintains the spermatid stage, and that activation is achieved through downregulation of SPE-6. A suppressor screen from Sam Ward’s lab with spe-27(it132) generated numerous suppressor mutations that restore partial fertility. Our work examines the suppressor mutations recovered in spe-6.

Null spe-6 mutations stall spermatogenesis at the transition from primary to secondary spermatocytes. In contrast, the spe-6 suppressor mutations cause premature spermatid activation, bypassing SPE-8 group signaling. The alleles we focus on are: hc166, hc176, hc188, hc190, zq10, zq11, and zq18. These mutations likely reduce function and are scattered across the gene, suggesting that lower of SPE-6 function initiates activation. One mutation, zq11, is an allele specific suppressor of spe27(it132). zq11 does not suppress spe-27(it110), indicating that SPE-6 and SPE-27 interact physically and suggest that SPE-6 is a target of SPE-8 group signaling.

Two other spe-6 alleles have intronic mutations. The hc190 and zq18 alleles are G to A substitutions of the 1st and 5th bases, respectively, of the 5’ end of the intron a highly-conserved site for splicing. These conditional mutations cause premature sperm activation and partial mRNA loss, although normal splices were present for both mutants at 25°C. Thus, loss of SPE-6 function through a simple drop of transcript abundance is sufficient to cause premature activation. Thus, SPE-6 function is necessary to maintain the spermatid stage. We have used CRISPR/Cas9 to induce similar intronic mutations in spe-44. However, neither mutation in spe-44 produces a phenotype. We are now attempting to create both mutations in the same intron.

384B Transgenerational requirement for C. elegans SET-2/SET1 in genome stability and DNA repair. Marion Herbette1, Marine Mercier2, Fanny Michal1, Cécile Bedet1, David Cluet1, Claire Burny1, Gaël Yvert1, Valérie Robert1, Francesca Palladino1 1) Laboratory of Biology and Modeling of the Cell, Ecole Normale Supérieure de Lyon, Université Claude Bernard Lyon 1, Lyon, France; 2) CGPhiMC, Université Claude Bernard Lyon 1, Villeurbanne, France.

Maintaining the integrity of genetic information across generations is essential for both cell survival and reproduction, and requires the timely repair of DNA damage. Specific post-translational modifications of histone tails play a central role in the DNA repair process through the recruitment of proteins and complexes with specific enzymatic activities, or by altering the chromatin state at the site of DNA lesions.

I will present data showing that SET-2, the C. elegans homologue of the conserved Set1 histone methyltransferase (HMT), is required for genome stability in the germline. SET1 family HMTs are responsible for methylation of histone H3 on Lysine 4 (H3K4), a modification universally found at transcriptional start sites and associated with gene transcription. The absence of SET-2 results is a strong reduction of H3K4 di- and tri-methylation in the distal part of the germline and correlates with a mutant phenotype and a sensitivity to DNA damage inducing agents which increases transgenerationally. Following exposure to ionizing radiation, set-2 mutant germlines show a delay in the repair of mitotic DNA double strand breaks (DSBs), and increased fragmentation in oocytes. These observations strongly suggest that SET-2 and H3K4 methylation play an essential role in genome stability in the germline of C. elegans.

Experiments performed to better understand the requirement of SET-2 and H3K4 methylation in genome stability show that the DNA damage response (DDR) pathway is still functional in the absence of SET-2 suggesting that SET-2 acts downstream or in parallel of the DDR to control genome stability in the C. elegans germline. Experiments designed to detect DSB repair defects at CRISPR- induced targets, or de novo mutations by whole-genome resequencing, will also be presented.

385C The SET-2/SET1 histone H3K4 methyltransferase maintains cell fate in the Caenorhabditis elegans germline. Valérie Robert1, Andrew Kekupa’a Knutson2, Andreas Rechtsteiner2, Gaël Yvert1, Susan Strome2, Francesca Palladino1 1) Laboratory of Biology and Modeling of the Cell, Ecole Normale Supérieure de Lyon, Université Lyon 1, Lyon, France; 2) Department of Molecular, Cell & Developmental Biology, University of California Santa Cruz, Santa Cruz, CA.
Maintenance of germ cell fate is of critical importance for species survival. In C. elegans, translational repression, P granules, chromatin factors and histone tail modifications have been shown to be essential to maintain germ cell identity. We previously showed that the conserved SET-2/SET1 histone H3K4 methyltransferase plays a crucial role in maintaining germline immortality and totipotency, two defining features of germline identity. set-2 mutant animals grown at 25°C become sterile over several generations, which is reminiscent of what happens in mortal germline (mrt) mutants. This sterility, which is reversible when animals are switched back to 20°C, is associated with a loss of germline-specific markers, transcriptional derepression of somatic genes, and transdifferentiation of the germline into soma. To look at how the transcriptional landscape changes in the absence of SET-2 across generations and to identify pathways involved in germline maintenance, we performed transcriptome analysis of germlines dissected from fertile animals grown for 2 generations at 25°C and on both fertile and sterile animals grown for 4 generations at 25°C.

We are currently analyzing these transcriptome data and performing RNAi to identify triggering events of transdifferentiation and transcriptional networks deregulated in transdifferentiated germlines. I will present preliminary results of this analysis.

386A Kinases PEK-1 and GCN-1/-2 are required for RNA granules formation during stress. L.S. Salinas, Gabriela Huelgas-Morales, Rosa Navarro Institute of Cellular Physiology, Department of cell biology and development, National University of Mexico (UNAM), México, Mexico City, MX.

In our laboratory we are interested in studying how the C. elegans gonad protects itself from stressful conditions. Previously, we and other groups have reported that during heat shock, starvation and oocyte arrest RNA granules are formed in the gonad. During stress, beside P granules, other types of RNA granules are present in the gonad; one type of RNA granules are formed in the core of the gonad and another are formed in the proximal gonad where oocytes are found. We suggest that those RNA granules are similar to stress granules because 1) their formation is induced by puromycin and 2) their formation is inhibited by cycloheximide. Both types of RNA granules have in common several RNA binding proteins like CGH-1, TIAR-1, CAR-1, MEX-3, etc. Until now RNA granules assembly, function, and regulation remain poorly understood. Recently we showed that only stress granules in the gonad core required the RNA binding protein TIAR-1 for their formation. In mammals, TIAR-1 homologs TIA-1 and TIAR are the main seeding proteins for stress granules formation. Stress granules are formed in mammalian cells due to translational arrest induced by the phosphorylation of the eIF2a by kinases like PKR, PERK, GCN2 and HRI which sense dsRNA, ER stress, amino acid starvation or arsenite/redox stress, respectively.

We are exploring the role of the kinases PEK-1, GCN-1 and GCN-2 in the formation of stress-induced RNA granules in the C. elegans gonad. The analysis of the formation of stress granules was carried out through immunostaining assays using an antibody that recognizes the CGH-1 protein in wt or pek-1, gcn-1 or gcn-2 mutants. We found that PEK-1 is necessary for the formation of stress granules in heat shock and starvation. While GCN-2 is partially required for RNA granules formation during heat shock but no starvation. A double mutant GCN-1, GCN-2 was unable to form granules under heat shock suggesting that these kinases are redundant. Our results suggest that PEK-1 and GCN-1/-2 are required for granules formation during heat shock but only PEK-1 is required to form granules during starvation.

387B Mechanisms of RNP granule assembly in the germ line. Andrea Montalbano¹, Michael Davis², Megan Wood¹, Jennifer Schisa². ¹) Biology, Central Michigan University, Mount Pleasant, MI; ²) Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI.

Germ granules share features with many other membraneless organelles, and recent evidence suggests that a liquid-liquid phase separation (LLPS) process drives their formation. The C. elegans adult germ line is an excellent model to study LLPS and its role in RNP granule assembly due to the dramatic cellular changes that occur in response to meiotic arrest or stress. During prolonged meiotic arrest that occurs in middle-aged hermaphrodites depleted of sperm and in females, germ granules recruit additional mRNAs and proteins to form large complexes termed RNP granules that are 20 times larger than typical germ granules. To identify regulators of RNP granule assembly, we performed an RNAseq screen that identified 319 positives, including two interesting families of chaperone proteins, the the TCP-1 Ring Complex (TriC)/ chaperone containing Tcp-1 (CCT) family and the DnaJ/Heat shock protein 40 kDa (Hsp40) family (Wood et al., 2016). We are currently exploring the specificity of these chaperone proteins in regulating different types of RNP granules as well as the related Hsp70 chaperone family.

Environmental stresses such as heat stress induce the assembly of large, germ line RNP granules that are similar, but not identical, to those induced by meiotic arrest. We have begun investigating the cellular response to glucose in the germ line and have determined that high concentrations of glucose induce the assembly of RNP granules. Interestingly, the RNP granules are maintained for up to three hours; however, they dissociate after longer periods of stress. Based on several lines of evidence, the germ line response to glucose largely appears to be an osmotic stress response, thus identifying osmotic stress as a trigger of LLPS. Although RNP granules are not maintained beyond three hours of osmotic stress, the quality of oocytes does not appear to decrease after longer periods of stress, suggesting a secondary adaptation in the germ line. We used an indirect marker of glycerol and observed high levels after five and twenty hours of glucose exposure. Moreover, in gpdh-1;gpdh-2 germ lines glycerol levels are reduced concomitant with RNP granules being maintained for an extended period. We speculate that increased glycerol levels may function as a secondary osmoregulatory adaptive response in the germ line, following a primary response of RNP granule assembly.


Notch signaling is the principal pathway that promotes cell proliferation in the C. elegans germ line. Here we report that two
members of the Derlin (Degradation in the ER) family of proteins, CUP-2 and DER-2 (R151.6), promote Notch signaling. cup-2(0); der-2(0) germ lines have fewer proliferative cells than wild-type germ lines. Depleting either one of these proteins in a germ line with enhanced GLP-1/Notch signalling suppresses the size of the proliferative zone. This suppression is stronger in cup-2(0) germ lines than in der-2(0). However, depletion of CUP-2 in two different Notch-independent tumorous genetic backgrounds is unable to suppress the tumour. This suggests that CUP-2 promotes proliferation through the Notch signaling pathway specifically as opposed to suppressing proliferation in general. We further asked if CUP-2 promotes Notch signalling in other contexts, and found that lack of cup-2 enhances the loss-of-function Notch phenotype in the ACVU decision in gonad development; therefore, CUP-2 is likely able to promote Notch signaling in general. To help determine how CUP-2 contributes to Notch signaling, we are analyzing its expression pattern. We have produced a V5::2XFLAG tagged form of CUP-2 by using the CRISPR/Cas9 system. We find that CUP-2 is expressed throughout the germ line. We are currently studying whether the CUP-2 expression pattern is altered in a variety of mutant backgrounds.

389A  CDK-1 and SCFSEL-10 promote the degradation of LIN-41 during the first meiotic division. C.A. Spike, G. Huelgas-Morales, T. Tsukamoto, D. Greenstein  GCD Department, University of Minnesota, Minneapolis, MN.

In many animals, oocytes enter meiosis early in their development but arrest in meiotic prophase I. Oocyte growth occurs during this arrest period, enabling the acquisition of meiotic competence and the capacity to produce healthy progeny. Meiotic resumption, or meiotic maturation, involves the transition to metaphase I (M phase) and is regulated by intercellular signaling and CDK-1 cyclin-dependent kinase activation. A key question is how meiotic maturation is spatially restricted in an all-or-none fashion to the ~1 oocyte. The highly conserved TRIM-NHL protein LIN-41 is a translational repressor that enables the production of high-quality oocytes and plays an essential role in controlling and coordinating oocyte growth and meiotic maturation. LIN-41 prevents the premature activation of CDK-1 thereby restricting M phase entry to the most proximal oocyte. Consistent with this role, LIN-41 is abundant in developing oocytes, but is eliminated in a CDK-1-dependent mechanism during the oocyte-to-embryo transition. Time-lapse video recordings demonstrate that GFP::LIN-41 levels decrease in a biphasic manner: a slow decline in LIN-41 levels followed by a rapid elimination such that LIN-41 is undetectable by the end of the first meiotic division. Elimination of GFP::LIN-41 during the oocyte-to-embryo transition requires the activities of multiple SCF subunits, including the substrate adaptor protein SEL-10/FBW7, suggesting that LIN-41 is a target of ubiquitin-mediated protein degradation. Deletions made using genome editing identify two non-overlapping regions in N-terminal intrinsically disordered domains that are individually necessary for the degradation of GFP::LIN-41 during the oocyte-to-embryo transition. Both regions contain potential CDK phosphorylation sites, and at least one of these sites is itself necessary for the degradation of GFP::LIN-41. Importantly, the two non-overlapping N-terminal regions of LIN-41 are sufficient, in combination, to mediate SEL-10-dependent degradation when transplanted into OMA-2. These data suggest that CDK-1 may directly phosphorylate LIN-41, resulting in multiple low-affinity SEL-10 binding sites that, at least in combination, lead to SEL-10-dependent ubiquitin-mediated protein degradation. Interestingly, the failure to eliminate LIN-41 from early embryos does not result in the continued translational repression of LIN-41 oocyte mRNA targets or, indeed, in any obvious embryonic defects. This result is surprising because LIN-41 is a potent inhibitor of protein translation and M-phase entry. These data suggest that LIN-41 must be both inactivated and degraded to promote the irreversibility of the developmental transition.

389B  ChIP-Seq reveals miRNA61-250 and deps-1 as Notch target genes in germline stem cells. Erika B. Sorensen1,2, Emma Doenier2, Elena P. Sorokin2, Doug F. Porter2, ChangHwan Lee2, Amy C. Groth3,4, Dan C. Noble2, Judith Kimble1,2  1) Department of Biology, Wabash College, Crawfordsville, IN; 2) Department of Biochemistry, UW-Madison, Madison, WI; 3) Howard Hughes Medical Institute; 4) Department of Biology, Eastern Connecticut State University, Willimantic, CT.

Notch signaling activates transcription of target genes and thereby regulates stem cells and differentiation broadly during animal development. The nematode C. elegans employs Notch signaling to maintain germline stem cells (GSCs) (1). Previous work identified two Notch targets with a candidate gene approach (2). Here we used chromatin immunoprecipitation followed by high throughput sequencing (ChIP-Seq) to identify GSC Notch targets on a genomic scale. ChIPs of the LAG-1/CSL DNA binding protein identified 384 significant peaks, of which 8 were also identified in parallel ChIPs of the GLP-1/Notch receptor. Two of these 8 common peaks belonged to the targets already known. In addition, we found peaks in the promoters of RNA regulators. One peak was in the promoter of mir-61 and mir-250, termed collectively mir61-250 (3). In situ hybridization demonstrates that these miRNAs are transcribed in GSCs and that their transcription is Notch-dependent. To ask if mir61-250 affects GSC maintenance, we used CRISPR-Cas9 (4.5) to delete the mir61-250 promoter. The resultant mir61-250 promoter deletion abolishes transcription of the miRNAs and therefore provides a loss-of-function mutant. Yet no GSC defect was seen. One explanation is that mir61-250 is functionally redundant with other genes. We are currently determining if miRNA overexpression or the presence of a miRNA sponge affects GSCs (6). Another ChIP peak was in the promoter of deps-1, a protein localized to the P-granule (7). Single molecule fluorescence in situ hybridization (smFISH) demonstrates that deps-1 expression in GSCs is Notch-dependent. We are exploring how these newly identified Notch target genes function as RNA regulators to control GSC maintenance.


391C  Uncovering key players in meiotic progression through mapping the gonad transcriptome changes. H. Achache1, E. Winter2, J. Gao3, T. Hashimshony1, I. Yanaí2, M.P. Colaiácovo5, Y.B. Tzur1  1) Department of Genetics, Silberman
The main cause for infertility, natural-miscarriages and congenital defects is mis-segregation of chromosomes during oocyte meiotic divisions, yet we know very little how this developmental program is genetically controlled. To uncover the entire transcriptome changes during oogenesis we analyzed the mRNA abundance of sequential segments of the C. elegans gonad, in which the nuclei are arranged in a perfect spatiotemporal order from the oogonial stem-cell to the mature oocyte. We found that most genes fall into two reciprocal expression profiles that switches expression levels at late pachytene. We observed that while the X chromosome is silenced throughout the first half of the gonad, some genes escape this control and are highly expressed throughout the germline. To find key players in oogenesis progression control we mined this database for genes which are upregulated at two critical transitions in oogenesis: the meiotic entry and the onset of chromosome remodeling. Combined with RNAi screen for genes which are required for correct localization of proteins on remodeled bivalents in the mature oocyte, we were able to focus on genes that are required for early and late oogonial processes. CRISPR-Cas9 full deletion of one of those genes, ogr-2, led to a delay in meiotic entry, and the proliferative zone length was increased. Although synopsis seemed unperturbed in this mutant, and double-strand breaks repair dynamics was only marginally shifted, apoptotic nuclei number was increased. Apoptosis analysis in ogr-2;spo-11, and ogr-2;syp-1 showed that the increase of apoptosis in ogr-2 mutant gonads is independent of the synopsis and DNA damage checkpoints. The localization of activated MPK-1, a driver of meiotic entry, was elongated in the gonads of ogr-2 mutants, suggesting a mechanism for the change in meiotic processes. This hypothesis is supported by our observation that the apoptotic increase in ogr-2 mutant meiocytes is dependent on mpk-1 and cep-1. Put together, we suggest that ogr-2 is required for oogonial progression and successes through the timely activation of MPK-1.


Germline stem cells are maintained in the distal region of the gonad. These cells undergo mitotic divisions and are maintained in this state through GLP-1 Notch signaling. The somatic distal tip cell (DTC) caps the end of the distal gonad and is essential for maintenance of the germline mitotic zone. As germ cells move away from the DTC they pass through the transition zone and enter early meiotic prophase. Here we present our work that identifies the Period protein homolog LIN-42 as a new regulator of germline development in C. elegans. Previous work in our lab and others has shown that LIN-42 is a transcription factor that regulates expression of both microRNAs and some mRNAs. LIN-42 is important for multiple developmental events including molting and hypodermal cell development. In addition, in the absence of LIN-42, late-stage specific events occur precociously in vulval precursor cells, sex myoblasts and the DTC. LIN-42 is expressed in all of these cells as well as multiple other cells in the muscles, neurons, intestines and hypodermis. Because of this we sought to understand the role of LIN-42, if any, in germline development. In lin-42(n1089) mutants we find that the mitotic zone size, determined by both the number of rows of cells and the total number of cells in the mitotic zone, is significantly reduced by 25% compared to wild type in young adult worms. We further find that mRNA levels of the GLP-1 Notch ligand, lag-2, are significantly decreased in lin-42(n1089) mutant worms relative to wild type. Thus, LIN-42 likely impacts germline development by regulating LAG-2 expression and subsequent GLP-1 Notch signaling. Current work aims to determine if LIN-42 acts directly or indirectly to control lag-2 levels. In Drosophila, the ATAXIN-2 protein is an important regulator of Period expression. In C. elegans, the ATAXIN-2 homolog axb-2 promotes germline proliferation. We find that lin-42 levels are significantly decreased in axb-2 mutant worms relative to wild type, suggesting that ATX-2 impacts germline development in C. elegans at least in part through its effects on LIN-42. Altogether our results establish a new role for the conserved, important Period protein homolog LIN-42 in regulating early germline development.

393B Regulation of Germline Stem Cell Proliferation by MRG-1 in C. elegans. K. Vanden Broek1, P. Gupta2, C. Wang1, D. Hansen* 1) Biological Sciences, University of Calgary, Calgary, Alberta, Canada; 2) Chemical and Systems Biology, University of Stanford, Stanford, California, USA.

Proteasomal mediated protein degradation has been identified by our lab as one mechanism that contributes to regulate the balance between stem cell proliferation and differentiation. Using the C. elegans germline as a model, we have shown that stem cell over-proliferation occurs when proteasomal activity is reduced. We found that stem cell over-proliferation also occurs when the activity of the E3 ligase, Ring Finger Protein 1 (RFP-1), is reduced. E3 ligases provide the specificity for the proteasome degradation pathway by directly targeting the substrate proteins for degradation. RFP-1 was found to regulate stem cell proliferation by targeting the proliferation fate promoting protein, Mortality Related Gene 1 (MRG-1), for degradation. The objective of this research is to investigate the role of MRG-1 in controlling the balance between stem cell proliferation and differentiation. To do this, I am using: 1) yeast 2-hybrid and bacterial co-immunoprecipitation with truncated versions of both MRG-1 and RFP-1 to identify the protein domains that allow for their direct interaction to occur; 2) RNAseq to investigate and characterize MRG-1’s role as a proliferation fate promoting protein by identifying genes that are up- and/or down-regulated in mrg-1 mutants; 3) RNA interference and/or mutant alleles to determine how the genes regulated by MRG-1 contribute to the balance between proliferation and differentiation in stem cells. These results will provide insight into the control of stem cells by identifying core factors necessary for their proliferation.

394C Can DLC-1 binding teach its partner some new tricks? X. Wang, E. Voronina University of Montana, Missoula, MT.

PUF and FBF (PUF) family RNA binding proteins are highly conserved translational regulators in eukaryotes. FBF-1 and FBF-2, two PUF family proteins in C. elegans, function redundantly in maintaining germline mitotic region. In addition to their common function in mitotic region maintenance and fertility, FBF-1 and FBF-2 have divergent functions. FBF-2 silences its
mRNA target through translational repression, while FBF-1 induces clearance of its target mRNAs (Voronina et al., 2012). Additionally, single mutations of fbf-1 and fbf-2 produce distinct effects on the mitotic region size (Lamont et al., 2004). One possibility is that different functions of FBF-1 and FBF-2 are due to association with distinct specific cofactors. Our earlier work identified DLC-1 as a cofactor for FBF-2, not for FBF-1. In vitro, DLC-1 interacts with FBF-2, not FBF-1, due to their sequence divergence. To see if DLC-1 binding is required for FBF-2 function in mitotic region maintenance and fertility, the fbf-2vrm mutant (losing DLC-1 binding sites) transgene was made and crossed into fbf-1 fbf-2 double mutant. We found that comparing to the wild type fbf-2, FBF-2vrm rescued fertility in only 80% of animals, suggesting that its function was compromised but not completely lost. Quantification of the mitotic zone number in the rescued gonads indicated that its size does not match either fbf-1 or fbf-2 single mutant. We hypothesize that DLC-1 binding is important for FBF-2-specific function regulating mitotic region size and that without DLC-1 binding, FBF-2vm functions similarly to FBF-1. In support of this model, we found that FBF-2vrm transgene did rescue fbf-1, but not fbf-2 mutant effect on mitotic region size. We are now testing whether FBF-2vrm promotes clearance of FBF target mRNAs.

PUF family phylogeny in five Caenorhabditis species suggests that FBF-1 and FBF-2 evolved from a gene duplication (Liu et al., 2012). Only one FBF homolog exists in both C. japonica and C. brenneri, therefore it would be interesting to explore whether DLC-1 binding to these FBF homologs is conserved, as a way to determine if this was the ancestral function for FBF. Our study develops a paradigm for how duplicated genes can diverge by modifying their interaction partners to fine-tune regulatory networks during animal development.

395A Mitochondrial ALH-6 is essential for sperm quality and regulates male reproductive senescence. C. Yen1,2, D.A. Lynn1,2,3, S.P. Curran1,2,4 1) Molecular & Computational Biology, University of Southern California, Los Angeles, CA; 2) Davis School of Gerontology, University of Southern California, Los Angeles, CA; 3) University of North Carolina, Chapel Hill, NC; 4) Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA.

Reproduction is essential to perpetuate life. Mitochondria integrity and functionality has been linked to proper sperm function across multiple species. Most studies have examined the negative impact of the environment or acute stress plays on sperm function and reproductive output. However, the mechanistic impact that normal cellular metabolism plays in the regulation of sperm quality and activity remains unclear. Here, we show that C. elegans with mutations in alh-6, a conserved proline metabolism gene, display early reproductive senescence. Loss of proline catabolism results in specific deficits in sperm number, size, and activation. These defects in sperm quality are linked to changes in mitochondria morphology, metabolic output, and reactive oxygen species (ROS) generation. Intriguingly, the reproductive defects in alh-6 mutants are not simply due to reduced flux through the proline catabolism pathway. Instead the premature reproductive senescence in alh-6 mutants is caused by aberrant ROS homeostasis and loss of energy storing metabolites; however the relative impact that altered levels of ROS and metabolic intermediates plays in the sperm number, size, and activation phenotypes is remarkably different. Finally, the expression of the mammalian ortholog of alh-6, Aldh4a1, is significantly reduced with age in mouse testes suggesting a potential conserved role of alh-6 in male reproductive fitness. Taken together, we have uncovered a novel role for a conserved and central amino acid catabolism pathway on normal sperm function and our work uncovers a new variable to measure which can predict and alter the rate of aging of the male reproductive system.

Development - Morphogenesis

396B PAR polarity proteins direct intracellular tube expansion through regulation of exocyst-mediated vesicle trafficking. J. Abrams, J. Nance NYU School of Medicine, Skirball Institute of Biomolecular Medicine, New York, NY.

Organs are comprised of various tubes with distinct cellular compositions. Seamless tubes are unicellular and lack cell junctions along their luminal surface. To form a lumen, seamless tubes undergo cell hollowing when intracellular vesicles coalesce to create an opening and then coordinated vesicle fusion expands and maintains the luminal structure. However, it is unknown what polarity cues direct vesicles to the luminal surface during seamless tube development. Previous studies of multicellular tube formation in MDCK cell cysts have linked the polarity proteins Par3-aPKC-Cdc42 to the exocyst, a protein complex required for vesicle trafficking. Our lab has shown in the C. elegans early embryo that PAR proteins act upstream of the exocyst, but the function of each protein complex and their epistasis during lumen expansion is not understood.

The C. elegans excretory canal forms a unicellular, seamless tube that functions in osmoregulation and expansion of the canal lumen relies on coordinated vesicle fusion. PAR proteins are expressed on the luminal surface of the canal and co-localize with exocyst components. To determine the role of PAR, exocyst, and their effectors during lumen expansion we have generated excretory canal-specific loss-of-function alleles. We made conditional alleles by knocking the ZF1 degron into the endogenous loci of polarity regulators or exocyst genes and degraded these proteins specifically in the canal. In these conditional alleles, we observed that PAR-3 and PAR-6 function distinctly in the canal, with loss of PAR-6 causing more severe lumen phenotypes while PAR-3 loss caused lumen defects at early larval stages but later resolves. Canal-specific loss of the exocyst component SEC-5 or the GTPase RAL-1, an upstream exocyst regulator, cause similar defects in lumen expansion that are more pronounced than PAR loss-of-function. We hypothesize that PARs function by directing exocyst recruitment and subsequent vesicle trafficking to the luminal surface. Consistent with this hypothesis, we have also observed that PAR-6 acts upstream of exocyst localization at the lumen, whereas SEC-5 loss-of-function fails to affect PAR’s apical polarity at the lumen. To determine how PAR is being concentrated apically at the luminal surface we investigated canal loss-of-function of the GTPase CDC-42, a PAR effector. Loss of CDC-42 in the canal caused defects in lumen expansion and basal canal outgrowth, suggesting a dual role in both luminal PAR recruitment and actin organization at the canal leading edge. We also determined that CDC-42 acts upstream of exocyst localization at the lumen but does not alter apical PAR-3, suggesting that CDC-42/PAR-6/PKC-3 and PAR-
3 may act independently during lumen development. Taken together, this work has identified an essential role for PAR proteins upstream of exocyst-directed vesicle recruitment during seamless tube expansion in vivo.

397C Functions, regulation, and composition of the epidermal apical extracellular matrix. Sarina Bernazzani, David Fay. Molecular Biology 3944, University of Wyoming, Laramie, WY.

During development, as well as later in life, the extracellular matrix (ECM) performs essential structural, protective, and physiological functions. We have shown that a fibrillin-like protein, FBN-1, is expressed in epidermal cells and is an important component of the apical extracellular matrix (aECM), termed the sheath in embryos and the cuticle in larvae and adults. FBN-1 acts in the embryonic sheath to prevent epidermal cell deformation caused by several distinct biomechanical forces, and is required in the cuticle for normal molting.

Our studies previously demonstrated that FBN-1 functions within a network that includes the highly conserved proteins SYM-3/FAM102B, a predicted membrane-tethered protein, SYM-4/WDRR44, a WD40 repeat protein, and MEC-8/RBPMS, a regulator of alternative splicing that is required for normal fbn-1 mRNA processing. Although viable as single mutants, animals that are doubly mutant for mec-8; sym-3, mec-8; sym-4, fbn-1; sym-3, or fbn-1; sym-4, display an anterior morphological defect termed the Pharynx Ingressed, or Pin, phenotype.

Our recent studies indicate that FBN-1 may serve as a link between epidermal cells and the aECM via attachment to integrins. Notably, integrins have not previously been reported to act at the apical surface of epidermal cells in C. elegans. Consistent with our model, mutations of the two RGD (integrin-binding) sites in FBN-1 lead to molting defects. In addition, fbn-1; sym-3, or fbn-1; sym-4, mutants exhibit a "notched-head" phenotype, which is also observed in integrin mutants. Furthermore, sym-3 and sym-4 mutants are hypersensitive to partial inhibition of ptf-3, the sole C. elegans b-integrin subunit.

Our work previously indicated that SYM-3 and SYM-4 act in a parallel pathway to MEC-8 and may promote the trafficking of structural proteins to the apical surface of epidermal cells. Functional full-length SYM-3 and SYM-4 fluorescent reporters localize to vesicle-like structures at or near the plasma membrane in epidermal cells. Furthermore, SYM-3 and SYM-4 act in a common genetic pathway with the RAB-11 GTPase, a known regulator of endocytic recycling and exocytosis. Interestingly, mammalian WD44 physically interacts with Rab11, although no functional link between these proteins has been demonstrated.

In other studies, we have sought to identify novel components of the aECM that may function with FBN-1. Partial inhibition of these candidate aECM components led to enhancement of the Pin phenotype in sym-4 mutants. Identified aECM components include, NID-1, an RGD-containing protein and, mlt-7, which encodes a collagen-linking enzyme that we have shown localizes to the aECM. Taken together, we have identified a network of genes that provide insight into the composition, regulation, and functions of the aECM during development.

398A Choose your favourite fuel provider! The energetic perspective of cell-to-cell heterogeneity during embryonic morphogenesis in C. elegans. Grégoire Bonnamour1,2,3, Emmanuel Martin1,2,3, Anya Mokrani1,2,3, Sarah Jenna1,2,3 1) Université de Québec à Montréal (UQAM), Montréal, Québec, CA; 2) Pharmaqam, Montréal, Québec, CA; 3) Biomed, Montréal, Québec, CA.

During late embryogenesis, Caenorhabditis elegans embryos elongate along their antero-posterior axis. This elongation is divided into an early phase, which is directed by morphogenetic events occurring within the hypodermis, and a late phase involving the collaboration between the hypodermis and the underlying body-wall muscles. Early elongation is controlled by two morphogenic programs: a RhoA-like program, involving ROCK/LET-502 and controlling the morphogenesis of lateral hypodermis, and a Rac1-like program, involving the Rac/Cdc42-specific effector PAK-1 and the Guanine-nucleotide Exchange Factor PX1 which control morphogenesis of dorsal and ventral anterior hypodermis. Our previous study established that RhoA/Rac1 antagonism defined cell-to-cell heterogeneity within the hypodermis at that stage. Morphogenesis is an extremely energy demanding process. However, the metabolic/catabolic pathways fueling cytoskeleton remodelling during embryonic morphogenesis has not been characterized in C. elegans. In order to better understand the molecular basis of cell-to-cell heterogeneity during early elongation, we dissected these energetic pathways at a single cell level. Using genetic mutants and transgenic animals expressing junctional fusion proteins, RNAi, quantitative DIC and confocal dynamic microscopy, we showed that genes coding for the energy sensor AMPK alpha-subunits aak-1/aak-2 and the genes controlling the autophagy Beclin/bec-1 and ATG14/eppg-8 are required for the proper elongation of the lateral hypodermal cells. Our data suggest that AMPK and autophagy are required for early elongation as part of the RhoA-like program and are dispensable for the Rac1-like program. We also showed that the elongation depends on functional mitochondrial respiration chain (MRC) complexes II, III, IV and ATP-synthase, glycolysis and glyoxylate cycle. Our data suggest that a functional MRC is required for the morphogenesis of lateral cells and that alteration of MRC complex IV has significant impact on the signaling pathways used by these cells to elongate. Altogether, these data suggest that cell-to-cell heterogeneity, previously observed within the hypodermis during early elongation, is characterized by the machineries remodelling apical junctions and controlling formation and shape of basolateral protrusions and by pathways providing cells with energy.

399B Branched actin defines Cadherin receptor dynamics in C. elegans embryonic epithelia. S. Borinskaya1,2, S. Sasidharan1,2, E. Larsen1,2, M. Solo1,2 1) Robert Wood Johnson Medical School; 2) Rutgers University.

During embryonic morphogenesis organs form and migrate to their final positions. This process requires continuous assembly and remodeling of adherens junctions (AJ). Our studies have identified Arp2/3 (branched actin nucleator) and its nucleation promoting factor, WAVE/Scar, as essential regulators of epithelial junctions. E-Cadherin/HMR-1 is a transmembrane receptor and an essential component of the AJ. Recent studies have shown that the turnover of Cadherin at the AJ is much more dynamic than previously thought. Adherens junctions rely heavily on the architecture and dynamics of the actin cytoskeleton. However, determining the function of the branched actin network at the junctions has proven elusive. Biochemical data have
shown that AJ component α-catenin/HMP-1 inhibits Arp2/3-dependent branched actin, which suggested that branched actin is only needed at the earliest stages of apical junction development. We have addressed the role of branched actin at epithelial junctions using Transmission Electron Microscopy and live imaging. We found that branched actin is continuously recruited during both the dynamic process of junction formation and the later process of junction maintenance. Our results demonstrated an essential role for branched actin regulators in apical junction maintenance, and interdependence between the branched actin regulators and the Cadherin-Catenin complex (CCC). Therefore, we propose that there is a feedback mechanism present in which AJ-to-WAVE crosstalk dictates how the branched actin network is functioning. To our surprise, the three main components of the CCC complex, including Cadherin/HMR-1, accumulated to higher levels in embryos depleted of WAVE. We turned to FRAP imaging technique and biochemical approaches on C. elegans embryos to address this observation. We predicted that the increased levels of HMR-1::GFP in WAVE mutants resulted from slower turnover of HMR-1 at the membrane. Instead, we observed higher HMR-1::GFP mobility in the intestinal and epidermal junctions of WAVE-defective embryos. Several competitive models could be used to explain these results. Our goal is to determine which of the proposed mechanisms or Cadherin turnover, lateral-to-apical diffusion or vesicular trafficking, is significantly disrupted in WAVE mutants. We will interpret the fluorescence recovery traces by kinetic modeling. In addition we will quantify HMR-1 levels and mobility in embryos that are devoid of trafficking proteins that regulate distinct trafficking steps. These studies will advance understanding of the branched actin role at the forming and established adherens junctions. We will elucidate how branched actin affects Cadherin turnover at the apical cell-cell contacts in an in vivo system.

400C  SPV-1, a RhoGAP with an F-BAR domain, regulates calcium signaling in the C. elegans spermatheca during ovulation events.  J. Boutillard1, R. Zaidel-Bar2, E. Cram1  1) Northeastern University, Boston, MA; 2) Mechanobiology Institute, National University of Singapore, Singapore.

The spermatheca is a 24-cell tube in the middle of the C. elegans reproductive system that functions as the site of fertilization, undergoing rounds of extreme stretch to accommodate entering oocytes followed by contraction to expel the fertilized egg. The contractility of the spermatheca is tightly regulated at the actomyosin level by calcium signaling and Rho activity. Many smooth muscle and non-muscle systems in other animals also exhibit actomyosin contractility regulated by calcium signaling and Rho activity, making the spermatheca a powerful model to study fundamental biological mechanisms regulating contractile tubes.

SPV-1, a RhoGAP with an F-BAR domain, has emerged as a key regulator of contractility in the spermatheca. When spv-1 is lost, the spermatheca contracts immediately upon oocyte entry, leading to faster egg transit and perturbed egg shapes. Calcium signaling has been shown to drive contraction in the spermatheca, so we used the genetically encoded calcium sensor GCaMP expressed in the cells of the spermatheca to determine if the loss of spv-1 alters calcium signaling. Using widefield fluorescence microscopy, we monitored calcium activity in the spermatheca during ovulation events.

We find that the loss of spv-1 results in a more rapid onset of calcium signaling upon oocyte entry, and elevated calcium levels throughout ovulation events. Overexpressing spv-1::mApple results in the expected opposite calcium phenotype, with slower onset of calcium signaling upon oocyte entry and decreased calcium levels throughout ovulation events. In addition to altered calcium levels, mutants with altered levels of spv-1 also lack proper spatiotemporal regulation of calcium signaling. High levels of SPV-1 result in retention of the embryo in the spermatheca, and preliminary data suggests that SPV-1 acts in a dose dependent manner to regulate the baseline calcium level. Studies using a mutated spv-1 which lacks RhoGAP activity exhibit calcium signaling similar to the loss of spv-1, suggesting that spv-1 controls calcium signaling primarily through GTPase activity.

Previous work established that contractility of the spermatheca is carefully regulated by calcium signaling and RhoA activity. This work suggests that there is significant crosstalk between these two regulatory networks. Ongoing work aims to identify effectors that SPV-1 uses to regulate calcium signaling, and to use quantitative microscopy and analysis to more deeply understand how SPV-1 is modulating calcium signaling.

401A  Novel genes involved in excretory canal structure and maintenance.  Travis Chiarelli, Hikmat Al-Hashimi, Erik Lundquist, Matthew Buechner  Dept. of Molecular Biosciences, University of Kansas, Lawrence, KS.

The excretory canal cell is the largest single epithelial cell within C. elegans, and its long fluid-filled hollow canals forms a useful model for understanding single-cell tubulogenesis. Our previous studies have identified a set of exc genes whose products regulate and maintain the narrow diameter of the canals during outgrowth. Some of these genes encode cytoskeletal proteins (including β-spectrin, formin, and intermediate filament proteins), and vesicular transport proteins (CRIP, IRG, and FGD proteins).

The Miller lab has made available a list of genes highly enriched in various tissues of C. elegans (www.vanderbilt.edu/wormdoc/wormmap/WormViz.html), including genes enriched in the excretory canal cell. We have gratefully built on these results to search for additional genes that affect excretory canal structure, as well as genes that interact with known exc genes. We designed and are conducting a feeding RNAi screen with ~200 candidate genes strongly expressed in the canal cell, and which encode proteins with good potential to interact with EXC proteins, with the purpose of understanding the network of structural and trafficking required to build and maintain a narrow tube in a growing, moving animal. Since the excretory canal cell may be refractory to RNAi, as it has some neural characteristics (long processes following neural pathways), we have been able to isolate and verify close to ten hits, some of which exacerbate...
canal defects, and excitingly, one which at least partially rescues the strongly cystic phenotype of the exc-5 mutant. These knockdowns offer insight into novel gene interactions as well as alternative pathways that have yet to be explored in regards to excretory canal maintenance. We are continuing the RNAi screen.


lin-28 was first characterized as a developmental timing regulator in C. elegans. lin-28 loss of function mutants skip L2 specific rates of the lateral hypodermal and vulva cell lineages, causing precocious development of these tissues. lin-28 mutants also exhibit reduced fertility. Our data show that lin-28 mutants not only produce far fewer embryos than wild type, but also ~72% embryos from lin-28 mutants are not viable at 25°C. We found that reduced fertility in lin-28 mutants is associated with abnormal morphogenesis of the somatic gonad, including defective structure of the spermathecal-uterine valve (Sp-Ut valve), which connects the spermatheca to the uterus in wild type animals. Consequently, many lin-28 embryos become trapped in the spermatheca after fertilization. Due to this blockage of spermathecal exit, some lin-28 oocytes contain endometiotic DNA, a characteristic of defective ovulation. Moreover, lin-28 embryos are more permeable to lipophilic dye than wild type embryos, indicating an abnormal egg shell integrity, which contributes to the lethality of embryos produced by lin-28 hermaphrodites. We found that the genes previously shown to act downstream of lin-28 in the regulation of lateral hypodermal and vulval developmental timing also act downstream of lin-28 in Sp-Ut valve morphogenesis and fertility. For example, loss of let-7,lin-29 or lin-46 suppresses abnormal Sp-Ut valve phenotype and fertility defects in lin-28 mutants. Also, wild type animals treated with hbl-1 RNAI also exhibit Sp-Ut valve morphogenesis defects similar to lin-28(lf) mutants. However, we found that the development of the somatic gonad, including the Sp-Ut valve, does not appear to be precocious in lin-28(lf) mutants, unlike the hypodermis. Moreover, somatic gonadal expression of lin-28 is not sufficient for rescuing Sp-Ut valve defects in lin-28(lf) mutants. Therefore we hypothesize that the abnormal somatic gonad morphogenesis of lin-28(lf) hermaphrodites results from temporal discoordination between the precocious development of hypodermal tissues and the essentially normal timing of somatic gonad development. We are now using tissue-specific rescue strategies to further investigate the anatomical focus of action of lin-28 in the control of somatic gonadal morphogenesis.

403C Development of amphids and other sensory epithelia in C. elegans. M.K. Chong1,2, B.M. Wierbowski1,2, M.G. Heiman1,2  1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Boston Children’s Hospital, Boston, MA.

Epithelia line nearly every surface of the body, establishing a barrier that resists breakage in the face of morphogenesis and growth. Some epithelia, called sensory epithelia, are innervated by neurons and act as a major interface where the organism receives information from its environment. These sensory epithelia are characterized by the presence of neurons and glia that are interposed among more conventional epithelial cells. We have turned to the sense organs of the C. elegans as a model for understanding the development of sensory epithelia. Each sense organ consists of a bundle of neurons that extend a single, unbranched dendrite to the nose. To access the environment, these dendrites project through a sensory epithelium made up of two glial cells that form a continuous sheet with the epithelium of the skin. During embryogenesis, neuronal dendrites elongate by remaining attached to this epithelium as the cell bodies migrate away in a process called retrograde extension. This process requires DYF-7, a ZP-domain-only protein that is secreted into the apical ECM during development. Without DYF-7, the sensory epithelium ruptures at glial junctions, causing a "short dendrite" phenotype. While all glial-ensheathed sensory dendrites are affected by loss of DYF-7, the severity of the phenotype varies with amphid neurons being the most strongly affected. We have taken a forward genetic approach to identify other factors that act together with or in parallel to DYF-7 in the amphid or in non-amphid sense organs. First, we performed a clonal enhancer screen in a hypomorphic dyf-7 background and isolated both an enhancer and a suppressor of dyf-7 that alter the incidence of short amphid dendrites from ~35% to ~90% or ~4%, respectively. Second, to understand the differences between sense organs, we performed a forward genetic screen for inner labial (IL) neurons with short dendrites. In contrast to dyf-7, which affects amphids strongly and ILs weakly, we isolated a mutant that strongly affects IL dendrite development, including both dendrite extension defects and dendrite "wandering" or defascication defects, while having much milder effects on amphid neurons. We predict that across the various sense organs, distinct factors either interact with DYF-7 directly or act in parallel to shore up cell adhesion at the epithelial surface.

404A Improved untwisting software for examining development in moving C. elegans embryos. R Christensen1, A Bokinsky2, A Santella3, M Moyle3, Y Wu1, M Guo1, E Ardiel1, B Harvey1, W Duncan1, M Levin1, E McCready2, W Mohler1, D.A. Colón-Ramos5, Z Bao5, H Shroff5  1) NIBIB, National Institutes of Health, Bethesda, MD; 2) Center for Information Technology, National Institutes of Health, Bethesda, MD; 3) Developmental Biology Program, Sloan-Kettering Institute, New York, NY; 4) CNNR, Department of Cell Biology, Yale University School of Medicine, New Haven, CT; 5) Center for Cell Analysis and Modeling, University of Connecticut Health Center, Farmington, CT.

The C. elegans embryo represents an excellent system in which to examine complex, systems-level developmental events, but rapid embryo movement and elongation confound inspection of events after embryos have begun twitching. This period of time encompasses numerous developmental processes, including cell specialization, neurite outgrowth and neuronal migration, and the beginning of functional activity in the nervous system. We recently reported (Christensen et al., eLife. 2015 4:e10070) a combined imaging and data analysis pipeline to enable developmental events during this period time to be studied. We now report improvements in our untwisting strain by incorporating a worm surface marker (lin-26p::vab-10(activating domain)::GFP, Gally C, et al., Development. 2009 136(18):3109-19) to provide more accurate information about the dorsal, ventral, and lateral boundaries of the developing embryo. We have adjusted the untwisting software to segment the surface marker, leading to less dorsal-ventral clipping and
better capture of embryo structure in bent regions compared to the original untwisting algorithm. We have also incorporated better segmentation of seam cell nuclei when untwisting, leading to reduced lateral clipping in untwisted images, and have added the ability to convert 3D positions between twisted and untwisted space for any volume. We apply the improved untwisting software to track the position of seam cell nuclei and E lineage intestinal nuclei. We plan to use this software to track the position of all nuclei in the embryo from the beginning of twitching until hatching, as well as the morphological development of the C. elegans nervous system during embryogenesis.

405B  A zona pellucida protein and a scavenger receptor play opposing roles in shaping a narrow tube.  J.D. Cohen, R. Forman-Rubinsky, M.V. Sundaram  Department of Genetics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA.

A lipid-, sugar-, and glycoprotein-rich apical extracellular matrix (aECM) shapes and protects the narrowest tubes in the body. For example, lung surfactant helps narrow Airways remain open, and a glycolyx (“sweet husk”) lines capillaries in the vascular system. Damage to the aECM may contribute to diseases of tube integrity such as chronic obstructive pulmonary disease and microvascular disease. Nevertheless, the roles that specific aECM components play in matrix organization and tube protection remain poorly understood.

To study aECM in narrow tube maintenance, our lab uses the C. elegans excretory system, a simple conduit composed of three tandem, unicellular tubes. In particular, we have focused on understanding the maintenance of the excretory system’s middle tube, the duct cell, as it elongates during embryogenesis. Genetic screening has identified apically secreted proteins that are required to maintain the duct lumen, including LET-653, which contains a Zona Pellucida (ZP) domain. ZP proteins are highly abundant in many aECMs, and ZP protein dysfunction is associated with human diseases, including chronic kidney disease and the microvascular disease hereditary hemorrhagic telangiectasia (HHT), but how ZP proteins protect tube integrity is still unclear. ZP domains consist of two parts, ZP-N and ZP-C. In vitro studies suggest that ZP-N domains can polymerize to form fibrils, while ZP-C domains regulate ZP-N polymerization and/or bind specific partners to alter signaling. However, we found that the LET-653 ZP-C domain is sufficient to rescue let-653 mutants and to form a stable (though transient) layer in the aECM; we are now testing if it can polymerize on its own and are seeking to identify its potential partners.

We sought to identify LET-653 interactors by screening for suppressors. Surprisingly, we found that loss of SCAV-2, a putative scavenger receptor, bypasses the requirements for LET-653 and several other aECM components. Scavenger receptors can transport lipids and the enzymes that process them; thus, scavenger receptors may regulate the amounts or types of lipids reaching the aECM. let-653 mutants show increased Dil staining of aECM lipids, suggesting that aberrant lipid accumulation may underlie let-653 phenotypes. Continuing work will identify the lipid classes that may interact with LET-653 in the aECM, and describe how SCAV-2 may regulate these lipids to alter the aECM.

406C  VPR-1 MSP domains have permissive and instructive signaling properties in different cell types.  T. Cole1, J. Schultz1, J. Vibeert1, P. Cotte1, H. Hoang1, S. Lee1, J. Chen2, S. Han3, M. Miller1 1) Department of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham ; 2) Department of Cell and Developmental Biology, Vanderbilt University; 3) Department of Neuroscience, Yale University.

The major sperm protein domain (MSP) is an evolutionarily conserved immunoglobulin-like structure with extracellular signaling and intracellular functions. The C. elegans genome encodes numerous proteins containing MSPs, including about 28 isoforms expressed specifically in sperm. vpr-1 encodes a type II endoplasmic reticulum protein with an N-terminal MSP, coiled coil motif, and C-terminal transmembrane domain. This broadly expressed, ancestral form has homologs in most animal species called VAPs. Human VAPB/ALS8 is associated with amyotrophic lateral sclerosis (ALS), a muscle disease caused by motor neuron degeneration. We have shown that the VAP MSP is proteolytically liberated from the transmembrane domain in the cytosol and secreted by an unconventional mechanism. In C. elegans, the secreted MSP interacts with the CLR-1 Lar-like receptor, thereby promoting mitochondrial localization to body wall muscle I-bands (Han et al. 2012 and 2013). Here we show that muscle mitochondrial abnormalities initiate during larval development. In addition to muscle defects, vpr-1 null mutants are sterile, due to arrested gonad development at the L2-L3 stage. Genetic mosaic and cell type specific expression studies suggest that the germ line, nervous system, and intestine are cellular sites of VPR-1 MSP secretion. Using Cas9 genome-editing technology, we show that endogenous CLR-1 is expressed throughout the muscle plasma membrane in larval and adult worms. MSP to CLR-1 signaling is sufficient to remodel muscle mitochondria during the L4 stage and adulthood. We used the binary Q system to temporally control VPR-1 expression. The results indicate that VPR-1 is specifically required for gonad development in a short time window around late embryogenesis or early L1. In mammals, VAP MSPs are found in blood and cerebrospinal fluid (Tsuda et al. 2008; Deidda et al. 2014). Similarly, our studies in C. elegans are consistent with VPR-1 MSP being secreted into the pseudocoelom. We are also characterizing mouse Vapb mutants, which have muscle mitochondrial abnormalities in fast-twitch skeletal muscle fibers. Taken together, our data support the model that secreted VAP MSP domains act on somatic gonadal and striated muscle cells at different times in postembryonic development.

407A  Developing tools to conditionally deplete proteins with combined spatial and temporal resolution using ZF1-mediated degradation.  L.B. Dojer, J. Nance  Developmental Genetics, Skirball Institute of Biomolecular Medicine, New York, NY.

In order to study the role of essential genes in specific developmental events, it is often necessary to have rapid control of gene function with both temporal and spatial resolution. Inhibition of gene function is most rapidly achieved by directly targeting protein gene product. Several methods have been described to deplete proteins by fusing them to a degron which can be
conditioned targeted for degradation. We previously described the ZF1-tagging method, wherein a protein can be rapidly depleted from cells by fusing it with the ZF1 degron and expressing the ZIF-1 adaptor protein; ZIF-1 targets the fusion protein for rapid destruction. A limitation of the ZF1-tagging method is that it is not currently possible to have both spatial and temporal control over protein degradation. We are developing a set of genetic tools that will allow us to bypass this limitation by adding a Cre-Lox recombination step that is needed for ZIF-1 expression. These tools will provide a powerful system for rapidly inactivating gene function in a particular set of cells at a particular time in development.

**408B  Tissue-specific activity of pathway components regulating *C. elegans* embryonic epidermal contraction.**  *E. Drewrik,* R. Smit, P. Mains  University of Calgary, Calgary, CA.

Epidermal morphogenesis plays a significant role in driving early elongation of the *C. elegans* embryo. Interactions between actin and myosin components result in contraction of the cytoskeleton, driving shape changes in epidermal cells that squeeze the worm from an ovoid embryo to a vermiciform larva. The actin cytoskeleton differs between lateral and dorsal/ventral (DV) cells, with an actin meshwork in the lateral epidermis and parallel bundles in dorsal/ventral epidermis. Contraction is thought to be strongest in lateral epidermal cells, whereas DV cells are proposed to play a more passive role, though still generate tension. I am investigating tissue-specific activity of the genetic components involved in regulating contraction using transgenic rescue and RNAi techniques.

Contraction/elongation is regulated by *let-502*/Rho-binding kinase and *mel-11*/myosin phosphatase working antagonistically in one branch of the elongation pathway, while *pak-1/p-21* activated kinase and *fem-2/PP2c* phosphatase work in a parallel pathway. *let-502* activity is regulated antagonistically by *rhgf-2*/Rho-GEF (Guanine exchange factor) and *rga-2*/Rho-GAP (GTPase activating protein). Both *rhgf-2* and *rga-2* are ubiquitously expressed in the epidermis; however, it has been shown by our lab that *rhgf-2* activity is sufficient in lateral epidermal cells, and shown by the Labouesse lab that *rga-2* is sufficient in DV cells (Diogon et al. Development, 2007). Evidence from our lab also suggests that *fem-2* is sufficient in DV cells. Although *let-502* and *mel-11* are expressed ubiquitously throughout the embryonic epidermis, it is yet to be determined in which cells the genes are necessary or sufficient.

I have designed constructs to test rescue of *let-502, mel-11,* and *pak-1* mutants in a tissue-specific manner under the control of lateral (*ceh-16*) and DV (*elt-3*) promoters. Additionally, I have generated constructs restricting RNAi activity in an *rde-1*(null) background to lateral or DV cell populations to test phenoicity. These methods will determine whether *let-502, mel-11,* and *pak-1* are sufficient and/or necessary in lateral, DV, or both cell types. Preliminary evidence for *let-502* suggests tissue-specific rescue following our model, where lateral embryonic epidermal expression rescues and DV expression does not.

**409C  A multi-cellular rosette mediates collective dendrite extension in the amphid.**  *L. Fan*1, L. Kovacevic1, M. Heiman2,3, Z. Bao1  1) Developmental Biology Program, Sloan Kettering Institute, New York, NY; 2) Division of Genetics, Boston Children's Hospital, Boston, MA; 3) Department of Genetics, Harvard Medical School, Boston, MA.

Coordination of neurite morphogenesis is crucial to the establishment of complex neural circuits, but the underlying cellular and molecular mechanisms remain poorly understood. We show an unexpected collective dendrite extension in the amphid sensory neurons in *C. elegans*. The amphid neurons first assemble into a multicellular rosette. The vertex of the rosette is attached to the anteriorly migrating epidermis and carried to the sensory depression, extruding the dendrites away from the neuronal cell bodies. Multiple adhesion molecules including DYF-7, SAX-7, HMR-1 and DLG-1 function redundantly in the attachment. PAR-6 is localized to the rosette center and dendrite tips, and required for DYF-7 localization and dendrite extension. Our results suggest a collective mechanism that coordinates neurite extension.

**410A  Lipocalins are required for apical extracellular matrix organization.**  *Rachel Forman-Rubinsky,* Jennifer Cohen, Meera Sundaram  Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA.

Biological tubes are lined by a poorly understood apical (luminal) extracellular matrix (aECM) or “glycocalyx” consisting of lipids, carbohydrates and glycoproteins. This aECM influences tube shape and integrity, and its disruption or loss can cause organ failure and disease. Very narrow tubes, such as capillaries and lung alveoli, appear particularly susceptible to aECM-related defects. We are using *C. elegans* to study the specific composition and structure of aECM and its tube-protecting functions. *C. elegans* external epithelia develop in the presence of a glycocalyx-like aECM that later matures to form the cuticle. Several components of the early glycocalyx are required for integrity of the narrow excretory duct and pore tubes. Others are required to inflate the larger vulva tube. By screening for mutants with excretory duct and pore defects, and then visualizing the discovered proteins in the vulva (where they are also present), we are beginning to learn about the multi-layered organization and dynamics of the protective glycocalyx.

EMS mutagenesis screens for lethal mutants with duct or pore cell abnormalities identified two lipocalins; *lpr-1* and *lpr-3*. Lipocalins are a family of functionally diverse, cup-shaped secreted proteins that bind and transport various lipophilic molecules. In humans, lipocalins are often found in luminal or aECM compartments such as blood plasma, urine or tear film, and they are used as biomarkers for detecting tissue damage. LPR-1 and LPR-3 are apically secreted into the glycocalyx, but they have different patterns of localization. LPR-1 localizes diffusely and can function tissue non-autonomously. LPR-3 localizes specifically to a matrix layer near the apical membrane, adjacent to the glycoprotein LET-653. In addition to the excretory phenotypes, both *lpr* mutants have other aECM related phenotypes such as defects in alae, cuticle permeability, or molting. We conclude that these lipocalins are required for aECM organization and its tube-protecting functions. Current studies are testing relationships between lipocalins and other aECM glycoproteins, and investigating whether lipocalins transport or sequester aECM lipids.
Basement membranes are evolutionarily ancient conserved structures that support tissue growth, development, and function. We have previously reported that two basement membrane proteins, Peroxidasin/PXN-2 and F-spondin/SPON-1, are essential for epidermal elongation in C. elegans (Gotenstein, et al. Development. 2010; Woo, et al Development. 2008). We performed large-scale forward genetic screens for suppression of embryonic lethality in pxn-2 or spon-1 mutant backgrounds. F-spondins are conserved ECM proteins whose mechanisms remain unclear. Peroxidasins are extracellular peroxidases thought to catalyze sulfhydryl cross-linking of collagen IV in the basement membrane (Bhave, et al. Nat Chem Biol. 2012); genetic interactions between collagen IV and pxn-2 mutants support this role for pxn-2 in C. elegans. Complete loss of function of PXN-2 causes nonconditional lethality and arrest in late embryogenesis. We report that such pxn-2 null mutant phenotypes can be suppressed to viability by gain of function mutations in a variety of extracellular matrix or cell-matrix receptor proteins. We identified over 30 suppressors, 12 of which result in missense alterations in the transmembrane protein myoctatin/LET-805. The LET-805 extracellular domain contains 36 Fibronectin type III repeats; our suppressor mutations cluster in two pairs of repeats, suggesting these repeats may play a critical role in LET-805 function. let-805 null mutants are nonconditional embryonic lethal, whereas our suppressors are viable and display no obvious phenotypes other than suppression. Localization of a LET-805::GFP knockin is essentially normal in the suppressor alleles, suggesting they have a subtle effect on LET-805 expression. let-805 suppressor alleles partially suppress lethality in loss of function but not null mutations in other basement membrane components, but do not suppress loss of function in intracellular epidermal cytoskeletal components. pxn-2 mutants have defects in locomotion that are not suppressed by let-805, and we will report our analysis of this possible neural role for PXN-2.

In summary, our results reveal novel compensatory mechanisms in basement membrane receptor complexes that allow key structural or enzymatic components to be bypassed.

### 411B Genetic suppression of basement membrane defects in C. elegans.

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Basement membranes are evolutionarily ancient conserved structures that support tissue growth, development, and function. We have previously reported that two basement membrane proteins, Peroxidasin/PXN-2 and F-spondin/SPON-1, are essential for epidermal elongation in C. elegans (Gotenstein, et al. Development. 2010; Woo, et al Development. 2008). We performed large-scale forward genetic screens for suppression of embryonic lethality in pxn-2 or spon-1 mutant backgrounds. F-spondins are conserved ECM proteins whose mechanisms remain unclear. Peroxidasins are extracellular peroxidases thought to catalyze sulfhydryl cross-linking of collagen IV in the basement membrane (Bhave, et al. Nat Chem Biol. 2012); genetic interactions between collagen IV and pxn-2 mutants support this role for pxn-2 in C. elegans. Complete loss of function of PXN-2 causes nonconditional lethality and arrest in late embryogenesis. We report that such pxn-2 null mutant phenotypes can be suppressed to viability by gain of function mutations in a variety of extracellular matrix or cell-matrix receptor proteins. We identified over 30 suppressors, 12 of which result in missense alterations in the transmembrane protein myoctatin/LET-805. The LET-805 extracellular domain contains 36 Fibronectin type III repeats; our suppressor mutations cluster in two pairs of repeats, suggesting these repeats may play a critical role in LET-805 function. let-805 null mutants are nonconditional embryonic lethal, whereas our suppressors are viable and display no obvious phenotypes other than suppression. Localization of a LET-805::GFP knockin is essentially normal in the suppressor alleles, suggesting they have a subtle effect on LET-805 expression. let-805 suppressor alleles partially suppress lethality in loss of function but not null mutations in other basement membrane components, but do not suppress loss of function in intracellular epidermal cytoskeletal components. pxn-2 mutants have defects in locomotion that are not suppressed by let-805, and we will report our analysis of this possible neural role for PXN-2.

In summary, our results reveal novel compensatory mechanisms in basement membrane receptor complexes that allow key structural or enzymatic components to be bypassed.

### 411C Identification of critical MAPK phosphorylation sites in LIN-31 and their effect on vulval morphogenesis in C. elegans.

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The development of the vulva in Caenorhabditis elegans is believed to be reliant on the phosphorylation of LIN-31 and LIN-1, two winged-helix transcription factors. LIN-31 and LIN-1 are part of the Ras/MAPK Kinase (MAPK) cell-signaling pathway. When MPK-1, a protein in the Ras/MAPK pathway upstream of the transcription factors, is phosphorylated, it enters the nucleus and phosphorylates LIN-1 and LIN-31. MPK-1 can phosphorylate any of the four consensus sites on LIN-31 or five major sites on LIN-1, which are a serine or a threonine adjacent to a proline. Our lab has found evidence that phosphorylation of these transcription factors may be connected to the morphogenetic events that result in the formation of the vulval structure, because a double phosphorylation-defective mutant produced a toroid fusion defect in the vulva. However, it is unclear which MPK-1 consensus sites on LIN-1 and LIN-31 are critical to vulval development. To determine the role of MPK-1 phosphorylation of LIN-1 and LIN-31 in vulval morphogenesis, we began by systematically knocking out different combinations of MAPK phosphorylation sites on LIN-31 using PIPE cloning. In order to integrate our mutations into the worm genome, we plan to employ a CRISPR/Cas9 strategy with self-excising cassette selection and a plasmid repair template containing our lin-31 mutations. Our lab originally created the phosphorylation-site knockout combinations in cDNA, but this CRISPR technique required plasmids with the mutations in genomic DNA. Using PIPE cloning, these mutations are being transferred into a genomic lin-31 backbone to be used as the repair template. Our lab is also currently attacking this problem from a slightly different direction using a co-CRISPR strategy with a single-stranded DNA repair template.

### 411A Eggshell plays a role in regulating body length of C. elegans at a late stage of development.

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A nematode embryo is surrounded by a chitinous eggshell. The functions of eggshell are to prevent polyspermy, support the spatial arrangement of blastomeres, and provide physical protection to embryo until hatching. It has been reported that eggs can develop from a 2-cell stage to hatching without the chitinous eggshell. However, cell lineages were followed only during the early stages (4~44-cell) to judge if the development went normally. Little is known about whether eggshell has a role in development at later stages besides the mechanical protection of the embryo.

In the present study, we examined the functions of eggshell at late stages of development. We removed eggshell of embryos at various stages in development with keeping the permeability barrier inside the eggshell, and then incubated them in egg buffer. The embryos hatched about 75% after the removal of eggshell even at Pretzel stage, which is the last stage of embryonic development. This rate is significantly lower than that of intact embryos with eggshell, which hatch more than 95%. In addition, the body length of L1 arrests hatched from the eggs without eggshell was about 15% shorter than that from intact eggs. These results imply that eggshell plays an important role in embryonic elongation. Mechanical support of embryos by hard eggshell may regulate precise epidermal morphogenesis.

### 411B Heterochronic pathway components lep-5 IncRNA and LEP-2/Makorin regulate LIN-28 stability to promote the juvenile-to-adult transition.

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During the juvenile-to-adult transition at the L4 stage, the tail tip cells in C. elegans males fuse, change shape and migrate anteriorly. The timing of this tail tip morphogenesis (TTM) is governed by the heterochronic pathway, best known for scheduling seam cell development. Previous work showed that heterochronic genes let-7 and lin-41 play a role in the timing of TTM, which is delayed into adulthood in let-7(lf) and lin-41(gf) mutants. We identified two new heterochronic pathway genes, lep-2 and lep-5.
Males mutant in either gene have fully unretracted ("leptoderan", Lep) tail tips that delay TTM into adulthood. Additionally, mutant adults undergo an additional molt and mutant lep-2 males show defective mating behavior, suggesting that the genes act in the body epidermis and nervous system as well as the male tail. Neither mutant has obvious defects in lateral seam development. lep-2 encodes a Makorin, a member of a family of conserved proteins with putative nucleic acid binding and E3 ubiquitin ligase activities. The active product of lep-5 is a cytoplasmically localized, long noncoding RNA (IncRNA), well conserved within Caenorhabditis but not yet identified outside of this genus. SL1-spliced lep-5 IncRNA is predicted to adopt a secondary structure with several stem-loops, at least two of which are necessary for its function. Using a photo-convertible LIN-28::Dendra2 fusion, we found that LEP-2 and lep-5 IncRNA promote degradation of LIN-28 protein, without affecting lin-28 mRNA levels. This is consistent with the predicted E3 ubiquitin ligase activity of LEP-2, which also has a predicted RNA-binding domain. Specifically, we hypothesize that the lep-5 IncRNA serves as a scaffold to bring LEP-2 and LIN-28 into close proximity to allow ubiquitination of LIN-28, tagging it for proteasomal degradation. We are currently testing this hypothesis. The proposed mechanism could be conserved in vertebrates. The human homolog of LEP-2, MKRN3, and human LIN28B, are both involved in the regulation of pubertal timing. Together with our findings in C. elegans, this suggests a conserved role of Makorins and LIN-28 in timing of the juvenile-to-adult transition. If a mammalian homolog of lep-5 IncRNA exists, it is expected to show structural similarity rather than sequence similarity; we are searching for such structural homologs. The fact that seam cell development is apparently unaffected by mutations in lep-2 and lep-5 suggests that developmental timing is regulated in a tissue-specific manner. Therefore, the male tail tip is a valuable model for studying the heterochronic pathway in all its complexity.

415C Tissue inhibitors of metalloproteinases (TIMPs) genetically interact with ADAMTS proteases and regulate gonadal development in C. elegans. Y. Kubota1, K. Nishiwaki2, A. Sugimoto1 1) Graduate School of Life Sciences, Tohoku University, Sendai, Japan; 2) Department of Bioscience, Kwansei Gakuin University, Sanda, Japan.

During development, extracellular matrix (ECM) supports proper development of tissues and organs by regulating cellular behaviors and tissue integrity. For proper ECM remodeling, spatiotemporal regulation of matrix metalloproteinase (matrix protease) activity is required. The ADAMTS family proteases are evolutionally conserved secreted zinc matrix proteases. In C. elegans, ADAMTS proteases, GON-1 and MIG-17 are required for gonadal morphogenesis (Blelloch R and Kimble J, 1999; Nishiwaki K et al, 2000). However, regulatory mechanisms of ADAMTS proteases in vivo remain to be explored.

Here, we focus on tissue inhibitors of metalloproteinases (TIMPs) that are small secretory proteins with a netrin domain, which have been shown to have negative regulate ADAMTS proteases in mammalian in vitro studies. The C. elegans genome encodes two TIMPs, K07C11.3/TIMP-1 and K07C11.5/CRI-2. Transcriptional and translational GFP/Venus fusion analyses indicated that TIMP-1 and CRI-2 were secreted and localized to the gonadal basement membrane and plasma membrane of the germ cells. The timp-1(tk71) deletion mutant, but not the cri-2(gk314) deletion mutant exhibited gonadal growth defects and sterility. These phenotypes of the timp-1(tk71) mutant were almost completely rescued by TIMP-1::Venus but not by TIMP-1(C215S)::Venus in which an essential Cys residue for inhibitor activity was substituted by Ser, suggesting that the protease inhibitor function of TIMP-1 is required for gonadogenesis and germ cell proliferation. RNAi knockdown and mutations of the timp-1 and cri-2 partially suppressed gonadal morphogenesis defects of the gon-1/ADAMTS and mig-17/ADAMTS mutants. In timp-1(tk71) mutant, localization of mCherry-tagged EMB-9, a1 chain type IV collagen, in the gonadal basement membrane was significantly decreased, and EMB-9::mCherry overexpression partially rescued the gonadal defects of the timp-1(tk71) mutant. Based on these observations, we propose that C. elegans TIMPs, TIMP-1 and CRI-2 act as negative regulators of the ADAMTS proteases to regulate basement membrane remodeling and gonadal development.

416A Extrinsic stress triggers an actin-based visco-plastic process to drive tissue elongation. Gabriella Pásti1, Alicia Lardennois2, Teresa Ferraro2, Julien Pontabry1, David Rodriguez1, Flora Liense2, Samantha Kim1, Christelle Gally1, Michel Labouesse1,2 1) Development and Stem Cells Department, IBGMC – CNRS UMR 7104/ INSERM U964/ Université de Strasbourg, 1 rue Laurent Fries, 67400 Illkirch, FR; 2) Université Pierre et Marie Curie, IBPS, CNRS UMR 7622, 7 Quai St-Bernard, 75005 Paris, FR.

Embryonic morphogenesis refers to changes in the shape and position of cells within the embryo. While it is clearly established that mechanical forces induced by molecular motors play a critical role in driving these changes, proper understanding of how cells respond to such forces is lacking. To understand the interplay between cell elasticity and its response to forces we are studying elongation of C. elegans embryos, which is initially driven by actomyosin contractility till muscle becomes active at the 1.7 fold stage, and then allow the embryo to elongate to reach 4-fold stage. Our main goal is to understand how the epidermis, an elastic material, acquires progressively its shape through cycles of muscle contractions. Previous work has positioned the kinase PAK-1 at the crossroads of hemidesmosomes and cytoskeleton remodeling. To better understand how PAK-1 acts, we performed a yeast two-hybrid screen and an RNAi screen in a strong pak-1 mutant background, which identified one factor: the a-spectrin SPC-1. We found that spc-1(-) pak-1(-) mutant embryos elongate up to the 1.5-fold stage and then retract to their initial shape. At the subcellular level, circumferential actin filament bundles appear discontinuous and not fully oriented perpendicular to the seam-dorsal junction. We performed a second RNAi enhancer screen in a spc-1(-) background and we identified three actin-binding proteins. Their absence, combined with the lack of the spectrin cytoskeleton, also induces embryos to retract. Interestingly, one of them is a formin with actin bundling properties. In a nutshell, we discovered a molecular network involved in stabilizing cell shapes in a system submitted to repeated mechanical stress. We modelled the embryo as a Kelvin-Voigt material submitted to two forces: one provided by the acto-mysin from epidermis and a second one contractile from muscles, which allowed us to predict the lengthening of the embryo over time. Altogether our data identify a cellular network that confers mechanical plasticity (in physical terms, it implies an irreversible deformation under stress) that stabilizes cell shapes during morphogenesis.
417B  KSP-like rKSP-like repeat protein CNP-2 supports reproduction by acting like the KSP domain of human neurofilament medium subunit (NF-M) in Caenorhabditis elegans. Hana Jung, Serpen Durnaoglu, Younsoo Hahn, Joohong Ahnn, Sun-Kyung Lee 1,2,3 1) Life Science, Hanyang University, Seoul, KR; 2) BK21 PLUS Life Science for BDR Team, Hanyang University, Seoul 133-791, Republic of Korea; 3) Research Institute for Natural Science, Hanyang University, Seoul 133-791, Republic of Korea; 4) Department of Life Science, Chung-Ang University, Seoul 06974, South Korea.

Repetitive amino acid sequences often confer proteins specialized in structure and function, playing a critical role in evolution of proteins. The KSP repeats of neurofilaments medium subunit (NF-M) are heavily phosphorylated, and regulate axonal diameter and neuronal conduction velocity in myelinated axons by extending their molecular backbones. The number of the KSP repeats varies among mammalian species and exhibits a positive correlation with larger axonal diameter in larger animals. However, the role played by differing numbers of KSP repeats in determining cellular structure and function has not been fully examined. Here, we report that a Caenorhabditis-specific protein CNP-2 is a substrate of tax-6/calcineurin (calcium/calmodulin-dependent serine/threonine phosphatase) and a KSP-like repeat protein rich in Lys, Arg, Ser, Thr, Pro, and Glu, which are frequently found in proteins that display flexible architectural features. cnp-2 is highly expressed in extensively elastic tissues such as the spermatheca, the spermathecal-uterus valve, and male tail. Also, cnp-2 is critical for both hermaphrodites and male reproductive processes. Bioinformatics analyses and experiments in transgenic worms revealed that the KSP repeat domain of unc-89/obscurin (a giant sarcomeric signaling protein) is functionally homologous with CNP-2, although it is distantly related and shows low sequence similarity and identity. In addition, expression of KSP repeats from human NF-M reversed reproduction defects in cnp-2 worm mutants in a repeat-number-dependent manner; a greater number of KSP repeats showed more efficient effects with respect to morphology and activity. Taken together, the data suggest that cnp-2 is a KRSTPE-rich repeat protein that shares common features with the KSP repeats of NF-M, including regulatory molecular extension to support inter-molecular interactions, and contractile and elastic cellular structures. This study also provides insight into how alterations in the number of amino acid repeats may play a role in the determination of cellular morphology and function as a molecular mechanism contributing to the evolution of species-specific traits.

418C  A candidate feeding RNAi screen reveals a novel role for tbcd-1 during embryonic epidermal morphogenesis. Dakota Hall, Stephanie Maiden  Biology Department, Truman State University, Kirksville, MO.

In Caenorhabditis elegans embryonic development, a dynamic series of events called epidermal morphogenesis surrounds the developing embryo in an intact epithelium and forms the shape of the animal through body elongation. This adhesion-dependent process requires the coordinated effort of numerous cytoskeletal systems to drive the cell migrations and cell shape changes occurring during this stage of development. While it has been shown that drug destabilization of the microtubule cytoskeleton perturbs body elongation in C. elegans, the function of this cytoskeleton in the developing epidermis is not well understood. To identify microtubule-associated proteins that may be important for epidermal morphogenesis, a feeding RNAi approach was used to screen candidate genes for body elongation defects in wildtype worms. Knockdown of the gene encoding tubulin folding cofactor D, tbcd-1, resulted in phenotypes consistent with defects in the epidermis. Initial screening found that 67.5% of tbcd-1(RNAi) progeny failed to hatch and 7.9% of progeny had larval body morphology defects. Further investigation by 4D time-lapse DIC confocal microscopy found that tbcd-1(RNAi) embryos exhibited defects in both ventral enclosure and elongation, two key morphogenetic events in the epidermis. Ventral enclosure defects, where the epithelial cells fail to properly surround the embryo before body elongation, resulted in extruded cells and ruptured embryos. Body elongation defects occurred at the comma stage and resulted in dorsal folds reminiscent of perturbations in cadherin-catenin adhesion proteins. These results support the hypothesis that tbcd-1 has a critical role in organizing the microtubule cytoskeleton during epidermal morphogenesis. Current experiments with fluorescent spinning-disk confocal microscopy are analyzing the patterns of microtubules in the epidermis of tbcd-1(RNAi) embryos, as well as identifying any cell shape or cell orientation defects. The goal of future work will be to determine specific sequences in tbcd-1 that impact proper epidermal morphogenesis.

419A  The gene regulatory network underlying differentiation of invasive behavior. T.N. Medwig, W. Zhang, D.Q. Matus  Department of Biochemistry & Cell Biology, Stony Brook University, Stony Brook, NY.

Invasion of cells through basement membranes (BMs) is a highly conserved behavior that is relevant to gastrulation, trophoblast implantation, leukocyte trafficking, and cancer metastasis. C. elegans vulval development provides a tractable in vivo model to study invasive differentiation. During this process, the anchor cell (AC), a specialized uterine cell, breaches the BM to connect the uterus with the underlying vulval epithelium. Prior research has identified five pro-invasive transcription factors (TFs) in C. elegans. These include the basic helix-loop-helix protein and E/Daughterless homolog hlh-2, the AP-1 proto-oncogenic subunit and sole Fos family homolog fos-1, and the nuclear hormone receptor and NR2E1/Tailless homolog nhr-67. In addition, two zinc-finger TFs, the EVI1/MEL1 homolog egl-43 and Krüppel-like protein mep-1, are known to mediate AC invasion as well. Notably, the first four of the aforementioned TFs share homology with mammalian counterparts associated with invasive behavior and oncogenesis, and the latter three have been shown to regulate proliferation of the AC. In order to map out the gene regulatory network that programs invasive differentiation, a series of molecular epistatic experiments were performed using GFP reporters paired with RNAi-induced gene silencing. The data acquired provide the first characterization of the regulatory relationships between these pro-invasive TFs.

420B  A Rho GAP with a curved membrane-binding domain regulates morphogenesis via CDC-42. H. Raduwan, M. Soto  Dept. of Pathology and Laboratory Medicine, Rutgers - RWJMS.

Rho GTPases are important in regulating cytoskeletal dynamics. Members of this protein family had been implicated in cell migration, polarization, nutrient intake, and epithelial morphogenesis. In C. elegans, loss of different Rho GTPases causes the embryo to die during different stages of development. While the processes regulated by Rho GTPases are subject to intense
research, the regulators upstream of these Rho GTPases are still poorly characterized. For example, C. elegans has 23 Rho GAPs but the roles of most of these are still not understood. In this study, we found genetic evidence that the Rho GAP Y34BA.8, which on its own has a mild embryonic morphogenesis phenotype, regulates Cdc-42 Rho GTPase, but not CED-10/Rac1. Besides the Rho GAP motif, Y34BA.8 also has a BAR motif, which binds to curved membranes. A vertebrate homolog, SH3BP1, has been implicated in cell migration and adhesion. We used CRISPR to generate a full deletion of Y34BA.8, and found a similar phenotype as depletion via RNAi. We also used CRISPR to tag Y34BA.8 and found a complex localization pattern. While most cells of the embryo and adult express low levels of this GTPase, it is strongly enriched in a few tissues that may give us a clue as to how it regulates development. For example, some of the strongest signal is at the apical pharynx and intestine, during the beginning stage of gut polarization. Y34BA.8 appears to become enriched basal to ERM-1, but apical to DLG-1/AJM-1 complex (DAC) on the intestinal lumen, yet we have not detected an effect in intestinal apical polarization. Y34BA.8 has slight enrichment at the front of migrating epidermal cells, similar to CDC-42, ARP-2 and epidermal F-actin. This suggests Y34BA.8 may regulate cell migration. Further experiments are underway to identify the processes affected by CDC-42 in Y34BA.8-dependent manner.

421C Uncovering interacting factors of Leukocyte Cell-Derived Chemotaxin 2 (lect-2) in dendritogenesis. M. Rahman1, C. A. Diaz-Balzac2, H. E. Bülow1,2 1) Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY; 2) Department of Genetics, Albert Einstein College of Medicine, Bronx, NY.

Dendrite development is essential for the transmission and processing of sensory stimuli. The mechanisms of dendritogenesis are not fully understood, but abnormalities in dendrite morphology have been found in several neurological disorders. We use the multi-dendritic C. elegans PVD neurons, which have complex menorah-like dendritic arbors, as a model to study the genes involved in dendrite development. Studies from our lab and others have shown that a conserved cell-adhesion complex, comprised of MNR-1/Menorin and SAX-7/L1CAM, acts from the skin to regulate PVD dendrite branching through the transmembrane receptor, DMA-1/LRR-TM, deemed the ‘menorin’ complex. Recently, we determined that the conserved gene, Leukocyte Cell-Derived Chemotaxin 2, or lect-2/Chondromodulin II, also functions to pattern PVD dendrites. Though implicated in immune response and a range of pathologies, the developmental functions of lect-2/Chm-II have not fully been characterized. Acting from an entirely different tissue—the body-wall muscle—LECT-2/Chm-II is a diffusible factor that is a key player in the ‘menorin’ complex. Its localization is fully dependent on SAX-7/L1CAM (but not MNR-1/Menorin or DMA-1/LRR-TM) and double mutant analyses show that lect-2 acts in the same genetic pathway as mnr-1/Menorin, sax-7/L1CAM, and dma-1/LRR-TM. We propose that LECT-2/Chm-II functions as not only a co-factor to the ‘menorin’ complex, but also in concert with other genes from potentially different tissues. In order to elucidate other binding factors of LECT-2/Chm-II, we are performing two forward genetic screens. In the first, we aim to isolate modifiers of a hypomorphic allele. In the second, we aim to isolate genes required for correct localization of LECT-2/Chm-II with the help of a FACS based Worm Sorter. In preliminary experiments, we have isolated novel alleles of not only sax-7/L1CAM, but of potentially novel interactors, which we aim to characterize in future experiments. Overall, we aim to gain a deeper and fuller understanding of lect-2/Chm-II and the interplay of different tissues and factors involved in dendrite development—eventually providing insight into developmental mechanisms and potential diagnostic approaches for neurodevelopmental disorders.

422A Discovery of angiogenic regulators by studying excretory canal cell tubulogenesis genes. Daniel Shaye1, Alison Kitajewski1, Jing Du1,2, Jan Kitajewski1, Iva Greenwald2 1) Dept. of Physiology and Biophysics, University of Illinois-Chicago (UIC), Chicago, IL; 2) Dept. of Pathobiology & Molecular Medicine, Columbia University, New York, NY; 3) Dept. of Biological Sciences, Columbia University, New York, NY.

The C. elegans excretory canal (EC) cell, which is required for osmoregulation, is a simple model to study biological tube formation, or tubulogenesis1. The EC extends long projections first dorsally, then anteriorly and posteriorly, each with an intra-cellular lumen. Previous genetic analyses have revealed conserved cell biological processes required for EC tubulogenesis: canal outgrowth requires some genes that also mediate neuronal outgrowth; lumen formation is driven by regulation of cell polarity, polarized vesicular trafficking, and fluid and ion transport; and lumen maintenance depends on the apical actin cytoskeleton. Many of these cell biological processes are also involved in vertebrate angiogenesis2. Moreover, several genes required for EC tubulogenesis, such as exc-4/Clic, ccm-3/PDCD10, gck-3/STK39 and wnk-1/WNK function in angiogenesis and are linked to vascular disease3-8. Therefore, the EC is a powerful model to identify and characterize novel conserved regulators of angiogenesis.

To identify new conserved regulators of EC tubulogenesis we performed a feeding RNAi screen and tested the 249 kinases conserved with humans9. We identified nine conserved kinases that caused EC phenotypes. Among these nine where those previously implicated in EC tubulogenesis (gck-1, gck-3, mrck-1 and wnk-1)1, indicating a low false-negative rate for the screen. Moreover, RNAi phenotypes of the newly identified kinases were confirmed by genetic mutants, indicating a low false-positive rate. To ask whether these kinases play a role in angiogenesis, we are performing an in vitro angiogenic sprouting assay called FIBA10, which uses human umbilical cord venous endothelial cells (HUVEC) coated onto collagen-dextran beads, embedded in fibrin, and stimulated to develop angiogenic sprouts. Our RNAseq analysis shows that orthologs of all of the kinases identified in our screen are expressed in HUVEC. Currently we are validating lentiviral shRNA clones to knock-down kinase expression in HUVEC, and positive clones will be used to assess the role of kinases in angiogenic sprouting.

423B  *cdc-25.2*, a *Caenorhabditis elegans* ortholog of *cdc25*, is required for the spermatheca development and its expression is regulated by NHR-6. E. Youn, H. Min, I. Kawasaki, Y. Shim Dept Biost & Biotech, Konkuk Univ, Seoul, KR.

Cell division cycle 25 (Cdc25) phosphatase promotes cell division by removing inhibitory phosphates of a Cyclin/CDK complex. In *C. elegans*, there are four *cdc-25* family members, *cdc-25.1, cdc-25.2, cdc-25.3* and *cdc-25.4*. Among *cdc-25* family members, only *cdc-25.2* has a ~5 kb long first intron where a regulatory element for gene expression may be located. To investigate whether the first intron of *cdc-25.2* regulates transcription, a transgenic strain containing either a P*cdc-25.2::GFP* or a P*cdc-25.2::intron1::GFP* transgene was generated and expression patterns of transgenes were examined. Interestingly, expression of *cdc-25.2* in the spermatheca was dependent on the presence of intron1 of *cdc-25.2*, suggesting that intron1 is necessary for the *cdc-25.2* expression in the spermatheca, and *cdc-25.2* is required for spermatheca development. Indeed, defective spermatheca morphology with the decreased number of spermatheca nuclei was observed in *cdc-25.2* mutants. Transcriptional regulators binding to intron1 were searched using database, ModEncode and MEME. Among seventy-eight putative transcription factors, CEH-1, CEH-39, DAF-12 and NHR-6 which are expressed in the spermatheca, were selected for further analyses. A loss-of-function *nhr-6* mutant showed defects in the spermatheca development, and RNAi depletion of *nhr-6* significantly suppressed expression of *cdc-25.2* in the spermatheca. Taken together, NHR-6 appears to activate expression of *cdc-25.2* by binding to intron1, and *cdc-25.2* promotes cell divisions during spermatheca development. This study was supported by grants NRF-2015R1D1A1A01057488.

424C  Alternative splicing of the formin *fhod-1*, results in tissue specific activities. R.B. Smit 1, O. Refai 1, S. Votra 2, D. Pruyn 2, P.E. Mains 1  1) Biochemistry and Molecular Biology, University of Calgary, Calgary, AB, CA; 2) Cell and Developmental Biology, SUNY Upstate Medical University, Syracuse, NY.

Myosin phosphorylation in the non-muscle myosin driven contraction of epidermal cells that drives embryonic elongation of *C. elegans* is well characterized. Rho-binding kinase (LET-502), and myosin phosphatase, (MEL-11) have opposing effects on elongation by phosphorylating or de-phosphorylating, respectively, myosin (MLC-4). LET-502 is regulated by RhoGEF (RHGF-2) activation in the lateral epidermal cells (seam cells) and RhoGAP (RGA-2) inactivation in the dorsal/ventral epidermal cells. MEL-11 is regulated by LET-502, presumably by phosphorylation. However, less is known about the non-myosin regulation of elongation.

*fhod-1*, a member of the formin family of actin nucleators and bundlers, encodes a protein homologous to human FHOD1 and FHOD3. Our genetic analyses suggest that *fhod-1* is likely the only formin acting during embryonic elongation and acts in a pathway downstream of LET-502/MEL-11 to set up the actin cytoskeleton on which myosin acts. Immunostaining shows that FHOD-1 appears to be expressed uniformly in early epidermal cells but later becomes more pronounced in the lateral epidermal cells. We also identified a novel isoform of FHOD-1 in embryos that omits exon eight, which encodes a portion of a predicted coiled-coil domain found in the formin family. Using CRISPR-Cas9, we isolated a seven base pair deletion within exon eight (sb123) that results in the loss of only the *fhod-1(long)* isoform. Genetic characterization of *fhod-1(sb123)*, in addition to isoform-specific rescue, suggests that, only the short isoform of FHOD-1 is functional in embryonic elongation. *fhod-1* is also involved in muscle development and maintenance. In contrast to the embryonic epidemis, the short isoform does not seem to be sufficient for promoting muscle sarcomere formation. We are also using tissue subtype-specific rescue constructs to confirm these results. Mammalian FHOD3 has a muscle specific isoform that is regulated by phosphorylation (Iskratsch et al., J. Cell Biol., 2010).

Although the alternatively spliced exons of FHOD3 and FHOD-1 are in different protein domains, an interesting possibility is that formins across species utilize alternative splicing, and possibly phosphorylation, to direct specific functions in multiple tissues.

425A  Vulva-specific knockout of the *lin-3 egf* gene leads to defects in dorsal lumen morphogenesis during vulval development. S. Spiri, L. Mereu, M. Morf, A. Hajnal  Institute of molecular life science, University of Zürich, Zürich, CH.

*lin-3* encodes the only embryonic growth factor (EGF) family in *C. elegans*. LIN-3 binds to and activates its receptor LET-23 (EGFR), which controls various essential processes during larval development, hermaphrodite fertility and behavior. One very well characterized function is the role of LIN-3 secreted by the anchor cell (AC) during primary (1°) vulval cell fate specification. However, after the induction of the vulva precursor cells, *lin-3* is also expressed in the inner-most 1° VulF cells to determine the uv1 subfate of ventral uterus cells. Since LIN-3 has many essential functions during early larval development and it is required in the AC to induce the vulval cell fates, it is challenging to detect potential later functions of LIN-3 during vulval morphogenesis. Moreover, due to the fact that *lin-3* is a large locus with three different transcriptional start sites and many splice variants, visualization of the wild-type expression pattern is rather difficult.

Using CRISPR/CAS9, we inserted an mNeongreen marker into the *lin-3* locus to create an endogenous and fully functional LIN-3 reporter. This reporter has enabled us to analyze the endogenous expression pattern and subcellular localization of LIN-3. Prior tissue-specific RNAi data have indicated that LIN-3 secreted by the VulF cells is required for dorsal lumen formation during vulval morphogenesis. To verify these RNAi data we generated a tissue-specific knockout strain by inserting into the *lin-3* locus two FRT recombinase sites flanking part of the EGF domain. By expressing the *flp* recombinase under control of the heat-shock promoter we can now induce the loss of LIN-3 function at different developmental stages. In addition, we are expressing the *flp* recombinase under the control of different promoters to investigate the tissue-specific functions of LIN-3 and to verify its role during dorsal lumen morphogenesis. At the same time we are generating a tissue-specific knockout reporter strain for the EGF receptor gene *let-23*. The combination of this two conditional knockout strains will allow us to explore novel functions of the receptor-ligand pair LIN-3/LET-23 at different stages of *C. elegans* development.

426B  Muscle to epidermis mechanotransduction, pathways involved in *C. elegans* embryonic elongation. S. Tak, Christelle Gally 2, Loïc Bourdon 1, Sarah Hoff-Yoessle, Agnes Aubry, Michel Labouesse 1,2  1) Development Biology, Institut de biologie Paris-Seine, Paris, FR; 2) IGBMC, 1 rue Laurent Fries, Illkirch, FR.
Force driven biological processes have long puzzled biologists. Little is known about how forces impact development of an organism. *C. elegans* provides a good in vivo system to study such a process. *C. elegans* embryonic elongation is driven by two forces i.e. actomyosin contractility and the tension provided by muscle contraction. We previously reported that the latter recruits GIT-1 to hemidesmosomes (HD), which in turn facilitates further elongation by activating proteins such as pak-1 (Nature 471, 99-103, 2011). Whereas muscle-defective embryos are paralyzed and arrest at 2-fold (Pat phenotype), git-1 null mutants are viable, suggesting that another pathway acts in parallel to the git-1/pak-1.

To identify genes involved in parallel pathways, we performed an enhancer RNAi screen to look for Body Morphology Defects (BMD) and 2-fold arrest in *git-1(tm1962)* animals. We found almost 100 candidates belonging to different cellular complexes. Genes such as *dnc-1*, *arp-1*, *cap-1* and *farl-11* give 80% 2-fold arrest and encode Dynein/dynactin subunits or regulators. To define their role for Shugoshin in ciliary function of adult worms. Here we describe the initial characterization of SGO-1 localization in *C. elegans*.

Why do egl-50; git-1 embryos arrest at 2-fold? What structures are affected? To answer the question I created a library of markers in egl-50; git-1 background to look for structural and dynamic defects. We did not observe any major organization defects of actin cytoskeleton. Using the HD marker LET-805::GFP (CRISPR construct) in egl-50; git-1, I found multiple HD structural defects, such as thickened HDs, locally disrupted or bifurcated HDs, and more rarely detached HDs. To define the cause for this defect, I used FRAP analysis of LET-805::GFP, revealing three kinds of Mobile Fractions in egl-50; git-1 mutants, which could be similar, lower or higher than wild-type.

I hypothesize that a higher mobile fraction makes the HD brittle, while a lower mobile fraction leaves HDs susceptible to not withstand the increasing muscle tension. Moreover, our data suggest that the Dynein/dynactin complex is essential to transport HD material.

427C A genetic screen for pharyngeal gland cell migration defects. S. Tkachuk, E. Bennici Clendinnen, O. Atta, M. Burg, J. Komish Department of Biological Sciences, University of Manitoba, Winnipeg, Manitoba, CA.

The *Caenorhabditis elegans* pharynx, or foregut, is used as a model organ to study morphogenesis during development. Its pharyngeal gland cells support a secretory and lubricating function in the digestive tract. The g1P gland cell has a cellular extension that spans most of the length of the pharynx. Of particular interest is how the g1P cell migrates through the organ and forms this extension. During embryonic development, the cell uses retrograde extension: part of the cell remains anchored at its origin in the anterior aspect of the pharynx and leaves a cellular projection in its migratory wake. The molecular pathway controlling this migration is not well understood, and appears to be genetically distinct from dendrite and glial extension in amphid neurons, which require *dex-1* and *dyf-7* to anchor their extensions (1).

cam-1 is the exclusive *C. elegans* Receptor tyrosine kinase-like Orphan Receptor (ROR), functioning as a non-canonical Wnt receptor. Deficiencies in this gene result in an under-migrated g1P, where the cell body stops prematurely in an anterior position. While RORs can be exclusive to nervous system development (sea slug and *Drosophila*), *cam-1* functions in multiple organs and tissues during *C. elegans* embryonic development, similar to vertebrate RORs. Differing from vertebrates, however, is requirement of the kinase domain for full ROR developmental functioning in the worm (2, 3). Down stream effectors of the kinase specific function are not well understood.

To better understand the retrograde extension mechanism and identify components of the *cam-1* pathway, a forward genetic screen was conducted. A *phat-1::*YFP strain was mutagenized with EMS, and 4,986 individual strains were screened for gland cell displacement. 60 strains with aberrant g1P position were isolated; 24 of these displayed an under-migration similar to *cam-1* loss-of-function mutations. A subset of these 24 are homozygous viable and were selected for preliminary genetic mapping. Six alleles have been mapped to chromosome IV with two complementation groups. In collaboration with the *C. elegans* Gene Knockout Consortium, we are identifying the causative mutations through whole genome sequencing. One complementation group is likely *cwn-2*, and the other *ham-1*.


428A Initial characterization of the embryonic functions of Shugoshin in *C. elegans*. B. Waddell, C. Carvalho University of Saskatchewan, Saskatoon, Saskatchewan.

In contrast to other species whose chromosomes harbor discrete centromeres, chromosomes of *C. elegans* are holocentric. For many species, the function of the Shugoshin protein family is essential for proper cell division. Interestingly, the sole Shugoshin protein in *C. elegans* (SGO-1) appears to be dispensable for cell division. We have previously identified a novel role for Shugoshin in ciliary function of adult worms. Here we describe the initial characterization of SGO-1 localization in *C. elegans* embryos. GFP and HA-tagged SGO-1 transgenic lines expressed in several dividing cells of the embryo, in which signal was detected on metaphase chromosomes and centrosomes. Importantly, SGO-1 was also observed in sensory neurons that extend dendrites to connect anteriorly to the developing sensory organ. An affinity purified polyclonal antibody raised against the conserved C-terminus region of SGO-1 confirmed these results and further highlighted the developing sensory channels, marking the putative positions corresponding to the 16 sensilla endings of the adult worm. Signal was also detected posteriorly in...
the excretory duct and phasmid region. Furthermore, we have implicated the tectorin-like protein DYF-7 involved in dendrite extension of sensory neurons in the antibody signals of the sensilla. Transgenic lines expressing SGO-1 do not reproduce the antibody signal in the sensory depression, leading us to suggest that it may recognize a protein other than SGO-1. We are currently generating a null sgo-1 allele to test this hypothesis. Overall these results suggest SGO-1 expresses in the developing sensory neurons and possibly glial cells at a time when the architecture of the sensilla is being established.

429B  Coordination of cell shape changes of different cell types during C. elegans elongation.  T. Wiesenfahrt, James McGhee, Paul Mains  University of Calgary, Calgary, AB, CA.

We are interested in how cells coordinate shape changes during embryonic development. The main force during cell shape changes is provided by the actin-myosin network, a component of the cytoskeleton. As a model to analyse how the contractile activity of different cell types is coordinated, we use the elongation of the C. elegans embryo, the process in which the embryo changes from a ball of cells into a worm-like shape. During early elongation a subset of epidermal cells, the lateral cells, contract while the dorso-ventral cells take on a more passive role. We uncovered two parallel pathways that mediate embryonic elongation. Mutations of genes in these pathways can lead to either hypo-contraction (short worms) or hyperr-contraction (burst embryos). We suspect that the dominant pathway drives contraction of lateral cells, while the second pathway drives contraction in the more passive dorso-ventral cells. The relative contraction of each set of cells should be altered differently in mutations affecting one or the other pathway. I measure the width of the lateral cells in different mutant backgrounds to test this model. Loss of RGA-2, a Rho GAP that inhibits contraction of dorso-ventral cells, leads to hyper-contraction, whereas loss of RHGF-2 a Rho GEF that triggers lateral contraction, leads to hypo-contraction. In double mutants, which is predicted to show decreased dorso-ventral contraction, the dorso-ventral cells seem to lose their contractility leading to narrower lateral cells. We are currently using ajm-1::gfp strains to analyse elongation in real time and to see if the changes in lateral cell shape are retained post-embryonically in rga-2; rhgf-2 and pak-1 mutants. Cell shape changes often display a pulsatilbe behaviour and I tested if this is true for lateral cells during C. elegans elongation, but could find no evidence for pulsatile contractions.

430C  The fax-1 and nhr-85 nuclear receptors of C. elegans function in gonad development.  B. Wightman1, K. Lightfoot1, E. Bayer1,2  1) Biology Department, Muhlenberg College, Allentown, PA; 2) Columbia University, New York, NY.

The nuclear receptors constitute a class of conserved transcription factors that function in regulating animal development. The NR2E subclass nuclear receptor fax-1 of C. elegans functions in the specification of neurons, including neurotransmitter receptor expression and axon pathfinding functions. The Drosophila ortholog of fax-1, unfilled, functions in the differentiation of mushroom body neurons, including their normal arborization properties. In addition, unfilled has been shown to regulate period in pacemaker neurons in cooperation with another evolutionarily-conserved nuclear receptor E75, which is Rev-erb-a (NR1D1) in vertebrates. The C. elegans ortholog of E74 is nhr-85, which prompted us to investigate similar cooperative functions between fax-1 and nhr-85. We have found that both fax-1 and nhr-85 are expressed in the migrating distal-tip cells (DTCs) of the hermaphrodite gonad from L2 through L4 stages. Loss of both fax-1 and nhr-85 results in a low-penetrance DTC migration defect. fax-1; nhr-85 double mutants display DTC migration defects that are similar, consistent with a model in which they act together in a linear pathway. Loss of fax-1 results in a significant reduction in brood size. The reduction in offspring appears to result from a defect in sperm production rather than oogenesis. This might possibly result from compromised DTC migration or function. We are currently investigating whether nhr-85 mutations cause a similar effect.

The vab-3 transcription factor plays a key role in regulating the migrations of the DTCs. In vab-3 mutants, the DTCs continue to migrate into adulthood and also fail to turn off fax-1, suggesting that expression of fax-1 is a property of a migrating DTC. A mutation in fax-1 does not suppress the vab-3 migration defect, indicating that fax-1 is not an essential downstream mediator of vab-3 function. We propose that fax-1 and nhr-85 function in DTC migration is a robustness phenomenon. Unlike Drosophila, fax-1 and nhr-85 function appear to overlap in a very limited number of cells—perhaps just the DTCs. This possibility limits the importance of co-regulation by FAX-1 and NHR-85 in C. elegans, indicating that evolution within ecdysozoa has led to different function roles for these nuclear receptors. Supported by NIGMS.


Morphogenesis is the fundamental process in biology that describes the formation of a tissue or organism. It encompasses multiple layers of controlling upon molecular basis (Turing, 1952) and cellular basis. Taking advantage of the invariant cell lineage in C. elegans ( Sulston, 1983), we asked how cells move to correct destinations. Previous studies proposed cell movement is guided by local cell-cell interaction ( Schnabel, 2006), which were supported by evidences in early-stage embryos and limited types of mutants. Here, we hypothesized that, upon cell-cell interaction, differential affinities between neighboring cells drive their next moves. With our collection of all cell tracks up to 350-cell stage in hundreds of wild-type and perturbed embryos (Du, 2015), we devised a computational model to optimize cellular configuration over time and infer cell-cell affinities for individual embryos. The comparison between wild-type and perturbed embryos demonstrated those affinities were specified by cell fate rather than cell lineage. This study provided a basis to systematically elucidate how affinity network can serve as a new paradigm to positional information and orchestration in complex tissues.
Alzheimer’s Disease is a neurodegenerative disease that affects more than 5 million people per year in the United States alone. One of the hallmarks used to diagnose the disease is the accumulation of senile plaques in the brain, whose major component is a byproduct of amyloid precursor protein (APP) processing. Due to the complexity of the human system, which contains three redundant APP genes, we are studying the Caenorhabditis elegans APP homolog, apl-1. APL-1 contains the highly conserved E1, E2, and cytoplasmic domains, but, like other family members, does not contain the Ab peptide present in APP. Through a suppressor screen we have identified a new APL-1 interacting protein MOA-1 that encodes a receptor protein tyrosine phosphatase-like domain.

MOA-1 is expressed in multiple tissues in both sexes, including the pharynx, neurons, ventral nerve cord, intestines, and hypodermal cells, as well as the hermaphrodite vulva. In addition, moa-1 has distinctive loss of function (lf) and gain of function (gf) phenotypes. In hermaphrodites, loss of moa-1 results in the premature termination of gonad arm migration (50%). Vulval development is also affected, leading to an egg-laying defective (Egl) phenotype in 5% of the mutants. In males, the loss of moa-1 leads to an over-retracted tail phenotype in 5% of the mutants, suggesting that tail tip morphogenesis is impaired and that MOA-1 affects developmental timing through the heterochronic pathway. As there are currently no known extracellular interactors with human APP, this new interaction between apl-1 and moa-1 may prove crucial in understanding apl-1 and APP gene function.

Development - Sex Determination

An unanticipated role of NHR-23 in C. elegans spermatogenesis. Guinevere Ashley, Londen Johnson, James M. Ragel, Raquel Martinez, Joseph Aguileria, Brian Sangalang, Keissie Sontay, Jordan D. Ward University of California Santa Cruz, Santa Cruz, CA.

A crucial event in the life of sexually reproducing organisms is the specification of sexual identity. C. elegans is an excellent system to explore sex specification, as the species has two sexes, XX hermaphrodites and XO males. Hermaphrodites have a female soma and a hermaphroditic germline that transiently produces sperm in L4 larvae then undergo a switch to oogenesis in the late L4 stage; sperm are stored within the spermatheca and used for self-fertilization. Germline sex is established by a complex network of repressive transcriptional and post-transcriptional regulation; however, how this network transforms the X chromosome to autosome ratio into sperm and eggs is still not clear. Through our studies on the role of the nuclear hormone receptor, NHR-23, in molting we generated a GFP-fusion at the 3′ end of the endogenous nhr-23 gene using CRISPR-mediated genome engineering. This knock-in also included a degron sequence that allows inducible, tissue-specific degradation when the small molecule auxin was administered (Zhang et al., 2015). Surprisingly, we observed a novel expression of NHR-23::GFP-degron-3xFLAG in the germline nuclei of hermaphrodites throughout the L4 stage. Using a transgene that permitted auxin-mediated depletion of transgenes specifically in the germline, we demonstrated that NHR-23 depletion in this tissue results in unfertilized oocytes. This phenotype appeared to be due to a defect in sperm production, as DIC microscopy revealed an absence of sperm following NHR-23 depletion in the germline. Moreover, the introduction of wildtype sperm was sufficient to rescue infertility following NHR-23 depletion in hermaphrodites, confirming that infertility was due to defective spermatogenesis. Taken together, our data indicate that NHR-23 is necessary for fertility and sperm development and that NHR-23 is critical for specifying sperm in hermaphrodites or for the very early stages of spermatogenesis. Ongoing efforts involve using a combination of ChIP-seq and RNA-seq to determine the direct targets of NHR-23 in the germline which promote sperm formation.

A correlation of germ cells and male somatic sex development in C. elegans. D. LIU1,2, B. YU1, P. LIU1, X. LI1 1) School of Life Science, Peking University, Beijing, CN; 2) Department of Biology, Southern University of Science and Technology, Shenzhen, CN.

Sex development/differentiation is a combinatorial effect of both genetic and environmental determinants. In a developing gonad, sex determinant gene and sex hormone coordinate to determine the sex of both germ and somatic cells, while maintenance of germ cell sex is regulated by signals from somatic gonad cells. Increasingly accumulated evidence reveals a role of germ cells in somatic sex development that is not restricted to the gonad, yet the underlying mechanism remains to be explored. To investigate the relationship between germ cells and somatic sex development in a well-defined system, we use germ line deficient mutant worm (C. elegans) and find that male-specific organs/tissues such as mating rays, trunk/tail diagonal muscles and somatic gonad are malformed in the absence of germ cells, whereas mutant hermaphrodites do not show any morphological defects. In addition, vitellogenin encoding genes are abnormally expressed in male intestine cells, indicating a feminized intestine. Thus, the presence of germ cells is essential for male somatic sex differentiation and/or maintenance. By profiling gene expression of germ-free worms and confirmation, germ cells appear genetically upstream of a key somatic sex determination pathway component, HER-1. RNAi screening of 58 nuclear hormone receptor (NHR) genes, which are also identified via analyzing profiled gene expression of male germ-free mutant, indicates that nhr-23 is downstream of her-1, regulating development of mating ray. While nhr-14 is negatively regulated by germ cells and upstream of mab-3, responsible for the ectopic expression of vitellogenin genes in male intestine. Therefore, germ cells likely coordinate male somatic sex development/differentiation, in which the steroid hormones are largely involved, although C. elegans worm lacks an endocrine system.
Self-fertile hermaphroditism arose independently multiple times in Caenorhabditis through the evolution of post-transcriptional gene regulation in the germline. Previous studies suggest that RNA binding proteins (RBPs) orchestrate the evolution of germline development by gaining and/or losing target transcripts. STAR domain RBPs are found across eukaryotes, and Caenorhabditis has a large nematode-specific family of them that includes the germline-specific GLD-1. GLD-1 has conserved, pleiotropic roles in meiotic commitment and oogenesis [1, 2] but has acquired a role in hermaphroditic sex determination in two androdioecious species, C. elegans and C. briggsae [2-4]. Its main known function with respect to sex determination in C. elegans is to bind and repress tra-2 mRNA in hermaphrodites [5, 6] allowing the production of sperm in an otherwise female soma. In contrast, Cbr-GLD-1 promotes oogenesis and does not detectably bind tra-2 in vivo. Despite GLD-1’s opposing roles in the two species, Cbr-GLD-1 can rescue a Ce-gld-1 null mutant, suggesting that mRNA targets are the changing entity [2].

C. briggsae and C. elegans GLD-1 have both shared and species-specific GLD-1 targets [7], and some C. briggsae GLD-1 binding sites appear to lack GLD-1 binding motifs. To better understand the dynamic evolution of GLD-1 targets, we are developing PAR-CLIP (photoactivatable-ribonucleoside-enhanced crosslinking and immunoprecipitation) to obtain the exact site of GLD-1 binding on C. briggsae target mRNAs. PAR-CLIP causes a recognizable sequence change reducing the possible binding site to a 20-40 bp region. Having a clear picture of GLD-1 binding sites in two independently derived hermaphrodites is essential to our understanding of this evolutionary mechanism. In addition, to provide further evidence that GLD-1 has recently gained the tra-2 regulator in C. elegans, we are performing a simpler mRNA-immunoprecipitation-qRT-PCR assays for GLD-1-ترا-2 mRNA association in C. remanei, C. brenneri and C. japonica (outcrossing). We expect that GLD-1 will only associate with tra-2 in C. elegans.


Development - Signaling Pathways in Development


Notch receptors are conserved transmembrane proteins that regulate key developmental processes and promote stem cell proliferation and renewal. Notch signaling remains active in the nervous system from birth to adulthood. Notch pathway genes are highly conserved across many species, including humans, and extensive work to decipher Notch signaling was initially done in Caenorhabditis elegans. We show that animals lacking the Notch metalloprotease sup-17/ADAM10 have significantly higher rates of ALM-AVM nerve ring breakage than wild type animals. Notch pathway mutants were crossed with animals with an integrated transgene that expresses GFP in mechanosensory neurons (zdls5) to visualize their nerve rings. Significantly higher rates of breakage were found in all Notch mutant animals when compared to wild type, which suggests that the Notch pathway is involved in ALM-AVM nerve ring development. Genetically identical Notch mutants did not exhibit different break rates across life stages. Therefore, the defect is likely to be developmental rather than degeneration or a developmental delay. We next need to identify which cells Notch works through to promote ALM-AVM nerve ring development. To do so, we are currently performing tissue specific rescue experiments for both the mechanosensory neurons and the surrounding glia. We are also assessing the functional role of the nerve ring connection in mechanosensory neurons with optogenetics experiments in Notch and wild type worms.

437A  Creating a C. elegans model for Human Antigen R (HuR).  Zhe Yang, Liang Xu, Matthew Buechner  Dept. of Molecular Biosciences, University of Kansas, Lawrence, KS.

Human antigen R (HuR) is an RNA-binding protein that binds to A-U-rich elements (ARE) at the 3’ UTR of mRNAs in order to stabilize them. HuR binds to many tumor-related genes, and is up-regulated in many cancer cell types such a breast cancer and colon cancer. We are studying the C. elegans homologue of HuR in order to help understand the function of this class of protein. The nematode homologue is EXC-7, which maintains the normal lumen diameter and length of the single-cell tube of the C. elegans excretory canal cell; mutants exhibit a wide lumen with many small cysts, and the electron-dense terminal web is detached from apical membrane at the tips of the canals. EXC-7 binds to the mRNA of sma-1 (encoding bHspectrin) and presumably stabilizes this and other essential cytoskeletal mRNAs. Both EXC-7 and HuR contain 3 RNA-recognition motifs surrounding a central hinge domain. We are using the mutant phenotype of the excretory canal cell as a readout to see the effect of HuR in C. elegans. HuR and EXC-7 have similar structure and function, so we hypothesize that HuR binding domains can replace those of EXC-7 in C. elegans, so that the worm can be developed as a model for study of HuR function for use in potential drug screens.

We are also examining the role of another RNA-binding protein, MSI-1, which has a structure similar to that of EXC-7, and...
whose homologues have been implicated in interactions with HuR. Preliminary evidence suggests that EXC-7 and MSI-1 proteins may cause similar phenotypes in *C. elegans* as well.

**438B Synthetic genetic interaction of lin-28/LIN28 with DRM/hDREAM complex in vulva development.** I. Selman Bulut 1–2, Alexander Gosdoschan 1, Baris Tursun 1 1) BIMSb, Max Delbrück Center (MDC), Berlin, DE; 2) Biology Department, New York University (NYU), New York, USA.

Previously, LIN-53 (RBBP4/7) was identified as a molecular barrier for converting germ cells into neuron-like cells in *C. elegans*. Its depletion allows transdifferentiation of germ cells into somatic cells such as neurons upon ectopic expression of a neuron fate-inducing transcription factor (e.g. CHE-1 or UNC-30) [1]. [2]. LIN-53 is a ubiquitously expressed histone chaperone protein and has been shown executing different functions in different tissues. While characterizing its function in somatic tissues, we tested whether there is a possible genetic interaction between *lin-53* and *lin-28* during vulva development, as *lin-53* and *lin-28* are the upstream regulators of Ras signaling [3]–[5].

When we combined the loss-of-function mutant *lin-28(n719)* with *lin-53* RNAi we assumed a genetic interaction due to a synergistic enhancement of the vulva phenotype which causes multiple protrusions anterior and posterior to the vulva. Surprisingly, during genetic crosses with vulva fate marker expressing strains we discovered that the strain *lin-28(n719)* used in this study and in many other reports must harbor a secondary background mutation, which is likely to play an important role in the fate decision of the vulva cells and for the observed genetic interaction with *lin-53*. Ongoing whole genome sequencing has identified a number of relevant loci for the secondary mutation and the final result concerning the identity of this background mutation will be presented during the meeting.

5. S. M. Johnson u. a., „RAS Is Regulated by the let-7 MicroRNA Family“, *Cell*, Bd. 120, Nr. 5, S. 635–647, März 2005.

**439C Regulation of communication between the spermatheca and the sp-ut valve.** P.G. Castaneda, E.J. Cram Northeastern University 360 Huntington Ave, Boston, MA 02115.

The Caenorhabditis elegans (C. elegans) spermatheca, the site for embryo fertilization, is a tube composed of a single layer of cells that undergo cyclic stretching, constriction, and relaxation as ~150 oocytes pass through the gonad and into the spermatheca. This feature makes the *C. elegans* spermatheca an ideal model in which to explore mechanotransduction machinery in smooth muscle-like cells. The spermatheca is made up of three regions: the distal constriction, the bag, and the spermatheca-uterine (sp-ut) valve. Immediately after oocyte entry, the sp-ut valve constricts. After ~10 minutes, the bag begins to constrict, and the valve relaxes allowing the fertilized embryo to exit the spermatheca and enter the uterus. We have identified several components that regulate oocyte transit through the spermatheca, including the phospholipase plc-1, which is required for the spermatheca to constrict, and the valve relaxes allowing the fertilized egg through the sp-ut valve into the uterus. However, very little is known about how the sp-ut valve and the spermatheca bag coordinate the appropriate response for successful egg transits. To address this, we expressed GCaMP, a genetically-encoded calcium sensor, under a sp-ut valve specific promoter (tag-312), as well as a spermatheca specific promoter (fkh-6). By conducting a candidate RNAi screen, we have identified inx-12, plc-1, and fln-1 as required for proper coordination between the bag and the sp-ut valve. We are currently using quantitative calcium analysis and spermathecal bag and sp-ut valve specific RNAi strains to characterize these genes.

**440A Functional analysis of DAF-16 isoforms in the parasitic nematode Brugia malayi.** Kirsten Crossgrove, Amanda Danno, Johnna Dykstra, Danielle Zgoba Dept Biological Sci, Univ Wisconsin, Whitewater, Whitewater, WI.

The mosquito transmitted parasitic nematode *Brugia malayi* causes lymphatic filariasis in humans. We are interested in understanding the molecular signals involved in molting of infective stage (iL3) parasites following transmission to humans. The iL3 stage is thought to be similar to the arrested dauer stage of the free-living nematode *Caenorhabditis elegans* since both are arrested L3 stages that require an environmental signal to molt to the L4 stage. A key regulator of dauer formation and recovery is the FOXO transcription factor DAF-16, a target of the insulin/IGF-1 signaling (IIS) pathway. Phosphorylation of DAF-16 by AKT proteins in response to IIS results in export of DAF-16 to the cytoplasm and the loss of target gene activation. The *C. elegans* daf-16 gene encodes multiple isoforms which vary in their N-terminal sequences in a similar way to the *C. elegans* isoforms. We expressed the *Bma*-DAF-16a and *Bma*-DAF-16b DNA binding domains in *E. coli* as fusion proteins with a six histidine tag and showed that the purified proteins bind to a canonical DAF-16 DNA binding element (DBE), but not to a non-specific DNA sequence. Full length *Bma*-DAF-16a and *Bma*-DAF-16b proteins activate transcription of a DBE-regulated luciferase reporter gene in a serum-dependent manner in NIH 3T3 cells. Both *Bma*-DAF-16 proteins contain three predicted AKT phosphorylation sites. We predict that mutation of these phosphorylation sites will prevent export of *Bma*-DAF-16 to the cytoplasm and result in high levels of reporter gene activity regardless of whether IIS is active. We will report on the effects of single and combination phosphorylation site mutations. A better understanding of the function of *Bma*-DAF-16 will help determine whether the parasite IIS pathway or its targets are good candidates for drugs to treat or prevent parasite
441B Identifying TGF-β regulators using the C. elegans model system. L. Faure1, K.K. Beifuss2, M.F. Lakdawala1, T.L. Gumienny1 1) Biology, Texas Woman's University, Denton, TX; 2) Lynntech, College Station, TX.

Transforming Growth Factor-beta (TGF-β) is a family of secreted cell signaling ligands. DBL-1, TGF-β superfamily member in the roundworm C. elegans, is secreted from nervous tissue, but must be received by receptors in neighboring cells to regulate body size, secretion of specialized extracellular matrix (surface barrier), and other processes. The control of TGF-β within the secreting cells is not well known in any system. We are using the C. elegans model system to identify new regulators involved in this conserved signaling pathway. For that, we are using two approaches to identify TGF-β regulators. First, we used a C. elegans strain expressing GFP-tagged DBL-1 in an RNA interference (RNAi) screen to determine candidates involved in the regulation for this pathway. We knocked down candidate genes by RNAi and assayed changes in GFP-tagged DBL-1 fluorescence intensity within the ventral nerve cord. We then asked if the candidate gene products found with this screening co-localized with GFP-tagged DBL-1. Our results show in vivo the implication of a novel protein secretory pathway for DBL-1 transport. Second, we are performing co-immunoprecipitation using GFP-tagged DBL-1 as a bait to identify the different proteins that directly bind DBL-1. Together, this work is expected to identify proteins that interact with DBL-1 and regulate it within the secreting cell and in the extracellular milieu between DBL-1-secreting and receiving cells.

442C A ticking clock: understanding oscillatory gene expression during C. elegans development. O. Filina, J. van Zon AMOLF, the Netherlands.

Larval development of C. elegans occurs in four stages that are punctuated by molts. Each molt is accompanied by a peak in expression of a broad spectrum of genes, referred to as molting cycle genes. Expression of these genes peaks at different times but with the same period as the molts. Such periodic gene expression suggests the existence of a timing mechanism capable of precise temporal regulation. The gene lin-42 is thought to be implicated in such a mechanism as its deletion causes severe developmental defects with extended larval stage duration and asynchronous molts. The goal of the current research is to understand the mechanism of temporal control by lin-42. In particular we are interested in how lin-42 controls oscillatory expression of the molting cycle genes. To address this, we examine the dynamics of molting cycle genes in lin-42(ok2385) mutant animals. For this, we use a newly-developed fluorescence time-lapse microscopy technique, which allows us to follow gene expression at a single-animal level over the entire course of development. Initially we focused on mlt-10, a gene that encodes for one of the cuticle components, whose expression exhibits peak ~ one hour prior to ecdysis. We found that even though in lin-42(ok2385) animals time of the mlt-10 peaks was delayed, their duration was same as in wild-type. Moreover, we found that the L2 peak in mlt-10 expression was absent in 30% of animals that reached the L2/L3 molt. Interestingly, presence of peak was not correlated with initiation of subsequent ecdysis and occurred in all-or-none fashion: L2 peak was either fully absent or exhibited wild-type amplitude. We use mathematical modelling to show that these phenotypes are consistent with regulation by a mixed incoherent feed-forward loop. Currently we are extending this analysis to other molting cycle genes that peak at different phases as well as other lin-42 alleles.

443A LIN-10 can promote LET-23 EGFR signalling independently of LIN-2 and LIN-7. K. Gauthier1,2, C. Rocheleau1,2,3 1) Department of Anatomy and Cell Biology, McGill University, Montreal, Canada; 2) Metabolic Disorders and Complications Program, Research Institute of the McGill University Health Centre, Montreal, Canada; 3) Department of Medicine, McGill University, Montreal, Canada.

The spatial organization of signal transduction cascades is critical for regulating cell signalling and function. This is particularly evident in Caenorhabditis elegans vulva precursor cells (VPCs), where Epidermal Growth Factor Receptor (LET-23 EGFR) signalling from the basolateral membrane is required for vulva cell fate induction. Basolateral localization of LET-23 is dependent on the LIN-2 CASK/LIN-7 Veli/LIN-10 Mint1 complex. Disruption of this complex results in exclusive apical localization of LET-23 and a vulvaless (Vul) phenotype; however, it is unclear how this complex regulates LET-23 localization. We previously identified a pathway consisting of ARF-1.2 and ARF-3 GTPases, AGEF-1 (a putative Arf guanine exchange factor), and the AP-1 clathrin adaptor complex that antagonizes basolateral localization of LET-23 and negatively regulates signalling. Interestingly, mammalian Mint proteins (LIN-10) bind Golgi-associated Arf GTPases and may serve as an adaptor protein analogous to the AP-1 complex. I hypothesize that the LIN-2/7/10 complex competes with the AGEF-1/ARF/AP-1 ensemble at the trans-Golgi network or recycling endosomes for LET-23 binding to mediate basolateral transport of LET-23. I found that GFP::LIN-10 localizes to cytoplasmic foci in the VPCs that may represent Golgi mini-stacks or recycling endosomes independently of LIN-2, LIN-7, and ARF GTIPases. This localization is mediated by the C-terminal domains of LIN-10 that also mediate Arf binding in mammalian Mint proteins. Preliminary analysis suggests that LIN-10 partially colocalizes with ARF-1.2 in the VPCs. Unexpectedly, I found that overexpression of LIN-10 rescues the lin-2 and lin-7 Vul phenotypes, and partly restores basolateral LET-23 localization in a lin-2 mutant, indicating that LIN-10 promotes signalling independently of its complex. Overexpression of LIN-7, but not LIN-2, can also partly rescue mutations in the complex components. Going forward, I will test the hypothesis that LIN-10 promotes the LET-23 signalling pathway by interacting with ARFs. The results will offer new insight into how trafficking and scaffolding components work together to carefully regulate cellular events by controlling the spatial organization of signalling proteins in polarized cells.

444B The DAF-7/TGFb cascade affects prostaglandin metabolism, sperm guidance, and sperm gene expression in the adult hermaphrodite gonad. M. Hu, M Miller Cell, Development and Integrative Biology, University of Alabama at Birmingham, Birmingham, AL.
Successful fusion of the sperm and egg is fundamental to the development of sexually reproducing animals and is critical for the maintenance of genetic diversity. It is well established that oocytes of certain marine species secrete chemotactants to promote sperm guidance. Accumulating evidence suggest that activated sperm of internally fertilizing animals also respond to chemotactic cues while searching for the oocyte in the female reproductive tract. In Caenorhabditis elegans, we have identified a group of structurally similar F-series prostaglandins (PGFs) that help guide the sperm towards the spermatheca. These PGFs are synthesized via a novel mechanism and are found in mammalian ovaries, suggesting that PGF regulatory mechanisms may be conserved. Previous studies showed that the DAF-7/TGFβ pathway is essential for sperm guidance and PGF levels (McKnight et al., 2014). The purpose of this project is to uncover the mechanism by which DAF-7/TGFβ regulates PGF levels. Using liquid chromatography tandem mass spectrometry, I measured PGF levels in wild-type, daf-1(m40), and daf-1(m40); daf-3(mgDf90) mutant adults. The data indicate that the DAF-3 co-SMAD transcription factor negatively regulates PGF biosynthesis or positively regulates PGF breakdown. As the type I TGF-β receptor daf-1 is partially required in the germ line, I hypothesized that DAF-3 transcriptional activity is critical in the germ line to affect PGF levels. To test this hypothesis, I conducted RNA-seq sequencing on wild type, daf-1(m40), and daf-1(m40); daf-3(mgDf90) mutant adults. I identified over 1000 genes that are significantly altered in the experiments. I focused on a set of 179 genes that are expressed in the germ line (Reinke et al. 2004). RNAi screening of these 179 genes identified 32 genes that might act downstream of DAF-3. Of particular interest, 25 of 32 positive RNAi clones encode for genes that are highly enriched in developing spermatocytes (i.e. sperm genes). My data thus far support the model that DAF-3 promotes increased sperm gene expression in the adult hermaphrodite germ line, thereby down-regulating PGF levels. Current efforts are underway to understand how these sperm genes affect PGF metabolism and fertilization.

445C  The molecular analysis of pat-3 integrin splice mutant reveals its interaction to unfolded protein response.  Z. Qiu, E. Yu, M. Lee  Dept Biol, Baylor Univ, Waco, TX.

Integrins are heterodimeric cell surface receptor mediating cell-extracellular matrix (ECM) interaction. Integrin control many cell behaviors such as adhesion and differentiation by modulating cell-ECM interaction. Mammalian β1 integrin has been studied for its role in tumor suppression in malignant tissue. A splice variant of β1 integrin is known to upregulate a tumor suppressor gene (TSG) and play an important role in cell cycle progression and proliferation. Caenorhabditis elegans is a free-living soil nematode that has been studied as a genetic model organism for molecular analysis of cell cycle gene interactions. pat-3 (sp), a splicing defective mutant in intron 7 of pat-3 gene cytoplasmic domain, shows elevation and mis-localization of cki-1::GFP. Cki-1 is a Caenorhabditis elegans TSG and the homolog of p27kip1, a cell cycle inhibitor, and its removal causes tumor-like defects as cell cycle failed in some tissues. In the previous analysis, we found that the role of pat-3 (sp) in regulating Cki-1/p27kip1 nuclear localization. Further RNAi analysis of this pat-3 (sp) mutant revealed that the Cki-1/p27kip1 localization is mainly dependent on the function of nuclear genes involved in unfolded protein response (UPR), a system buffering cell stress upon malformed proteins, such as regulated IRE-1 dependent decay (RIDD) and protein degradation. To gain further insight of the pat-3 integrin, we have performed highthroughput mRNA sequence analyses and confirmed that expression of genes involved in ER stress and UPR were increased or decreased in pat-3 (sp). Taken together, we expect that the mutant form of β integrin cytoplasmic tail may cause ER stress and interfere with UPR in Caenorhabditis elegans.

446A  C. elegans SMA-10 regulates BMP Receptor Trafficking.  Kelvin Liao1, Ryan Gleason3, Mehu Vora1, Ying Li4, Nanci Kane1, Richard Padgett1,2 1) Waksman Institute, Piscataway, NJ; 2) Cancer Institute of New Jersey, Rutgers University, Piscataway NJ; 3) Johns Hopkins University, Baltimore, MD; 4) Life Science Institute, Chongqing Medical University, District Yuzhong, Chongqing China.

Signal transduction of the conserved BMP signaling pathway functions through two distinct serine/threonine transmembrane receptors, the type I and type II receptors. Endocytosis orchestrates the assembly of signaling complexes by coordinating the entry of receptors with their downstream signaling mediators. Recently, we showed that the C. elegans type I bone morphogenetic protein (BMP) receptor SMA-6, part of the transforming growth factor-β (TGFβ) superfamily, is recycled through the retromer complex unlike the type II receptor, DAF-4. From genetic screens in C. elegans aimed at identifying new modifiers of BMP signaling, we reported on SMA-10, a conserved LRG (leucine-rich and immunoglobulin-like domains) transmembrane protein. It is a positive regulator of BMP signaling that binds to the SMA-6 receptor. Here we show that the loss of sma-10 leads to aberrant endocytic trafficking of SMA-6, resulting in its accumulation in distinct intracellular endosomes including the early endosome, MVB, and the late endosome with a reduction in signaling strength. Our studies show that trafficking defects caused by the loss of sma-10 are not universal, but affect only a limited set of receptors. Likewise, in Drosophila, we find that the fly homolog of sma-10, lambik (lkb), reduces signaling strength of the BMP pathway, consistent with its function in C. elegans and suggesting evolutionary conservation of function. Loss of sma-10 results in reduced ubiquitination of the type I receptor SMA-6, suggesting a possible mechanism for its regulation of BMP signaling.

447B  HSP90 co-chaperones PHF-5 and HIP-1 promote GLP-1/Notch signaling during C. elegans embryogenesis.  J. Lissemore1, O. Zucaro1, E. Maine2 1) Biology, John Carroll University, University Heights, OH; 2) Syracuse University, Syracuse, NY.

The GLP-1/Notch transmembrane receptor is required for mitotic proliferation of germline stem cells (GSCs) and for early embryonic development in C. elegans. We identified daf-21(om40), formerly called ego-3(om40), in a screen for genetic enhancers of a glp-1 temperature sensitive allele, glp-1(bn18ts). daf-21 encodes the molecular chaperone HSP90, thus the discovery of this daf-21/glp-1 interaction reveals an important role for HSP90 in GLP-1/Notch signaling and germline development in C. elegans.
development in *C. elegans*. Proper HSP90 function requires the activity of numerous co-chaperones; at least two dozen co-chaperones are known from various organisms and most have orthologs in *C. elegans*. An obvious question, then, is which, if any, of these co-chaperones play a role in GLP-1/Notch signaling in *C. elegans*. Therefore, we are using the glp-1 (bn18ts) sensitized background as a tool to identify additional components of the HSP90 molecular chaperone system required for proper GLP-1/Notch function in germline and embryonic development. We are using RNAi by feeding to systematically knock down the expression of all known *C. elegans* co-chaperone orthologs in wildtype N2 and glp-1 (bn18ts) worms. In the initial stage of this project, we are examining the effects on brood size and embryonic/early larval viability. To date, we have done RNAi knockdown of four co-chaperone genes for which there is evidence of germline expression: pph-5 (encodes PP5), hip-1 (encodes Hip), C01G10.8 (encodes Aha1), and C56C10.10 (encodes Aip). Preliminary analysis of these data shows that pph-5 and hip-1 knockdowns significantly reduced embryonic and early larval viability in glp-1 (bn18ts) compared to the same knockdowns in N2. We did not detect a significant reduction in brood size for knock down of any the tested genes. We hypothesize that the HSP90 co-chaperones PPH-5 and HIP-1 promote GLP-1/Notch signaling during embryonic and early larval development.


Environmental conditions experienced during animal development have sustained impact on maturation and adult lifespan. We recently showed that in *C. elegans* developmental rate and adult lifespan depend on larval population density and that this effect is mediated by excreted small molecules. We established the time point of first egg laying as ultimate and easy accessible marker of developmental pace and for full maturity (= egg laying assay). We found that wildtype hermaphrodites raised under high density conditions developed significantly faster than animals raised in isolation. Furthermore, larval population density but not population density during adulthood, strongly affected *C. elegans* lifespan. Population-density dependent developmental acceleration (Pdda) was dramatically enhanced in fatty acid ß-oxidation mutants that are defective in the production of dauer-inducing ascarosides. In contrast, Pdda is abolished by synthetic ascarosides or steroid hormones that act on the nuclear hormone receptor DAF-12. However neither ascarosides nor any of the known steroid hormones are required for Pdda, instead it is mediated by another larval population density-dependent signal (Ludewig 2017, in press) that antagonizes the effects of the dauer pheromone.

We further show that the effects of high larval population density persist through adulthood, as *C. elegans* larvae raised at high densities exhibit significantly reduced adult lifespan and respond differently to exogenous chemical signals compared to larvae raised at low densities, independent of density during adulthood. Our results demonstrate how inter-organismal signaling during development regulates reproductive maturation and longevity.

449A  Characterization of IncRNA C30E1.9 in *C. elegans*. A. Mariani, C. Link University of Colorado at Boulder, Boulder, CO.

Nuclear Enriched Abundant Transcript 1 (NEAT1) is a long non-coding RNA necessary for the formation of nuclear bodies called paraspeckles (composed of about 40 proteins) in mammals. NEAT1 is expressed as two isoforms (3.7kb and 23kb). This transcript has been implicated in different neurodegenerative diseases (including ALS/FTD and MS), brain viral infection (Japanese encephalitis virus and rabies virus), in addition to playing an oncogenic role in most solid tumors. In regards to ALS, it has been shown that NEAT1 is directly bound by TDP-43 and that its expression is highly up-regulated in human motor neurons presenting ALS pathology. In our transcriptome analysis of TDP-1 knock-out and FUS knock-out *C. elegans* we noticed a significant overexpression of a transcript (C30E1.9), which presents itself in two isoforms of length ~4kb and 23kb. We hypothesized that this transcript was the NEAT1 ortholog in nematodes. We performed smRNA FISH experiments and observed the formation of nuclear bodies in both N2 and TDP-1 knock-out worms: no statistical significance was noticed in the number of nuclear bodies between the two strains. As is the case for NEAT1 in mammals, we did not observe this transcript in the germine. We performed smRNA FISH in strains expressing human TDP-43::GFP and TDP-1::GFP in order to determine whether the lncRNA C30E1.9 co-localizes with TDP-43 and TDP-1: no strong co-localization was found. Feeding RNAi against the C30E1.9 transcript has so far been inconclusive, as no phenotypes have been observed, but clear reduction in the C30E1.9 nuclear bodies has not been observed. Ultimately, given that NONO protein knockdown reduces embryonic and early larval viability in *C. elegans*.

450B  Multidrug resistance protein 5 (MRP-5) transports vitamin B12 from the intestine to the gonad. Huimin Na1, Peng Zhang2, Gabrielle Giese1, T. Keith Blackwell2, Albertha J.M. Walhout1 1) Program in Systems Biology and Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA 01605, USA; 2) Research Division, Joslin Diabetes Center, Boston, United States. Department of Genetics, Harvard Medical School, Boston, MA, USA.

Parental micronutrient status has profound effects on developing offspring. However, how micronutrients are transported to developing embryos is unclear. We recently identified a new transporter that transports vitamin B12 from the intestine to the gonad in *Caenorhabditis elegans*. Using a transgenic reporter strain, we screened an RNAi transporter sub-library and identified *mrp-5* as a potential vitamin B12 transporter. Knockdown of *mrp-5* leads to embryonic lethality and vitamin B12 deficiency in *F*1 embryos. Direct injection of vitamin B12 into the gonad rescues embryonic lethality of both the *mrp-5* knockdown and mutant animals. Additionally, *mrp-5* knockdown reduces embryonic S-adenosyl methionine (SAM) content, which is vital for embryonic
development. This study identifies a critical link as to how vitamin B12 is transported from the parent to the developing offspring.

451C A transcriptomic approach to understand complex (Wnt) signaling dynamics in Q neuroblast lineage progression and migration. E.E. Fernandes Póvoa, L. Rella, A.L.P. Ebbing, M.C. Betist, H.C. Korswagen Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences (KNAW) and University Medical Centre Utrecht, Utrecht, The Netherlands.

Wnt signaling plays a major role in regulating cell migration, not only during metazoan development, but also in the context of human disease. Frequently, these regulatory mechanisms comprise intricate interactions and cross-talk between different signaling pathways. For instance, a shift from canonical to non-canonical Wnt signaling has been reported to underlie a "phenotypical switch" that induces melanoma cells to adopt a migratory and invasive phenotype.

In the nematode *C. elegans*, the migration of the QR neuroblast and its descendants can be divided into four sequential steps, three of which are mediated by distinct Wnt signaling mechanisms. However, little is known on how these mechanisms are regulated and how they interact to achieve the correct temporal regulation of QR lineage progression and migration. We are using this single cell migration model system to deepen our knowledge on how such types of complex interactions can be regulated.

We have combined FACS-based Q neuroblast sorting and RNA-sequencing (CEL-seq) to better understand the temporal transcriptional dynamics occurring during QR lineage progression and migration. Here, we show that our approach is capable of capturing expression dynamics of multiple Wnt-related genes previously described in literature.

Based on the data generated, we are currently testing and characterizing a list of prominent candidate genes for their roles in these Wnt signaling switches, and their underlying relevance for QR lineage progression and migration.

452A The molecular analysis of pat-3 integrin splice mutant reveals its interaction to unfolded protein response. Z. Qiu, E. Yu, M. Lee Biology, Baylor University, Waco, TX.

Integrins are heterodimeric cell surface receptor mediating cell-extracellular matrix (ECM) interaction. Integrin control many cell behaviors such as adhesion and differentiation by modulating cell-ECM interaction. Mammalian β1 integrin has been studied for its role in tumor suppression in malignant tissue. A splice variant of β1 integrin is known to upregulate a tumor suppressor gene (TSG) and play an important role in cell cycle progression and proliferation. Caenorhabditis elegans is a free-living soil nematode that has been studied as a genetic model organism for molecular analysis of cell cycle gene interactions. pat-3 (sp), a splicing defective mutant in intron 7 of pat-3 gene cytoplasmic domain, shows elevation and mis-localization of cki-1::GFP. CKI-1 is a C. elegans TSG and the homolog of p27kip1, a cell cycle inhibitor, and its removal causes tumor-like defects as cell cycle failed in some tissues. In the previous analysis, we found that the role of pat-3 (sp) in regulating CKI-1/p27kip1 nuclear localization. Further RNAi analysis of this pat-3 (sp) mutant revealed that the CKI-1/p27kip1 localization is mainly dependent on the function of nuclear genes involved in unfolded protein response (UPR), a system buffering cell stresse upon malformed proteins, such as regulated IRE-1 dependent decay (RID) and protein degradation. To gain further insight of the pat-3 integrin, we have performed highthroughput mRNA sequence analyses and confirmed that expression of genes involved in ER stress and UPR were increased or decreased in pat-3 (sp). Taken together, we expect that the mutant form of β integrin cytoplasmic tail may cause ER stress and interfere with UPR in C. elegans.

453B The Rheb-TORC1 signaling axis is necessary for developmental progression. Tam Duong, F. Sefakor Mote, David Reiner Institute of Biosciences and Technology, College of Medicine, Texas A&M University, Houston, TX.

Activation of Target of Rapamycin Complex 1 S/T kinase (TORC1) by the Rheb small GTPase is a major signaling axis that is conserved from yeasts to humans. TORC1 integrates inputs from both local and systemic nutrients to promote anabolism and growth. Activated TOR complex 1 (TORC1; defined by the presence of DAF-15/Raptor) promotes biosynthesis of proteins and other macromolecules while inhibiting autophagy/catabolism. TORC1 signaling has been mainly studied using in vitro cell culture, and Rheb itself has received relatively little attention. Here, we use *C. elegans* as a model to dissect the Rheb-TORC1 signaling axis in vivo. A few *C. elegans* genetic reagents for the Rheb-TORC1 axis have been defined: null or strong loss-of-function mutations in *let-363/TOR* and *daf-15/Raptor* cause developmental arrest at the 3rd larval stage. We found that deletion alleles of *rheb-1* conferred arrest at a size much smaller than the L3 arrest described for disruption of Raptor or TOR. We interpret the Rheb mutant arrest to be at the L2 stage, and we confirmed this result by assaying the HLH-8::GFP M-lineage reporter in the *rheb-1* mutant. We hypothesize that DAF-15/Raptor and LET-363/TOR mutations are maternally rescued, and preliminary depletion of maternal DAF-15/Raptor led to a range of arrest phenotypes, including putative L2. Arrested *rheb-1* mutants move and pump, and they survive for a week after arrest. Mutation of DAF-16/FOXO significantly decreases *rheb-1* mutant arrest lifespan, while mutation of InsR/DAF-2 extends the *rheb-1* mutant lifespan almost three fold. Neither *daf-16* nor *daf-2* mutations alter stage of *rheb-1* mutant arrest. These results suggest that Insulin/IGF signalling (IIS) functions in parallel to TORC1. We hypothesize that the *rheb-1* null phenotype is a diapause/arrest rather than lethality, suggesting a TORC1 requirement in developmental progression. To avoid perturbing membrane-targeting sequences at the Rheb C-terminus, we used CRISPR to N-terminally tag endogenous Rheb, and observed expression in all tissues, with strong localization to lysosomes and modest localization to plasma membranes. RHEB-1 co-localizes with lysosome markers such as LMP-1::GFP and GFP::RAB-7, but not with GFP::RAB-5 (a marker of early endosomes). We also used CRISPR to C-terminally tag the endogenous DAF-15/Raptor. We observed Raptor in all tissues, localized to the cytosol and lysosomes. We will use co-localization of Rheb and Raptor to investigate Rheb-dependent recruitment of Raptor to the lysosome, comparing between well-fed and dietary restricted animals. Further, we also developed a putative constitutively activated TORC1 by knock-in of membrane targeting sequence of RHEB-1 into the DAF-15/Raptor C-terminus, and we hypothesize that this protein fusion might be able to rescue the arrest phenotype of the *rheb-1* mutant.

Asymmetric cell division is essential to generate cellular diversity in multicellular organisms. In C. elegans, many asymmetric cell divisions are regulated by Wnt signaling and we have focused our analysis on DSH-2, a key intracellular phosphoprotein downstream of the Frizzled receptor. Loss of both maternal and zygotic dsh-2 function results in asymmetric neuroblast division defects and embryonic/larval lethality, while loss of zygotic dsh-2 function affects asymmetric division of the somatic gonadal precursor cells, Z1 and Z4. We had evidence suggesting that lethality maybe a secondary consequence of earlier asymmetric division defects. Therefore, to identify genes that function with dsh-2 in asymmetric division, we undertook a genetic screen to isolate suppressors of dsh-2 lethality. Over 60 suppressors were isolated, the majority of which also suppressed asymmetric neuroblast division defects. We have focused our analysis on Sup245, a strong dominant suppressor of both asymmetric neuroblast and Z1/Z4 division defects. Z1 and Z4 divide asymmetrically to produce distinct proximal and distal daughter cells that adopt different cell fates. The proximal (unsignalated) daughter has a high POP-1/TCF: low SYS-1/Lrb-catenin ratio while the distal (Wnt signaled) daughter has a low POP-1:high SYS-1 ratio resulting in the specification of distal cell fate. dsh-2 mutants disrupt both POP-1 and SYS-1 asymmetry and as a result the distal daughter is transformed into another proximal daughter, a hallmark of reduced Wnt signaling. Using both SYS-1 and POP-1 GFP reporters, we determined that Sup245 partially reestablished both POP-1 and SYS-1 asymmetry which was sufficient to restore distal cell fate. In order to determine the molecular identity of Sup245, we performed a series of genetic mapping experiments in combination with whole genome sequencing. Through this analysis, we have mapped Sup245 to a small genetic interval on the right arm of chromosome I. There are 3 candidate mutations in this region for Sup245; 2 intronic mutations and 1 exonic mutation in the gene W04A8.6. W04A8.6 is a novel gene and there is no sequence information that lends insight into its function while knockdown of Sup245 results in embryonic lethality. To unambiguously prove that the mutation in W04A8.6 corresponds to Sup245, we will reintroduce the Sup245 specific mutation into the wild-type W04A8.6 gene using CRISPR/Cas9 gene editing technology and assay for suppression of dsh-2. We are also undertaking experiments to further analyze W04A8.6 localization and interactions with POP-1 to determine its role in Wnt pathway function.

455A Analysis of the roles of EXC-4 and human CLICs during tubulogenesis in C. elegans and endothelial cells. Anthony Arena1, De Yu Mao1,2, Jan Kitajewski1, Daniel Shaye1 1) Dept. of Physiology and Biophysics, University of Illinois-Chicago (UIC), Chicago, IL; 2) Dept. of Pharmacology, Columbia University, New York, NY.

Biological tube formation (tubulogenesis) is a key process during vascular development and angiogenesis, and its disruption leads to disease. The chloride intracellular channel (CLIC) family was first implicated in tubulogenesis by the discovery that EXC-4, a worm CLIC, is required for development of the C. elegans excretory canal (EC) cell1; a single-celled tube that is a proven model for studying conserved regulators of tubulogenesis2. We, and others, have shown that two human CLICs, CLIC1 and CLIC4, are expressed in vascular endothelial cells and are required for lumen formation during angiogenesis3-5. Moreover, CLIC1, when localized to the apical plasma membrane, rescues the exc-4 null (0) phenotype, suggesting conservation of function between EXC-4 and CLIC16.

In the EC EXC-4 is constitutively localized to the apical plasma membrane1. This localization is critical for EXC-4 function1,6, and the ability of CLIC1 to rescue exc-4(0) was dependent on apical membrane targeting5. Therefore, membrane localization is an important determinant of EXC-4/CLIC function. In contrast to EXC-4, human CLICs accumulate in the cytoplasm and are only transiently recruited to the plasma membrane upon activation of G-protein-coupled receptors (GPCRs). Additionally, our results suggest that CLIC1 and CLIC4 influence different pathways downstream of GPCRs in endothelial cells, suggesting distinct functions for these CLICs. We are investigating whether CLIC4, like CLIC1, can rescue exc-4(0) when targeted to the apical EC membrane. Results from these experiments will address whether both CLICs have similar, or distinct, functions in tubulogenesis. Dependent on these results, we will undertake structure-function analyses to further define the functions of CLIC1 and CLIC4 in the EC and in human endothelial cells. We are also addressing whether EXC-4 can replace CLIC1 and/or CLIC4 in human endothelial cells, and whether exc-4 and G-protein signaling also interact in CeEC tubulogenesis.


The study of the EGL-15 fibroblast growth factor receptor (FGFR) in C. elegans has long served as a paradigm for understanding principles of receptor tyrosine kinase (RTK) signaling. Defects in the processes mediated by EGL-15 result in striking phenotypes that provide powerful genetic tools that have been used to discover many components that mediate RTK signaling. One example is the regulation of fluid homeostasis - hyperactivation of EGL-15 results in a Cir (Clear) phenotype characterized by excessive accumulation of clear fluid within the worm’s body. Hyperactivation of EGL-15 signaling is typically accomplished by a mutation in the gene clr-1, which encodes a receptor tyrosine phosphatase that negatively regulates the EGL-15 signaling pathway. The isolation of suppressors of the Cir phenotype, termed Suppressor of Cir (soc) mutants, has led to the identification of many of the core components of EGL-15 signaling. For example, the Grb2/SEM-5 adapter protein that links RTK activation to the activation of the RAS GTP-binding protein was identified in the original set of soc mutations.

Although SEM-5 is required for the regulation of fluid homeostasis via EGL-15, a key signaling component that links activated
EGL-15 to SEM-5 has yet to be identified. While activated EGL-15 can recruit SEM-5 via phosphorylated tyrosines in its carboxy-terminal domain (CTD), this mechanism cannot explain the SEM-5 requirement for fluid homeostasis, since an egl-15 mutation, n1457, that truncates the CTD and eliminates these binding sites does not confer a Soc phenotype.

To identify these missing components, we conducted a modified, “enhancer” soc screen in an egl-15(n1457 DCTD) background and identified 33 new soc mutations. Five of these are alleles of known, major components of the EGL-15 signaling pathway: four alleles of egl-15 itself, and one allele of soc-1. Preliminary characterization of eleven additional new soc enhancer mutations revealed that two are autosomal; the other nine are X-linked and define at least two new soc genes. We have identified multiple alleles of one of these genes; each of these alleles has a weak Soc phenotype on its own, but a strong Soc phenotype in the enhancer background. The enhancing effect suggests that this new gene likely cooperates with other components of the EGL-15 signaling pathway. Whole-genome sequencing will be used to identify the molecular identity of these new signaling genes. The continued characterization of these soc mutations will help further our understanding of the molecular mechanisms by which RTKs promote specific biological responses to intercellular signals.

457C  Studies of the cellular focus of IIS pathway genes in maintenance of somatic gonad and germline stem cell quiescence in dauer larvae.  C. Tenen, I. Greenwald  Columbia University, New York, NY.

During dauer diapause, precursor cells in developing tissues must remain quiescent and maintain their developmental potential over long periods of time. The canonical insulin/insulin-like signaling (IIS) pathway is a key regulator of dauer diapause; DAF-18/PTEN is an inhibitor of IIS, and DAF-16/FoxO is the main target. Dauer larvae lacking these components can be generated in daf-7 mutants, which constitutively form dauer due to abrogation of a parallel dauer entry pathway (Vowels and Thomas 1992; Larsen et al. 1995). We will report our progress towards understanding the roles of these IIS components in maintaining quiescence and developmental potential in the somatic gonad and germline in dauer.

First, in continuous development, LIN-12/Notch signaling is active in the Ventrual Uterine precursor cells (VUs) from their birth until they divide in the mid-L3 stage; however, in dauer larvae, VUs do not divide and the LIN-12 transcriptional target mir-61 is not expressed. These results suggest that LIN-12 signaling is blocked in the quiescent VUs, as it is in quiescent Vulval Precursor Cells (Karp and Greenwald 2013). Furthermore, mir-61 is expressed in VUs in dauers lacking either DAF-18 or DAF-16 activity, suggesting that a canonical IIS pathway, in which DAF-18 activates DAF-16 through inhibition of IIS, is responsible for the block to LIN-12/Notch signaling in dauer. Second, we have observed that IIS components appear to have different roles in the maintenance of VU quiescence. In daf-18 null mutant dauers, the VUs divide, indicating loss of quiescence, and their descendants express markers for the pi cell type, another indication that the block to LIN-12/Notch signaling is relieved (Newman et al. 1995). In contrast, the VUs rarely divide in daf-16 null dauers, suggesting that DAF-18 does not regulate somatic gonad precursor cellular quiescence in dauer exclusively through IIS inhibition, or, alternatively, that IIS targets other than DAF-16 are required for regulating somatic gonad quiescence in dauer. Finally, we have been investigating the cellular focus for the requirement for daf-18 in maintaining quiescence of somatic gonad precursors and the germline stem cells (GSCs) in dauer (Narbonne and Roy 2006, and our unpublished observations). Our mosaic analysis, tissue-specific rescue, and tissue-specific knockout experiments together suggest that DAF-18 non-autonomously coordinates both VU progression and germline quiescence in dauer from another cellular source.


Asymmetric cell division, the unequal distribution of cell fate determinants between daughter cells, is a critical system underlying the development and maintenance of varied tissue types in a multicellular organism. Despite widespread utilization of such divisions, the mechanisms responsible for induction of asymmetry are less understood due to the complex, pleiotropic, and often functionally redundant signaling systems involved. Throughout C. elegans development, the Wnt/beta-catenin asymmetry pathway induces the asymmetric distribution of Wnt signaling components to polarize a mother cell and therefore differentially regulate Wnt target genes in its daughter cells. Despite this asymmetry in its regulation immediately post-division, cytoplasmic SYS-1 localizes symmetrically to mitotic centrosomes in a manner consistent with well conserved beta-catenin localization. The subfunctionalization of C. elegans’ 4 beta-catenin homologs allows us to investigate the role of this phenomenon with particular focus on the transcriptional regulation of the Wnt/β-catenin asymmetry pathway. Recent data indicate that centrosomal localization of SYS-1 serves as a clearance mechanism to increase the robustness of Wnt-mediated polarity. However, the method and regulation of SYS-1 trafficking to and accumulation at the centrosome is unknown. In the early embryo, this centrosomal localization of the SYS-1 is dependent on the centrosomal scaffolding protein RSA-2. Loss of RSA-2 by RNAi prevents SYS-1 centrosomal loading, forcing it to accumulate in the approximate region of kinetochore microtubules. This localization suggests that SYS-1 may be directly transported to centrosomes along microtubules via the action of molecular motors. Given the minus-end directed movement implied by the localization at a microtubule organizing center, we focused on dynein and dynactin components as the primary minus-end directed motor. Preliminary data from microtubule-destabilizing and ATP-depleting chemical treatment indicate that both ATP and microtubules are important for SYS-1 centrosomal localization. A temperature sensitive allele of dynein heavy chain show a disproportionate effect on SYS-1 localization. Additionally, an RNAi screen aimed at depleting these microtubules and microtubule transport proteins has identified several dynein light chain subunits that promote proper centrosomal
localization of embryonic SYS-1/beta-catenin. Together, these results suggest an active role for the multimeric dynein/dynactin cargo binding complex in SYS-1/beta-catenin regulation and signaling status.

459B LIN-14 transcriptionally regulates lin-4 and let-7 family miRNAs, which in turn negatively regulate LIN-28. J. Tsialikas1, M. Romens2, A. Abbott3, E. Moss1 1) Cell Pathology, The Children’s Hospital of Philadelphia, Philadelphia, PA; 2) Molecular Biology, Rowan University, Stratford, NJ; 3) Biological Sciences, Marquette University, Milwaukee, WI.

The heterochronic pathway is a network of genes that regulates the proper succession of cell fates during larval development in C. elegans. These genes encode miRNAs and proteins whose activation and repression specify stage-appropriate behavior in progressively differentiating cell lineages. LIN-28 is an RNA-binding protein which acts early in this pathway to promote execution of L2 cell fates. Although its 3'UTR contains predicted lin-4 and let-7 family binding sites, it was previously only shown to be a target of lin-4. In this work we used multiply mutant strains to reveal that lin-28 is genetically downstream of the let-7 family miRNAs, mir-48, mir-84 and mir-241, as well as the lin-4 family miRNA, mir-237. Furthermore, we found that these miRNAs are inhibited by the transcription factor LIN-14, another member of the heterochronous pathway. qRT-PCR and reporter data further suggest that LIN-14 targets these miRNAs by directly affecting their transcription. This work addresses a number of long-standing issues in the field, including the mechanism by which LIN-14 positively regulates LIN-28, as well as identifying LIN-14’s relevant targets in the heterochronous pathway. What’s more, this work offers an explanation for the profound effect lin-4 mutations have on development, compared to the relatively weak effect of most miRNAs. When lin-4 is null, LIN-14 is constitutively expressed late in development. As we now know, this dysregulated LIN-14 is also repressing the transcription of several other miRNAs in the pathway. Therefore, removing lin-4 effectively removes at least 4 other heterochronous miRNAs.

460C Dissecting the roles of the ADAM10 metalloprotease SUP-17 in the BMP signaling pathway in Caenorhabditis elegans. Lin Wang, Zhiyu Liu, Herong Shi, Jun Liu Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

BMPs (Bone Morphogenetic Proteins) belong to the TGFbta superfamily of ligands, and mediate a highly conserved signal transduction cascade. Upon ligand binding, type II receptors phosphorylate type I receptors, which in turns phosphorylate R-Smads (receptor regulated Smads). Phosphorylated R-Smads then complex with co-Smad (common mediator smad) and enter the nucleus to regulate gene transcription with different co-factors. BMPs play important roles in developmental and physiological processes. Malfunction of the pathway in humans can cause various disorders, such as skeleton diseases, heart diseases and cancers. So it is critical to strictly regulate the level of BMP signaling spatiotemporally. Using a highly specific genetic screen, we have identified several modulators of the BMP pathway in C. elegans. These include the Neogenin homolog UNC-40 [1], two paralogous tetratraspan proteins, TSP-12 and TSP-14, and an ADAM10 metalloproteinase (A Disintegrin and Metalloproteinase 10) SUP-17 [2]. We use the CRISPR/Cas9 system and tagged the endogenous TSP-12, SUP-17 and UNC-40 proteins with different fluorescence tags. We found that TSP-12 is localized to the cell surface and intracellular vesicles, and that TSP-12 can bind to SUP-17 and promote its cell surface localization. We have genetic evidence and preliminary biochemical evidence showing that UNC-40 is one, but not the only, substrate of SUP-17 in BMP signaling. We are currently identifying additional substrate(s) of SUP-17 in BMP signaling. Our work highlights the importance of intracellular signaling and protease processing in the regulation of BMP signaling.


461A Developmental and Ageing-related roles of Homeodomain-Interacting Protein Kinase (HPK-1) in C. elegans. M.E. Wood, S. Berber, M. White, S. Cordwell, H Nicholas School of Life and Environmental Sciences, The University of Sydney, Sydney, AU.

Proteins of the Homeodomain-Interacting Protein Kinase (HPK) family regulate an array of processes in mammalian systems, such as the DNA damage response and cellular proliferation. Members of this protein family are serine/threonine kinases that are predominantly localised to the nucleus. Caenorhabditis elegans expresses a single HPK protein, called HPK-1. The HPK-1 protein is expressed in many cell types in the nematode and, like its mammalian counterparts, is localised to nuclear speckles, suggesting that it may be involved in analogous cellular processes. Previous studies have implicated HPK-1 in longevity control and we have recently suggested that this protein may be regulated in a stress-dependent manner as animals carrying a fosmid-based fluorescent reporter exhibit increased levels of HPK-1 following heat stress. To expand these observations, we have been investigating the role of HPK-1 in various processes, such as development, longevity, and in the response to stress, by conducting phenotypic analyses on a worm strain carrying a deletion mutation within the hpk-1 gene. HPK-1 appears to be required for normal somatic and germine development, as animals lacking HPK-1 are reduced in size compared with their wildtype counterparts, and show a decrease in brood size. We have previously shown that HPK-1 is required for normal longevity, with loss of HPK-1 function leading to a faster decline of physiological processes that reflect premature ageing. Loss of HPK-1 function also renders worms hypersensitive to heat and oxidative stress, suggesting that HPK-1 contributes to survival following exposure to these stressors.

In order to investigate the mechanisms involved in these processes, a nuclear phosphoproteomic study is underway to identify potential HPK-1 phosphorylation targets. Putative nuclear targets such as transcription factors for developmental, ageing and

Entomopathogenic nematodes (EPNs) are specialized insect parasites that infect and kill insect hosts. Previous research has shown that EPN infective juveniles (IJs) responded to hosts at various stages of infection, being attracted to uninfected or recently parasitized hosts, and repelled from late-stage, resource-deficient cadavers. Using gas chromatography and mass spectrometry, we found several odors associated with late-stage, resource-deficient cadavers, including prenol (3-methyl-2-buten-1-ol). We found prenol to be strongly repulsive to EPN IJs. Further investigation into the relationship between prenol and EPNs revealed that prenol may serve as a dispersal cue for EPN IJs that are still in contact with, or have recently emerged from, the parasitized cadaver. Our findings suggest that adults C. elegans may be more sensitive to prenol than dauers. Moreover, C. elegans dauers appear to be more sensitive to prenol than EPN IJs – despite being attracted rather than repelled – displaying a strong behavioral phenotype and greater participation in chemotaxis behavior than IJs. Our findings suggest that C. elegans has evolved to detect and respond to odors associated with prenol-parasitized hosts.

Phenotypic switching between nematode mutualism and insect pathogenesis by the bacterium Xenorhabdus nematophila. Mengyi Cao1, Matthew D Stilwell2, Elizabeth A Hussa3, Douglas B Weibel2, Heidi Goodrich-Blair1 1) Department of Bacteriology, University of Wisconsin-Madison, Madison, WI; 2) Department of Biochemistry, University of Wisconsin-Madison, Madison, WI; 3) Department of Biology, Millsaps College, Jackson, MS; 4) Department of Microbiology, University of Tennessee-Knoxville, Knoxville, TN.

The entomopathogenic nematode Steinernema carpocapsae mutualistically associates with the symbiotic bacterium Xenorhabdus nematophila. During transmission stage, the infective juvenile (IJ) nematodes carry and release bacterial symbionts into insects. X. nematophila symbionts help kill the insect and convert its nutrients to support nematode reproduction. Upon nutrient depletion, progeny nematodes re-associate with the bacteria and develop into IJs that emerge from the cadaver and seek new insect hosts. X. nematophila phenotypic variation, termed VMO (virulence modulation), is characterized by a switch between two cell types: one type (V+M-) is virulent in insects but defective in nematode mutualism and the other (V-M+) is attenuated for virulence in insects, but engages in normal mutualism with nematodes. Varying levels of the global transcription factor Lrp (leucine responsive regulatory protein) control the VMO switch: High Lrp levels result in the V-M+ phenotype, while low Lrp levels cause the V+M- phenotype. To assess the population dynamics of VMO, we used an Lrp-dependent fluorescent reporter of gene expression. We discovered that nutrient limitation induces low-Lrp expression and heterogeneity within the bacterial population. Based on these data, we hypothesize that symbiotic bacteria in the nutrient-limiting nematode IJ express heterogeneous levels of Lrp, and that these cells are adapted to express virulence traits in the next life stage within the insect. To pinpoint the specific step of bacteria-nematode interaction in which the mutualism (high Lrp) to pathogenesis (low Lrp) switch occurs, we investigated the influence of bacterial Lrp on interactions at each nematode developmental stage. Our data revealed that high Lrp is optimal for early stages of bacteria-nematode association prior to the IJ stage. However, low-Lrp expressing cells, pre-adapted for virulence, arise during transmission: a low-Lrp expressing strain exhibits a higher number of bacteria CFU per IJ than does a high-Lrp strain. These data suggest that the mutualism-to-pathogenesis switch occurs in the IJ nematodes. To track Lrp-dependent switching of symbiotic bacteria in the individual nematodes, we developed microfluidic devices and monitored colonization in multiple living IJ nematodes over 5 days. We will present our progress investigating the Lrp-dependent symbiosis switch in vitro and in vivo.

The very hungry Caenorhabditis elegans: What single component does all axenic media require? M. Flavel1, A. Mechler2, M. Shahnin2, E. Mathews1, A. Franks1, W. Chen2, D. Zanker2, S. Tegegne1, C. Doneski1, M. Jois1 1) Physiology, Anatomy and Microbiology, La Trobe University, Bundoora, Victoria, AU; 2) La Trobe Institute of Molecular Sciences, La Trobe University, Bundoora, Victoria, AU.

The benefits of culturing C. elegans on an axenic diet rather than a bacterial, monoxenic diet include the ability to control the exact nutrients delivered to worms and the removal of a complex, metabolically active organism such as E. coli from the experiment. For decades attempts to develop a chemically defined, synthetic liquid medium have been made, but have had limited success. C. elegans are filter feeders that actively eject liquid and particles smaller than bacteria, whilst ingesting particles similar in size to bacteria or bacterial clusters. Therefore, they should not be able to derive nutrients from a liquid source. However, studies that use liquid medium suggest that they do. Our study investigated what component enables commonly used axenic media (CeHR, CeMM, AXM) to initiate worm growth.

This question was explored by addressing why UHT skim milk is added to CeHR medium. We compared normal CeHR medium to CeHR medium without milk supplementation. When milk wasn’t added to CeHR, L1 arrest was observed. This confirmed that the addition of milk to CeHR was essential and led us to identify whether the essential component present in milk could be.
detected in all axenic protocols. We hypothesised that UHT skim milk was essential due to one or a combination of the following factors: 1. It provided a source of bacterial contamination. 2. It provided an otherwise excluded, but essential nutrient; or 3. It provided a particulate, delivery vessel of nutrient media inside the worm.

UHT is a process likely to kill all bacterial cells. However, it was unclear whether dead cells remained in the milk. We analysed the UHT skim milk used in our experiments by PCR and found bacterial numbers were below detectable limits. It therefore appeared that microorganisms were not acting as a major growth component of the UHT milk supplemented CeHR medium.

We used fluorescence-activated flow cytometry (FACS) analysis to detect whether small particles were available in these media. FACS analysis detected $8.5 \times 10^5$; $6 \times 10^7$ and $8 \times 10^7$ particles per ml of CeHR, CeMM and AXM media respectively. Both CeMM and AXM do not receive milk supplementation, yet contain more particles per ml than CeHR. All media contained particles within a size range that matches the size of bacteria. The removal of formed particles by filtering, centrifugation or solubilisation led to arrest at L1 lifestage.

Our results indicate worm development in axenic media is dependent on the presence of particulate matter in the culture medium. However, what these particles are composed of is undefined, therefore the nutrients nematodes receive in these conditions remains undefined. This poses a major challenge to the development of a defined medium for the culturing of C. elegans.

465B Cryptic S.M.S. (Small Molecule Signaling) between Caenorhabditis and Pristionchus. P. Gudibanda1,4, P. Loi4, N. Movahed4, A. Antebi2, R.J. Sommer2, F.C. Schroeder4 1) Department of Molecular Biology and Genetics, Cornell University, NY; 2) Max Planck Institute for Biology of Ageing, Cologne, Germany; 3) Max Planck Institute for Developmental Biology, Tübingen, Germany; 4) Boyce Thompson Institute, Ithaca, NY.

The primary mechanism for organisms to react to rapidly changing environments is the alternation of gene transcription profiles by modulating extra- and intracellular small molecule signaling (SMS). The formation of dauers, a stress-resistant, developmental arrest stage that is induced under unfavorable growth conditions in both Caenorhabditis elegans (free living nematode) and Pristionchus pacificus (necromenic nematode), represents an evolutionarily conserved strategy to counter adverse environmental conditions. Although transcriptional profiling suggests a high degree of conservation of the underlying signaling networks, including the roles of transcription factors DAF-16 and DAF-12, it is unclear whether the chemical structures of the steroid hormones regulating C. elegans development are broadly conserved in nematodes. In this study, we identify the endogenous small molecule ligands of the NHR Ppa-DAF-12 using an unbiased metabolomics approach, which demonstrates conserved steroid-based SMS in C. elegans and P. pacificus.

For our unbiased, endogenous ligand screen, metabolome fractions generated from WT and daf-22 mixed-stage P. pacificus extracts were tested for activation of a hybrid NHR, consisting of the ligand binding domain of Ppa-DAF-12 and the DNA-binding domain of Cel-DAF-12, in an HEK-cell culture based luciferase assay. Using high-resolution mass spectrometry, we determined extracts were tested for activation of a hybrid NHR, consisting of the ligand binding domain of Ppa-DAF-12 and the DNA-binding domain of Cel-DAF-12, in an HEK-cell culture based luciferase assay. Using high-resolution mass spectrometry, we determined that active fractions contained the exact same three dafachronic acids (DAs), D7DA, D1,7DA and 3aOH-D7DA, that had previously been characterized in C. elegans. The de novo biosynthesis of these DAs in P. pacificus was confirmed by $^{13}$C-$^2$ labeled cholesterol feeding experiments. We further showed that, like in C. elegans, accumulation of dauer-inducing ascarosides in P. pacificus liquid culture counteracts DA biosynthesis. As a result, dauer pheromone-defective Ppa-daf-22 mutant worms produce larger quantities of DAs than Ppa WT, like in Cel-daf-22 mutant worms. Taken together, these results demonstrate extensive conservation of SMS between C. elegans and P. pacificus, including the DAF-12 ligand’s chemical structures and the regulation of their biosynthesis via ascarosides. We further show that DAs are excreted in physiologically relevant concentrations by both C. elegans and P. pacificus, raising the possibility of inter-species regulation of development. We currently investigate potential conservation of other components of DAF-12 signaling, including interactions with corepressors (e.g. the Ppa homolog of Cel-DIN-1) and other NHRs (e.g. NHR-8).

466C Markov chain Monte Carlo simulation of the dauer larva formation decision-making and signalling in populations of Caenorhabditis elegans. A.J. Hills1, M.E. Viney2, S.C. Harvey1 1) Biomolecular Research Group, Department of Geographical and Life Sciences, Canterbury Christ Church University, Canterbury, UK; 2) School of Biological Sciences, University of Bristol, Bristol, UK.

Phenotypic plasticity allows species to respond to environmental changes. In the wild, populations of the nematode Caenorhabditis elegans develop and grow in nutrient-rich, ephemeral habitats. Movement between these ephemeral patches, and long term genotype survival, depends on the development of dauer larvae. Within these natural populations, survival is therefore dependent upon a critical development decision, as worms must commit to either a reproductive fate to increase local numbers, or a migratory fate to find new resources to exploit. Failure to disperse at the correct time will result in loss of the local population with the resources in the ephemeral habitat.

Under laboratory conditions, the decision between dauer and non-dauer larval development is driven by ascaroside signalling – which acts as a proxy for population size – by food availability, and by temperature. However, phenotypic differences between genotypes in reaction to these factors is substantial. For example, ascaroside production profiles vary between genotypes as do the responses to specific ascarosides and to mixtures of ascarosides. There is also evidence that suggests that ascaroside signalling by worms may be manipulative.

Here we present the results of Markov Chain Monte Carlo (MCMC) simulations of modelled C. elegans genotypes optimising the developmental switch under different environmental pressures. The simulation results are compared with a number of wild-type

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strains. The evolution of ascaroside signalling is examined with the additional effects of exposure to signal distortion and noise as may be present in heterogeneous wild habitats. The model is presented in the form of a generalizable method for studying developmental decisions and other phenotypically plastic traits under a variety of environmental conditions.

467A Surveying the species correlation between nematodes and nematode-trapping fungi in natural habitats. Ching-Ting Yang, Siou-Ying Lin, Yen-Ping Hsueh. Institute of Molecular Biology, Academia Sinica, Taipei, TW.

Nematodes and nematode-trapping fungi are both ubiquitously present in the environment. However, little is known about how these predatory fungi interact with their nematode-prey in nature. Our previous findings showed that the nematode-trapping fungi of the Arthrobotrys species eavesdrop on the nematode pheromone ascarosides as an indication of the presence of the nematode prey (Hsueh et al. 2013). We observed that different Arthrobotrys species exhibit different ascaroside-specificity and hypothesized that this might result from the co-evolution between the different species of nematodes and nematode-trapping fungi. To understand what species of nematodes and nematode-trapping fungi co-exist in the natural environment and to acquire material to test our hypothesis, we collected wild-isolates of nematodes and nematode-trapping fungi from different locations in Taiwan and determine the identity of the fungi and nematodes at the species or genus level based on their ITS or 18S rDNA sequences. In total, we successfully isolated both nematodes and nematode-trapping fungi from 13 of the 22 locations sampled. Seven species of Arthrobotrys have been identified to share the same niches with nematodes of the genera Oscheius, Cervidellus, Rhabditis, Caenorhabditis, Pelodera, Diploscapter, Meyerozyma, Acrobeleoides, and Mesorhabditis, suggesting that Arthrobotrys species are likely to prey on a broad range of nematode species in nature.


468B Statistical measurements of phenotypic variation to study genome and environment interactions C. elegans. David Jordan, Eric Miska. The Gurdon Institute, University of Cambridge, Cambridge, Cambridgeshire, GB.

Phenotypic diversity is the raw material for natural selection. If such variations can be inherited, they can play a role in evolutionary change. The study of phenotypic diversity must begin by quantifying such variation. For example, one may ask how many phenotypes can be generated from a single genotype in a fixed environment. However, quantifying phenotypes may be ill-posed, e.g. if traits are continuous, the answer may infinite. Instead, we propose to ask a related question, how many parameters are necessary to describe the space of phenotypic variation. To do this, we have collected a variety of phenotypic measurements from small populations of C. elegans, including details of their development, growth rate, movement, brood size, and lifespan. We collect these measurements with a set of custom video microscopes that can record and track the movements and sizes of 40 animals from eggs to adults (~3 days). Starting with the simplest case, a single genotype, in a fixed environment, first we explore the local phenotypic space with perturbations in temperature, food availability, and stress. We then expand both the genetic diversity, using a collection of C. elegans strains isolated from the wild, as well as the environmental diversity, using a variety of bacterial food sources also isolated from C. elegans natural habitats. To understand the relationships, if any, between these phenotypic measurements, we propose to use methods of dimensionality reduction to directly answer the question of how many parameters are necessary to capture the observable phenotypic diversity. Furthermore, we intend to explore how that changes under both genetic, and environmental perturbations. While the mechanism of inheritance of phenotypic variation due to genetic change is well understood, whether variation resulting from other sources can be inherited, and if so what the mechanism might be, is less well understood. Using the map of phenotypic space we have constructed, we intend to explore how populations move in this space in response to environmental stresses, such as heat shock, starvation, osmotic stress, and pathogen infection. We can then monitor the relaxation of the population distribution to its unperturbed state to assess whether any such variation can be inherited and if so, on which timescales. We hope that these studies will add both new methods, new data, and new ways of thinking to evolutionary, and ecological studies.

469C Investigation into the Inheritance Pattern of Resistance to a Nematocide that Results in Terminal Embryogenesis. Daniel Kazerski, Tess Renahan, Ray Hong. California State university, Northridge, Northridge, CA.

Developmental arrest in nematodes can be associated with the presence of biological and chemical factors known as teratogens. Not much is known about the naturally occurring teratogens that can penetrate the eggshells of nematodes and cause significant developmental problems. (Z)-7-tetradecen-2-one (ZTDO), a pheromone produced by the oriental beetle, Exomala orientalis, has been shown to cause irreversible and terminal embryotic arrest in the embryos of its associated nematode, Pristionchus pacificus. Some strains of P. pacificus show partial resistance to ZTDO, including the strain RS5278 from Bolivia. In an effort to determine the inheritance pattern of ZTDO resistance, crosses were performed between a resistant and non-resistant strain of P. pacificus. We crossed the resistant RS5278 strain with a dumpy marker, and performed a 4-hour ZTDO drop assay following a 2-hour embryo synchronization. The results showed that all F1 progeny were susceptible, representing the maternal phenotype. However, the F2 cross progeny exhibited a level of ZTDO resistance similar to the paternal phenotype. The absence of ZTDO resistance in the F1 but not in the F2 offspring leads us to form the working hypothesis that the inheritance pattern is maternal and that the resistance allele is dominant. We hope to use this model to perform further tests using different strains of P. pacificus to confirm the inheritance pattern of ZTDO resistance.

Gut microbiota plays important role in various host physiological process, including toxic response. *C. elegans* is a useful model to study the microbiome host response relationship, as it is bacterivore and possess simple body plan with the intestine as the major body cavity. Moreover, diverse gut microbiota can be easily established by exposing germ-free hatchlings to different microbial environments and its self-fertilizing potentiality provides genetically homogenous populations onwards. In this study, to investigate the role of gut microbiome on host defense mechanism toward chemical exposure, *C. elegans* was first fed with diverse microbiota extracted from enriched korean soil and subsequently was exposed to environmental chemicals, such as, cadmium. Survival, reproduction and life span of *C. elegans* were investigated and the results were compared with that from the worms fed with OP50 in lab condition. It was found that soil-microbiota-fed worms showed faster growth rate, compared to OP50-fed worms. The response to cadmium exposure was also found to be different between soil microbiota- and OP50-fed worms. Our results suggest gut microbiome affects response of *C. elegans* to cadmium exposure, highlighting the importance of gut microbiome on host stress response mechanism. Further metagenomics analysis on soil microbiome, as well as, *C. elegans* transcriptomics analysis will provide molecular mechanism on how gut microbiome affect *C. elegans* toxic response.

471B  Determining the role of TGF-β signaling in innate immune response to a variety of immune challenges by the nematode model organism *C. elegans*.  B.J. Madhu, L.K. Hanson, T.L. Gumienny Texas Woman's University, Denton, TX.

The innate immune response coordinates several molecular activities, including a cell-cell signaling pathway called TGF-β (Transforming Growth Factor-β). This response is conserved in species from simple animals to humans. In *C. elegans*, the DBL-1/TGF-β signaling pathway is required for an innate immune response to fight infection by fungus and bacterial strains *P. aeruginosa*, *S. marcescens*, *E. faecalis*, and *P. luminescens*. To determine if DBL-1/TGF-β is generally required to mount an effective innate immune response, we challenged normal and TGF-β mutant nematodes with a range of human opportunistic pathogens, both Gram positive (*B. megaterium*, *E. faecalis*, and *S. epidermidis*) and negative (*E. cloacae*, *K. oxytoca*, and *S. marcescens*) strains. We determined how *C. elegans* responds to these human pathogens by quantitating and comparing avoidance behavior, nematode survival, and intestinal pathogen colonization in both wild-type and DBL-1 variant backgrounds. While the response to fungus is mediated by a non-canonical TGF-β pathway, the specific TGF-β pathway that responds to bacterial infection is unknown. To test if canonical signaling (which uses SMA-2, SMA-3, and SMA-4) or non-canonical signaling (which uses SMA-3 but not SMA-2 or SMA-4) is required to mount an effective response to these bacterial strains, we will determine if the expression levels of immune response genes vary between wild-type and *sma-2, sma-3*, and *sma-4* mutant immune-challenged animals. We will also assay avoidance behavior and survival of *sma-2, sma-3*, and *sma-4* mutant animals on these pathogens. In addition, we will look for cross-regulation between TGF-β, MAPK signaling, and insulin-like signaling pathways, which are all involved in mounting an innate immune response. It has been previously reported that the targets of p38 MAPK pathway are upregulated upon infection by *S. marcescens*, *E. faecalis*, and *P. luminescens*, and are potentially regulated by DBL-1. To determine cross-regulation, we will quantify changes in the expression levels of the immune response genes as well as the TGF-β, MAPK, and insulin-like signaling pathway genes upon exposure of DBL-1 pathway variants to our test bacterial strains. This work will expand our knowledge about the molecular mechanism of the DBL-1/TGF-β signaling pathway in response to a variety of bacterial challenges.

472C  Nematodes as important source for omega-3 long-chain fatty acids in the soil food web and the impact in nutrition for higher trophic levels.  Ralph Menzel, Henrik Chrzanowski, Diana Geweiler, Liliane Ruess  Humboldt-University at Berlin, Berlin, Germany.

PUFA (Polyunsaturated fatty acids) content of food resources, in particular omega-3 long-chain (LC) PUFAs with three or more double bonds, are considered essential for growth, reproduction, and neural development of higher animals. Surprisingly, and in contrast to aquatic ecosystems, omega-3 LC-PUFAs seem not widely available in terrestrial food webs. Far-reaching ideas indeed proclaim aquatic ecosystems as the principal source of these LC-PUFAs in the whole biosphere, including inhabitants of terrestrial ecosystems. Interestingly, *de novo* synthesis of omega-3 LC-PUFAs, which requires the presence of *?12* (fat-2) and omega-3 (fat-1) desaturases absent in vertebrates, has been observed in *Caenorhabditis elegans* and other nematodes. This calls for studies to assign how this remarkable metabolic capability enhances or even determines the availability of omega-3 LC-PUFAs in the soil food web. Our project addresses the following questions: How common is the ability for *de novo* synthesis of omega-3 LC-PUFAs across different functional groups of nematodes, whether or not is this capability related to their diet, and how efficiently is the transfer of these PUFAs to higher trophic level, such as microarthropods? Using a genetic approach by doing PCR with degenerated primers we identified so far two novel fat-2 like genes in the free-living soil nematodes *Acrobeleoides buetschlii* and *Oscheius dolichuroides*. Sequence alignments with already known nematode fat-2 genes supports the idea that already an ancestral nematode did contain such gene rather than a horizontal gene transfer to only a subgroup of nematodes. By using GC/MS analyses we observed the trophic transfer of nematode LC-PUFAs to nematophagous Collembola, as *Folsomia candida* and *Protaphorura fimata*. In fact, Collembola reflected the PUFA-richness or -poorness of their nematode prey, achieved by feeding *C. elegans* mutants with defects in the PUFA biosynthesis, in their neutral lipid fraction. In contrast, Collembolas PUFA-content of the phospholipid fraction remained unchanged, suggesting that these invertebrates also possess the metabolic capability to *de novo* synthesize PUFAs, including omega-3 LC-PUFAs.

473A  Investigating the tropism of vertically transmitted bunya-like viruses in *Caenorhabditis*.  Aurélien Richaud1, Lise Frézal1, Kevin Chen2, Hongbing Jiang2, David Wang2, Marie-Anne Félix1  1) Institut de Biologie de l’Ecole Normale Supérieure
Ceratosolen pollinating fig wasps. The fig and wasp host specificity of this pheromone. We previously reported a class D GPCR serpentine receptor (SRD-1), a receptor in AWA neuron, is responsible for pheromone through the GC-MS analysis, signal transduction pathway mutant strain studies and also the demonstration of upon induction by both candidates separately. We concluded that these two chemicals are the active components of this stage-, and species-specific attractiveness on wild-type males. Noticeably, these compounds could neither attract receptor-

Interestingly, we did not find so far any structural genes corresponding to bunyaviruses (or any other viruses) in these strains, but only that coding a RdRp gene, thus we still do not know whether viral particles may be active in these

We tested whether these strains were deficient in RNAi, which could explain the replication and persistence of virus-like RNA fragments. Indeed, we found by injecting unc-22 dsRNA in the gonad of JU1396 and QG551 that they were unable to mount a RNAi response. Additionally, we are sequencing the small-RNAs from these two strains to see whether we can detect siRNA targeting the viral fragments and determine a specific small RNA size distribution and base composition pattern.

We are able to detect viral particles. We will also clone the fragment coding for one of the RdRp and inject it into a strains. To address this question, we plan to perform transmission electron microscopy on JU1396 and QG551 to see whether we are able to detect viral particles. We will also clone the fragment coding for one of the RdRp and inject it into a C. elegans strain insensitive to RNAi to test the hypothesis that this virus could be vertically transmitted through the germ line cytoplasm even lacking both glycoprotein and nucleoprotein genes.

Identification of nematodes volatile sex pheromone and the molecular basis of its perception. Xuan Wan, King L. Chow Division of Life Science, Hong Kong Univ Sci & Technol, Hong Kong, HK.

Nematodes rely on their sensory modalities to locate mating partner. Volatile sex pheromone attracts potential mating partners from afar. Intriguingly, sexually matured females C. remanei and self-sperm exhausted hermaphrodites C. elegans produce a volatile attractant that is detectable by both co-specific and its relative adult males, qualifying this attractant as a long-range sex pheromone. We previously reported a class D GPCR serpentine receptor (SRD-1), a receptor in AWA neuron, is responsible for detecting this pheromone and several components in the olfactory pathway are involved in this pheromone signal transduction. The GC-MS results of chemo-attractants from female C. remanei verified several volatile chemicals. Two of them exhibited sex-, stage-, and species-specific attractiveness on wild-type males. Noticeably, these compounds could neither attract receptor-defective mutant nor pheromone-perception-defective mutant males. We also visualized AWA neuron excitation by employed a calcium indicator, GCaMP5. The excitation of the AWA neurons was observed only in wild-type males, but not in srd-1 mutants, upon induction by both candidates separately. We concluded that these two chemicals are the active components of this pheromone through the GC-MS analysis, signal transduction pathway mutant strain studies and also the demonstration of ligand-receptor relationships in calcium imaging and behavioural assays. (The study is supported by Research Grants Council, Hong Kong.)

The natural history of a fig-associated Caenorhabditis. G. C. Woodruff1, N. Kanzaki2, P. C. Phillips1 1) Institute of Ecology and Evolution, University of Oregon, Eugene, OR; 2) Forest Pathology Laboratory, Forestry and Forest Products Research Institute, Tsukuba, Japan.

Biotic interactions are ubiquitous and require information from ecology, evolutionary biology, and functional genetics in order to be completely understood. However, study systems that are amenable to investigations in such disparate fields are rare. Figs and fig wasps are a classic system for ecology and evolutionary biology with poor functional genetics; C. elegans is a classic system for functional genetics with an historically poorly-described ecology. In order to help bridge these disciplines, here we describe the natural history of a close relative of C. elegans, C. sp. 34, that is associated with the fig Ficus septica and its pollinating Ceratosolen wasps. To understand the natural context of fig-associated Caenorhabditis, fresh F. septica figs from four Okinawan islands were sampled, dissected, and observed under microscopy. Caenorhabditis was found in all islands where F. septica figs were found. Caenorhabditis was routinely found in the fig interior and almost never observed on the outside surface. DNA sequencing of fig-derived animals revealed that they share nearly identical cytosome oxidase I sequence with C. sp. 34. Caenorhabditis was only found in pollinated figs, and Caenorhabditis was more likely to be observed in figs with more foundress pollinating wasps. Actively reproducing Caenorhabditis dominated younger figs, whereas older figs with emerging wasp progeny typically harbored Caenorhabditis dispersal (likely dauer) larvae. Additionally, Caenorhabditis was observed dismounting from plated Ceratosolen pollinating wasps. Caenorhabditis was never found on non-pollinating, parasitic Philotropysus wasps. And, Caenorhabditis was only observed in F. septica figs among six Okinawan Ficus species sampled. These observations suggest a natural history where C. sp. 34 proliferates in young F. septica figs and disperses from old figs on Ceratosolen pollinating fig wasps. The fig and wasp host specificity of this Caenorhabditis is highly divergent from its close
relatives and frames hypotheses for future investigations. This natural coincidence of the fig/fig wasp and Caenorhabditis study systems sets the stage for an integrated research program that can help to explain the evolution of interspecific interactions.

477B  Species-specific toxicity to the bacterial metabolite violacein in bacteriovorous nematodes is ameliorated by oleic acid.  K. Yoon¹, J. Choi², S. Choi³, R. Mitchell⁴, J. Lee¹  1) Division of Biological Science and Technology, Yonsei University, Wonju, 220-710, South Korea; 2) School of Life Sciences, Ulsan National Institute of Science and Technology, Ulsan, 689-798, South Korea.

The bacterial metabolite violacein is produced by various bacteria in diverse environments. Violacein is toxic to certain types of bacteria and even certain cancer cells, and has garnered interest as an antimicrobial and anticancer therapeutic. However, the mechanism of violacein’s toxicity and specificity in certain organisms is not known. Here, we exposed several species of bacteria-consuming nematodes to violacein. Violacein stunts the growth of Caenorhabditis elegans, Caenorhabditis briggsae and Pelodera sp. resulting in developmentally-arrested animals, but C. remanei is partially resistant and Pristionchus pacificus is completely resistant to the toxicity. Adult C. elegans exposed to violacein show intestinal defects and decreased fat reserves, and hold their eggs in their body resulting in internal hatching of larvae and eventual death of the animal. Interestingly, larvae that are hatched within the mother’s body are quite resistant to violacein toxicity. We found that lipid extracts from the mothers’ carcasses can ameliorate the effects of violacein. Screening through several types of fats, we show that supplementation with oleic acid and polyunsaturated fatty acids derived from oleic acid can counter the effects of violacein in both C. elegans and C. briggsae. We are currently investigating the role fatty acid plays in violacein resistance.

Ecology and Evolution - Evo-Devo, Evolution of Mating Systems

478C  Regulation of the Sperm to Oocyte Transition in C. briggsae by cbr-met-2.  Aaron Berenson, Scott Baird  Biological Sciences, Wright State University, Dayton, OH.

Within Caenorhabditis, three species reproduce primarily through self-fertilization: C. elegans, C. briggsae and C. tropicalis. Hermaphrodites from these species produce and store sperm during L4. During adulthood, the gonad undergoes a permanent transition from spermatogenesis to oogenesis. The timing of this transition limits the number of sperm produced leading to an overall restriction on the total achievable brood size in these populations. In C. elegans the transition is regulated through post-transcriptional control of fem-2 and fem-3. Both genes are required for spermatogenesis in C. elegans hermaphrodites. However, the orthologs of these genes are not required for sperm development in C. briggsae. We have identified cbr-met-2 as a regulator of this transition in C. briggsae. cbr-met-2 mutant hermaphrodites transition from spermatogenesis to oogenesis around the time of the L4 molt whereas wildtype animals remain spermatogenic until 2.5 to 3 hours into adulthood. This early transition results in decreased brood sizes and in an early cessation of egg laying. Wildtype (AF16) C. briggsae hermaphrodites have an average brood size of 325.9 ± 21.8 whereas three strains containing cbr-met-2 mutations (xoe1, xoe2 and bd28) had broods of less than 200 animals. Interestingly, the reduction in brood size, the early cessation of egg laying and the premature transition to oogenesis associated with mutations in cbr-met-2 had little impact on intrinsic growth rate. The early transition observed in cbr-met-2 mutant animals leads to a modest increase in progeny on the first day of egg laying. Since intrinsic growth rate is dependent upon the number of progeny as well as the timing of their appearance, it is possible that the decrease in brood size is offset by the early onset of reproduction.

479A  Genome architecture and evolution of the unichromosomal asexual nematode, Diploscapter coronatus.  Hélène Fradin¹, Karin Kiontker¹, Charles Zeger¹, Michelle Gutwein¹, Jessica Lucas¹, Mikhail Kvitun², David Corcoran², Ryan Baugh³, David Fitch³, Kristin Gunsalus³, Fabio Piano³  1) Department of Biology, New York University, New York, NY; 2) Duke Center for Genomic and Computational Biology, Duke University, Durham, NC; 3) Department of Biology, Duke University, Durham, NC.

Long-lived clades of animals that reproduce exclusively asexually are rare, presumably because lack of variation in such species results in high extinction rates. Longevity of asexual clades appears to be correlated with the maintenance of heterozygosity across generations. To understand how successful asexual lineages evolve while maintaining heterozygosity, we investigated the reproductive biology and genome sequence of the nematode Diploscapter coronatus. D. coronatus is a species that belongs to the Prororbhabditis group, the sister group to Caenorhabditis. We confirmed the existence of a long-lived (approx. 18 million years) asexual clade within Prororbhabditis and resolved its phylogeny. We found that all asexual species in the clade have an unusual karyotype: a single pair of chromosomes. This karyotype evolved once from an ancestor with six chromosome pairs. This drastic drop in the number of chromosomes coincides with the transition from sexual to asexual reproduction. We determined the D. coronatus genome structure and sequence, and find evidence in the genome assembly that the single chromosome resulted from the fusion of ancestral chromosomes. This fusion is associated with extensive rearrangement among neighboring regions, which we used to infer a partial spatial order in which ancestral chromosomes fused. The genome can be organized into two divergent homologous haplotypes, confirming that heterozygosity is maintained in this species despite the asexual reproduction. Interestingly, two adjacent fused regions, corresponding to ancestral chromosomal domains I and X, show lower levels of heterozygosity than the other domains. These data are consistent with a scenario in which an initial X-I fusion became a neo-X chromosome in a sexual ancestor. This neo-X would have had a reduced effective population size and thus reduced heterozygosity and increased linkage disequilibrium relative to the autosomes. Consistent with chromosomal fusions, we find no evidence of typical nematode telomeres in the D. coronatus genome. Parthenogenesis is likely evolved after chromosomal fusion. Cytological observations indicate that D. coronatus reproduces with a modified meiosis that skips Meiosis I, synapsis and recombination and results in a diploid embryo without fertilization. Consistent with this model, certain key conserved genes with roles in homologous pairing and recombination were not found in the D. coronatus genome. Also,
oogenesis without Meiosis I is one way in which parthenogenetic organisms can maintain heterozygosity. As a prelude to functional studies, we also show that D. coronatus is amenable to experimental manipulation by RNAi.

480B  **FUSEXINS are ancient fusion proteins necessary for viral, somatic and sexual cell fusion.**  C. Valansi1, D. Moi2, E. Leikina3, E. Matveev1, M. Graña4, L. V. Chernomordik5, H. Romero6, P. S. Aguilar2, B. Podbielwicz1 1) Dept Biol, Technion-IIT, Haifa, Israel; 2) Laboratorio de Biologia Celular de Membranas, Instituto de Investigaciones Biotecnologicas "Dr. Rodolfo A. Ugalde" (IBI), Universidad Nacional de San Martin (UNSAM), Buenos Aires, Argentina; 3) Section on Membrane Biology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, Bethesda MD; 4) Unidad de Bioinformatónica, Instituto Pasteur Montevideo, Montevideo, Uruguay; 5) Laboratorio de Organización y Evolución del Genoma, Unidad de Genómica Evolutiva, Dpto. Ecología y Evolución, Facultad de Ciencias/C.U.R.E., Universidad de la República, Montevideo, Uruguay.

Cell fusion is essential for fertilization and organ formation. Two cell fusion families have been shown to be both essential and sufficient for somatic cell-cell fusion: the FF (fusion family) fuse one third of all cells generated in C. elegans and the Synctins that fuse billions of cells in the placenta. The FF2(GCS1) are membrane glycoproteins essential for gamete fusion in plants, Chlamydomonas, Plasmodium, Tetrahymena, and Dyctostelium. However, HAP2 homologues in Fungi, vertebrates and C. elegans have not been identified. To determine whether HAP2 is not only essential but also sufficient for cell fusion, we expressed the Arabidopsis sperm HAP2 in cultured mammalian cells that normally do not fuse. We found that HAP2 expression in heterologous cells results in the formation of giant cells via cell fusion. Genetic analyses support that HAP2 has to be present in only one of the fusion partners (usually the male gamete). However, we found that when expressed in heterologous cells, HAP2 is sufficient to fuse them only when expressed in both fusing cells. Thus, HAP2-mediated plasma membrane merger occurs via a bilateral mechanism reminiscent to intracellular fusions mediated by SNAREs. Furthermore, expression of HAP2 on the surface of pseudotyped vesicular stomatitis virus results in HAP2-dependent virus-cell fusion but only to cells that also express HAP2. Structural modeling of the HAP2 protein family predicts that it is homologous to EFF-1 from C. elegans and class II viral fusion proteins (e.g. Zika). The crystal structure of Chlamy HAP2 demonstrates structural homology with EFF-1 and class II viral fusion proteins. Interestingly, cell-cell fusion also occurs between C. elegans EFF-1 and HAP2-expressing cells. HAP2-mediated fusion is a process that occurs via a hemifusion intermediate in which the outer membranes merge before the cytoplasm. We name this superfamilly FUSEXINS: FUSion proteins essential for sexual reproduction and EXoplasmic merger of plasma membranes. We propose that enveloped virus entry into cells, sexual reproduction, and somatic cell fusion had a common ancestor. The holy grail of fertilization is to identify the fusion proteins that merge the sperm and the egg in worms and also in humans.


481C  **Establishing genetic techniques to study Caenorhabditis sp. 34, a sister species of C. elegans.**  Asako Sugimoto1, Kenji Tsuyama1, Yuki Hoshi1, Satoshi Namai1, Ryohei Kumagai1, Natsumi Kanzaki1, Taisei Kikuchi2 1) Graduate School of Life Sciences, Tohoku University, Sendai, Japan; 2) Forestry and Forest Products Research Institute, Tsukuba, Japan; 3) Parasitology, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan.

Caenorhabditis sp. 34 is a sister species of C. elegans recently isolated from the syconia of the fig Ficus septica on Ishigaki Island, Japan (see abstract by T. Kikuchi, et al.). C. sp. 34 is gonochoric and shares typological key characters with other Elegans supergroup species, but strikingly, adults are nearly twice as long as C. elegans. The optimal culture temperature for C. sp. 34 is significantly higher (27°C) than that of C. elegans (20°C). Young adult males and females tend to form clumps, and Dauer larvae are rarely observed in laboratory culture conditions. Recently the C. sp. 34 genome assembly was produced into six chromosomes (see abstract by T. Kikuchi, et al.). The marked differences from C. elegans in morphology, behaviors and ecology, and the availability of the complete genome sequence make C. sp. 34 highly attractive for comparative and evolutionary studies. To make C. sp. 34 genetically tractable, we have been developing genetic and molecular techniques and tools. Stable transgenic lines of C. sp. 34 could be obtained by microinjecting marker plasmids commonly used in C. elegans, although the efficiency was lower than that in C. elegans. Both soaking and feeding RNAi was as effective as in C. elegans. A panel of antibodies against C. elegans proteins successfully recognized expected structures in C. sp. 34 by immunofluorescence. Thus, many of the rich genetic and molecular resources for C. elegans can be directly used for C. sp. 34 studies. We well present some of the comparative analyses of gene functions regarding the body size, germ cell formation and sex determination.

482A  **The shape of things to come: elucidating the mechanisms of Developmental System Drift associated with axis formation in Caenorhabditid nematode embryos.**  Nicholas Testa, Annalise Paaby  Biological Sciences, Georgia Institute of Technology, Atlanta, GA.

Developmental system drift (DSD) occurs when selection for a stable phenotype allows for developmental mechanisms—including genes, pathways, and cellular and sub-cellular processes—to diverge over evolutionary time even as the gross developmental phenotype remains stable across taxa. This phenomenon, first described formally in 2001, has been referenced routinely in the literature and has received some modest theoretical treatment, but little experimental investigation. Here, we investigate DSD and the underlying mechanisms associated with anterior-posterior axis determination and early embryogenesis.
in 10 nematode species within the genus *Caenorhabditis*.

*Caenorhabditid* nematodes are an exemplary model of DSD with highly stereotyped and near-invariant early embryonic cell divisions. Previous studies have demonstrated that, despite their near-indistinguishable developmental regime, *Caenorhabditid* worms may differ in several cellular and developmental phenotypes during early embryogenesis. Furthermore, many of the gene sequences associated with axis determination in early development (especially par-2) have diverged significantly across taxa.

In this study, we precisely quantify subtle differences in early embryonic shape and cell-size caused by species-specific differences in axis determination. For each treatment, approximately 30 individual videos of embryogenesis were collected. Young adult worms were dissected, and resulting embryos mounted on agar pads and imaged on a spinning disk confocal DIC microscope; subsequent videos start in the single-cell stage and progress past 4-cells. We then collected images from these videos at precise time points corresponding to both 2-cell and 4-cell embryos.

Using geometric morphometrics with a combination of 12 and 18 landmarks/semi-landmarks, we have demonstrated differences in embryonic shape for 2- and 4-cell embryos (respectively) of N2 (*elegans*) and AF16 (*briggsae*) at both 20°C and 30°C. If the underlying mechanisms that regulate embryonic shape remained the same across species, we would expect to see both species react similarly to stress during development. Instead, our data demonstrate that developmental shape trajectories diverge in stressful environments, revealing cryptic genetic variation of their mechanisms. Our data confirm that DSD has occurred between at least two taxa within *Caenorhabditis*. Future data will include as many as 10 different *Caenorhabditid* species.

**Ecology and Evolution - Evolution of Complex Traits, Quantitative Genetics**

**483B  Natural variation in stochastic programmed cell death in the adult male tail is associated with several QTL.  Melissa Alcorn1, Davon Callander1, Yamila Torres Cleuren1,2, Joel Rothman1  1) MCD Biology, University of California, Santa Barbara, Santa Barbara, CA; 2) Computational Biology Unit, University of Bergen, Bergen, Norway.**

Somatic programmed cell death in *C. elegans* is thought to occur by a largely invariant pattern. However, we have found an exception in the development of the male copulatory structure, which exhibits variability (reduced fidelity) in the total number of sensory rays. *C. elegans* males typically develop eighteen bilaterally symmetric rays, in which each ray is comprised of the dendritic processes of two neurons and a structural support cell. We have found that in the laboratory N2 strain, ~20% of males are missing one or more rays as a result of stochastic, inappropriate programmed cell death (PCD). This stochastic cell death varies widely across 94 unique wild isotype strains, ranging from 0 to ~35% of males with missing rays. The absence of rays in selected strains is the result of inappropriate PCD; as ray losses are suppressed in egl-1 (RNAi) animals. A genome-wide association study identified one quantitative trait locus (QTL) on chromosome I underlying this variation in stochastic PCD. In addition, genetic mapping strategies using RILs identified several independent QTL on chromosomes II, III, and X that contribute ~55% of the phenotypic variation seen in the RILs and most likely other isotypes. Concurrent with *in silico* analysis, we are validating candidate regions in different genetic backgrounds to test their effect on the propensity for cell death and to identify causal genes that determine the molecular mechanism(s) of stochastic PCD.

**484C  Evidence that machine learning tools provide a more sensitive strategy than conventional mapping methods for identifying loci underlying genetically complex traits.  Melissa Alcorn1, Yamila Torres Cleuren1,2, Matthew Cieslak2, Joel Rothman1  1) MCD Biology, University of California, Santa Barbara, Santa Barbara, CA; 2) Psychological and Brain Sciences, University of California, Santa Barbara, Santa Barbara, CA; 3) Computational Biology Unit, University of Bergen, Bergen, Norway.**

Genome-wide association studies (GWAS) and quantitative trait loci (QTL) mapping have been successful in identifying genetic variants in humans and model organisms. However, for complex traits, in which multiple loci contribute, genomic variants with small individual effects may be missed by these methods owing to limited statistical power from testing thousands of single-nucleotide polymorphisms (SNPs). Here we present a case-study of the application of machine learning algorithms, specifically ElasticNet regression, as a complementary approach to conventional GWAS and QTL mapping techniques, with the goal of identifying major loci and others that may contribute minor effects on their own, but collectively drive substantial phenotypic diversity. As a test of the approach, we applied ElasticNet regression to identify loci that control the plasticity in the gene regulatory network for *C. elegans* endoderm development. Our phenotype analysis has revealed large variation in the requirement for SKN-1 in endoderm development: while the laboratory N2 strain shows a partially penetrant phenotype loss of gut (30% of embryos produce gut) in *skn-1(-)*, we found that this varies widely across 94 wild isolates, ranging from 0% to ~60%. GWAS using efficient mixed-model analysis (EMMA) identified a single highly significant peak on chromosome IV that accounts for at least some of the variation across these strains. QTL mapping using RILs obtained from crosses between N2 and the MY16 isolate (in which only ~2% of embryos make gut in *skn-1(-)*) identified a significant QTL on chromosome IV, likely corresponding to the locus identified by GWAS, as well as three additional QTL on chromosomes I, II, and X. We found that the ElasticNet machine-learning tool applied to the same dataset not only effectively identified all four regions found by conventional QTL mapping, but also uncovered additional loci on chromosomes III and V (R² = 0.50, p = 1.95x10⁻⁷), revealing that this method may provide a more sensitive strategy for identifying genomic variations responsible for complex genetic traits. We are currently testing the novel regions identified by the machine learning approach in near-isogenic lines of different genetic backgrounds to assess their impact on the observed variation in the endoderm gene regulatory network.
485A Transcriptomes as phenotypes: synMuvs as a case study. D. Angeles-Albores1,2, H. Schwartz1,2, P.W. Sternberg1,2 1) Division of Biology and Biological Engineering, Caltech, Pasadena, CA; 2) Howard Hughes Medical Institute.

Quantitative genetic analysis relies on measuring phenotypes of single and double mutants. If two genes interact, the double mutant will have a phenotype that is not the sum of the phenotypes of the single mutants that were combined to make up its genotype. Although epistatic analysis lies at the heart of genetic analysis, its generalization to multi-dimensional quantitative phenotypes has proven very difficult. Transcriptional profiles are a particularly appealing complex quantitative phenotype, given the ease of obtaining RNA and the accuracy with which RNA levels can be measured. Despite its quantitative resolution of transcript levels, RNA-seq is used mostly as a qualitative tool with which to identify genes that are responsive to a perturbation relative to a control. Recently, we have developed tools that enable us to consider transcriptomes as phenotypes by comparing how gene expression levels change across two single mutants and comparing them with the expression levels in a double mutant. We can summarize these results in a single number, called the transcriptome-wide epistasis coefficient, which accurately reflects the underlying genetic architecture. As a case study, we chose genes associated with the synthetic Multivulva (synMuv) phenotype. Briefly, synMuv genes can be separated into two functionally redundant classes named A and B. Class B largely consists of general chromatin modifiers, such as epc-1 enhancer of polycomb, hpl-2 heterochromatin protein 1, and lin-35 Rb. Class A is composed of nematode-specific genes with poorly understood molecular functions, but that act redundantly with the class B synMuv genes to prevent ectopic expression of lin-3 EGF. In synMuv AB double mutants, ectopic expression of lin-3 induces ectopic vulval development, the synMuv phenotype. How or why this happens is not well understood despite work on the synMuv genes since the 1980s. We have sequenced several synMuv class A and class B single mutants and AA and AB double mutants to understand how these two redundant pathways interact in a whole-organism framework.


Caenorhabditis elegans typically feeds on rotting fruit and plant material in a fluctuating natural habitat, a boom-and-bust lifestyle. Moreover, stage specific developmental responses to low food concentration suggest that starvation-like conditions are a regular occurrence. In order to assess variation in the C. elegans starvation response under precisely controlled conditions and simultaneously phenotype a large number of individuals with high precision, we have developed a microfluidic device that, when combined with image scanning technology, allows for high-throughput assessment at a temporal resolution not previously feasible. Under these conditions worms exhibit a markedly reduced adult lifespan with strain-dependent variation in starvation resistance, ranging from <72 hours to ~120 hours. Initial results contrasting wildtype individuals with lipase mutants suggest a significant role for processes of fat metabolism in mediating resistance to starvation stress. Application of this device to a well-defined polymorphic population of C. elegans allows for mapping of the relationship between natural variation in genotypes and differences in starvation resistance.

487C Identification of the gene underlying variation in Caenorhabditis elegans bleomycin sensitivity. S.C. Brady1,2, D.E. Cook1,2, R.E. Tanny3, S. Zdraljevic1,2, E.C. Andersen1,2 1) Interdisciplinary Biological Sciences, Northwestern University, Evanston, IL; 2) Department of Molecular Biology, Northwestern University, Evanston, IL.

Individual cancer patients exhibit differential responses to anti-neoplastic treatments. In many cases, a portion of this variability is attributable to genetic factors that impact drug responses. Bleomycin is an effective anti-tumor antibiotic that causes double-stranded DNA breaks and subsequent cytotoxicity. However, patients with increased bleomycin sensitivity experience potentially lethal pulmonary toxicity and other less severe side effects. Although bleomycin sensitivity is heritable, genetic variants that underlie differences in bleomycin response have not been discovered yet. By leveraging genetic diversity in Caenorhabditis elegans and an established high-throughput fitness assay, we identified a quantitative trait locus (QTL) that is highly correlated with bleomycin response. Within the confidence interval of this QTL, we identified a single candidate gene that contains one non-synonymous variant between sensitive and resistant strains. We will present evidence from CRISPR/Cas9-generated loss-of-function alleles and reciprocal allele replacements to causally connect this gene to the differences in bleomycin sensitivity.

488A Quantitative genetic mapping of difference in oxygen and carbon dioxide-evoked behaviors that are dependent on environmental growth conditions. Richard Campbell, Mark Lowder, Patrick McGrath School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA.

The basis of behavioral variation in populations of animals is rooted in genetic, environmental and neuronal diversity. Since C. elegans is amenable to controlling each of these factors, we are seeking to understand how environmental and genetic diversity gives rise to natural differences in behaviors. Wild isolate strains such as the Hawaiian strain, CB4856, are very sensitive to small changes in atmospheric O2 and CO2 and exhibit a robust increase in turning and increase in speed in response to a shift from 20% O2/1% CO2 to 21% O2/0% CO2. Interestingly, this behavior is dependent on environmental growth conditions. If animals are grown on uniform lawns lacking an O2 gradient this response disappears. We asked if these O2 and CO2 responses are similar across different wild isolates. We found that there was variation among a representative panel of 12 wild isolates with some strains showing robust responses and some showing little to no change. To understand the genetic basis for this variation in O2 and CO2 behaviors, we are taking two approaches. First, we will perform QTL mapping on recombinant inbred lines we generated between MY14 (which does not show the sharp change in speed and turning behavior) and a modified N2 strain that contains the ancestral alleles of npr-1 and glm-5 (which shows the sharp change and speed and turning behavior). We will also perform GWAS on a large panel of wild isolates collected and sequenced by the CeNDR resource. We anticipate finding causal variants in other loci that are responsible for growth-dependent O2 and CO2 behaviors.
The development of C. elegans is precise and stereotyped, including patterns of cell division in early embryogenesis. Nevertheless, natural genetic variation in wild-type isolates can cause dramatic differences in phenotype following single-gene perturbations, indicating that different wild-type genotypes harbor functional variation in critical gene networks. These same wild isolates also show extreme variation in the efficacy of germline RNAi. What are the genetic, molecular and cellular mechanisms that govern these differences? And how do they evolve when stabilizing selection ensures that phenotypic development remains stable and stereotyped?

Here we use single-molecule FISH to quantitatively measure the gene expression at specific locations and time points in early development. By characterizing the temporal and spatial heterogeneities of mRNA transcript numbers in the first few cell divisions, we can connect sub-cellular phenotypes to known variations in early embryonic pathway function and germline RNAi. We use a high-throughput, semi-automated pipeline to acquire precise transcript counts at precisely staged embryos, including implementation of the machine learning spot-counting software A2.

Despite near-invariant cell division phenotypes, wild isolates show significant differences in transcript abundance for critical embryonic genes. These differences in gene expression do not fully explain differences in embryonic lethality following gene knockdown, as neither wild-type gene expression nor transcript abundance following RNAi correlates perfectly with patterns of embryonic lethality. Notably, we observe significant difference in transcript abundance variance following RNAi among wild-type isolates, suggesting inefficiency of RNAi may be controlled by stochastic thresholds. Currently, we are scaling up the experiments using a microfluidic chip specifically designed for worm embryos in order to test hypotheses with high statistical rigor.

References:
2- Aro: a machine learning approach to identifying single molecules and estimating classification error in fluorescence microscopy images.

Natural variation underlying geomagnetic orientation in C. elegans.
B.L. Clites1, D.E. Cook2, B. Palacios1, E. Andersen2, J. Pierce1 1) Institute of Cellular and Molecular Biology, University of Texas at Austin, Austin, TX; 2) Department of Molecular Biosciences, Northwestern University, Evanston, IL.

Animals from diverse taxa use the Earth’s magnetic field to orient and migrate. Despite decades of study into magnetosensitive behaviors, the molecular and genetic mechanisms that contribute to magnetosensation have remained elusive. This is partially due to the dearth of easily manipulable model organisms that historically displayed magnetosensitive behaviors. We previously demonstrated that the C. elegans uses magnetic field information to orient burrowing up or downward or crawling to an artificial magnet in a Petri dish in a new behavior dubbed “magnetotaxis” (Vidal-Gadea et al., 2015). We also found that twelve strains isolated from different sites across Earth varied in their magnetotaxis in interesting ways. The worm’s magnetotaxis response varied with the magnetic field properties at the site from where the specific strain was isolated. For example, worms from Hawaii, where the magnetic field is weak, show a weaker magnetotaxis response than did worms from Bristol, where the magnetic field is stronger. The variation in behavior is stable across generations, suggesting a genetically encoded difference in behavior. The discovery of magnetosensitive behaviors in C. elegans opens up new and powerful avenues for finally elucidating the genetic and molecular bases of magnetosensation. Our lab, with help from the Andersen Lab at Northwestern University, is using complementary genetic approaches to uncover how this novel magnetotaxis behavior works in nematodes. First, we are testing the roles of candidate genes in magnetotaxis. Preliminary data suggest that pkc-1 plays a role in magnetotaxis. pkc-1 mutant worms show an increased magnetotaxis response compared to wild-type N2 animals. In parallel, we are pursuing a genome-wide association study as a complimentary approach to uncover quantitative trait loci responsible for variation in magnetotaxis across ~130 wild-type isolates that have already been sequenced by the Andersen group. We have expanded our set of measured magnetotaxis phenotypes to more than 50 strains from across the globe. We see that the strength of magnetotaxis positively correlates with the strength of the magnetic field at a strain’s site of isolation. Progress on these studies will be reported.

Exploring the genetic architecture of acute-intoxication in C. elegans.
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About 1 in 8 Americans will be afflicted with alcohol dependence at some point during their lifetime. The CDC estimates that in 2006, excessive alcohol consumption cost the US economy 223.5 billion dollars. These data clearly demonstrate that alcohol use disorders are a looming health problem with serious consequences.

Familial and twin-studies estimate the heritability of alcohol dependence to be around 50%, suggestive of a significant genetic contribution to the disorder. Understanding the genetic components underlying alcohol use disorders then, is instrumental in solving the problem. A spate of human genome wide association studies (GWAS) have identified risk loci associated with...
alcohol dependence, however, replication of has been difficult to achieve in most cases, excepting a small number of genes involved in alcohol metabolism. The failure to identify and replicate novel loci associated with alcohol use disorders is due, at least in part, to the extreme variance that exists in human populations.

To circumvent these limitations, we are using the model organism *C. elegans* to identify novel loci involved in ethanol response. Our lab, with the help of the Andersen Lab at Northwestern University, is conducting a GWAS study on acute intoxication, using about 130 *C. elegans* strains isolated from across the globe. Our unbiased quantitative genetic approach is likely to identify novel loci that have been difficult or impossible to identify in human quantitative genetic studies for the reasons mentioned above. Thus far, we have found that there is significant natural variation in acute intoxication across the panel of wild-type strains. Previous work from our lab has demonstrated a significant role of the BK-potassium channel in ethanol response (Davis et al., 2014). Concordant with those findings, certain strains harboring unique coding differences in slo-1, the gene which encodes the BK-potassium channel, display acute intoxication phenotypes that differ from the mean. These data, however, are still only correlative. Confusingly, other strains harboring early stop codons in slo-1 are still able to become intoxicated, suggesting major roles for genes other than slo-1 in the ethanol response of *C. elegans*. In the long term, our approach might also provide valuable insights into the evolution of ethanol response phenotypes in wild populations. We hope that by elucidating the genetic architecture of acute intoxication in *C. elegans*, we will discover novel risk factors that also underlie alcohol use disorders in human populations.

492B  Leveraging the power of *Caenorhabditis elegans* to dissect quantitative traits across a diverse collection of drug responses.  K.S. Evans1,2, S.C. Brady1,2, D.E. Cook1,2, E.C. Andersen1,2  1) Interdisciplinary Biological Sciences, Northwestern University, Evanston, IL 60208; 2) Department of Molecular Biosciences, Northwestern University, Evanston, IL 60208.

Within natural populations, most phenotypes are determined by the contributions of multiple genetic loci and/or interactions with the environment. Additionally, genetic factors can act independently and/or interact to control trait differences across populations. Studies of yeast and *Arabidopsis* found that the majority of phenotypic variation is attributable to independent additive quantitative trait loci (QTL). By contrast, studies of *Drosophila* and humans suggest that most traits are controlled by interacting genetic loci. Here, we leverage the genetic power of a panel of 265 recombinant inbred advanced intercross lines (RIAILs) of *Caenorhabditis elegans*, derived from the Bristol strain (N2) and a wild isolate from Hawaii (CB4856), to reconcile this debate in a highly powered statistical study. For this panel of strains, we use a high-throughput fitness assay to analyze strain responses to 16 different conditions, including heavy metals, antineoplastic drugs, pesticides, and neuroactive compounds. Using linkage mapping, we identify a diverse set of genomic regions that underlie differences in responses to these compounds. We will present the relative contributions of additive QTL and higher-order genetic interactions across various growth parameters in responses to these 16 drugs. Additionally, we identified four genomic regions that impact responses to multiple classes of compounds. We generated chromosome-substitution strains (CSSs) and near-isogenic lines (NILs) to experimentally validate three of these “QTL hotspots”. For many conditions, these strains recapitulate the phenotypic effect predicted using the recombinant inbred lines, validating the power of our efforts. Furthermore, the discovery of these QTL hotspots may be indicative of pleiotropic loci that control responses to multiple conditions.


Nematodes can be a good model for evolution of novel traits because they have extremely divergent phenotypes. We are interested in the question of how a novel trait emerges and evolves, which is one of the fundamental questions in evolutionary biology. To answer this question, we use a new species of nematodes, *A. freiburgensis*. This nematode shows nictation behavior, a dispersal behavior, and interestingly, it can use its own cuticle of the previous molt for tail support instead of fungi or any rough surface, which is the case of *C. elegans*. To understand how changes in the genome affect evolution of this new behavior, we are sequencing its genome and planning to perform forward and reverse genetics.

494A  The effect of the genetic background on seam cell patterning.  Sneha Latha Koneru, Michalis Barkoulas  Department of Life Sciences, Imperial College London, London, GB.

The seam cells are lateral epidermal cells that show intriguing stem cell-like properties. However, seam cell development has only been studied so far in the laboratory reference strain N2, thus it remains unclear whether what we currently know about seam cell patterning is indeed representative for the species. As a first step towards addressing this question, we have introgressed a seam cell marker (*wls51*) into divergent *C. elegans* isolates. We found that the terminal seam cell number is robust to standing genetic variation. It has been shown that cryptic genetic variation can be uncovered by sensitizing a developmental system by introducing mutations. We have used CRISPR/Cas9-mediated genome editing and genetic introgressions to generate *de novo* or transfer existing mutations in known seam cell gene network components into divergent *C. elegans* isolates. We found that mutations in *lin-22, eff-1* and *bro-1* did not show consistent differences in mutation expressivity in the different isolates tested. However, we discovered that both the expression of the GATA transcription factor *egl-18* in the seam and the expressivity of a null mutation in this factor vary significantly among isolates. We will discuss here these first experiments and our planned strategy to identify the genetic modifiers involved. Taken together, these results provide the first evidence linking the composition of the genetic background to seam cell patterning.
Dissecting genetic modifiers of mating behavior in *C. elegans*. T. Lemus Vergara\(^1\), L. Kruglyak\(^{1,2}\) 1) Human Genetics, University of California Los Angeles, Los Angeles, CA; 2) Howard Hughes Medical Institute.

Sexual reproduction shuffles genetic variation, maintains diversity, and removes deleterious alleles, facilitating adaptation to new environments or stress conditions. In strictly sexual species, mating is essential for reproduction and displaying the correct mating behavior is critical. However, in species where sexual reproduction is optional, the role of mating behavior is unknown. In these species, it is unclear what are the molecular pathways involved in the modulation and variation of mating behavior under normal and stress conditions, and whether the genes involved in mating behavior are subject to selective pressures. I aim to answer these questions by mapping genetic variants that influence mating rates in the facultative outcrossing nematode *C. elegans*.

To date, the majority of mating behavior studies in *C. elegans* focus on the function of specific genes in mating, leaving aside the genetic contributions from the rest of the genome. These studies are also center on male behaviors, such as mate searching, and male reproductive drive, disregarding hermaphrodite contributions. In addition, most of the research on mating behavior mechanisms has been done only in the laboratory strain. This is partly because the methods to measure mating behavior, and specifically mating rates, are labor-intensive and time-consuming. I am currently developing methods that allow the measurement of mating rates in multiple strains and large sample sizes in a short period of time. I will then perform a genome-wide scan for genetic variants that influence mating rates using mating protocols that incorporate the contributions of both hermaphrodites and males.

The variants identified will provide information about how genetic and environmental variation modifies mating behavior, which pathways are involved in the modulation of mating rates under control and stress conditions, whether there is a bias in sex-specific functions, and if genes involved in mating rates show signals of selection. In addition, genes harboring genetic variants can potentially be targets for the inhibition of mating behavior in related parasitic nematodes.

496C Polygenicity and epistasis underlie fitness-proximal traits in the *Caenorhabditis elegans* multiparental experimental evolution (CeMEE) panel. Luke Noble\(^1\), Ivo Chelo\(^2\), Thiago Guzzella\(^3\), Bruno Alfonso\(^3\), David Riccardi\(^1\), Patrick Ammerman\(^1\), Adel Dayarian\(^3\), Sara Cavalho\(^2\), Anna Crist\(^3\), Ania Pino-Querido\(^4\), Boris Shraiman\(^{4,5}\), Matthew Rockman\(^1\), Henrique Teotonio\(^3\) 1) Center for Genomics and Systems Biology, New York University, NY, USA; 2) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 3) Institut de Biologie, École Normale Supérieure, Paris, France; 4) Kavli Institute for Theoretical Physics, University of California, Santa Barbara, CA, USA; 5) Department of Physics, University of California, Santa Barbara, CA, USA.

Understanding the genetic basis of complex traits remains a major challenge in biology. Polygenicity, phenotypic plasticity and epistasis contribute to phenotypic variance in ways that are rarely clear. This uncertainty is problematic for estimating heritability, for predicting individual phenotypes from genomic data, and for parameterizing models of phenotypic evolution. Here we report a recombinant inbred line (RIL) quantitative trait locus (QTL) mapping panel for the hermaphroditic nematode *Caenorhabditis elegans*, the *C. elegans* multiparental experimental evolution (CeMEE) panel. The CeMEE panel, comprising 507 RILs, was created by hybridization of 16 wild isolates, experimental evolution at moderate population sizes for 140-190 discrete generations, and inbreeding by selfing for 13-16 generations. The panel contains around 22% of single nucleotide polymorphisms known to segregate in natural populations, and complements existing mapping resources for *C. elegans* by providing high nucleotide diversity across >95% of the genome, and fine-scale mapping resolution. We apply it to study the genetic basis of two fitness components, fertility and adult hermaphrodite body size, with high broad sense heritability in the CeMEE. While simulations show we should detect common alleles with additive effects as small as 5%, at gene-level resolution, the genetic architectures of these traits does not feature such alleles. We instead find that a significant fraction of trait variance, particularly for fertility, can be explained by sign epistasis with weak main effects. Multiple interactions involving *egl-18* (and potentially the functionally related flanking genes *egl-4* and *elt-6* as well) are highly influential for fertility, consistent with selection for embryo retention during experimental evolution. Phenotype prediction, while generally poor (\(r^2<10\%\)), requires modeling epistasis for optimal accuracy, with most variance attributed to the highly recombinant, rapidly evolving chromosome arms.

497A Microarray profiling of gene expression in Alpha-Synuclein aggregations and its alteration by natural genetic variation in *Caenorhabditis elegans*. Y.A. Wang\(^{1,2}\), B.L. Snoek\(^1\), M.G. Sterken\(^1\), J.A.G. Riksen\(^1\), D.E. Cook\(^2\), R.E. Tanny\(^3\), E.C. Andersen\(^3\), J.E. Kammenga\(^3\), S.C. Harvey\(^4\) 1) Laboratory of Nematology, Wageningen University, Wageningen, The Netherlands; 2) Biomolecular Research Group, School of Human & Life Sciences, Canterbury Christ Church University, Kent, United Kingdom; 3) Department of Molecular Biosciences, Northwestern University, Evanston, United States.

Neurodegenerative diseases (NGDs), such as Alzheimer’s diseases (AD) and Parkinson’s diseases (PD), are characterized by progressive degeneration in the human nervous system. The nematode *C. elegans* is an excellent model in which to study NGDs due to the high level of conservation of gene functions compared to humans. However, *C. elegans* research largely relies on a single worm genotype — the canonical N2 strain — limiting the ability to explore how naturally varying alleles alter pathological mechanisms in NGDs. In order to identify how genetic variation acts on NGDs, we analyzed transgenic animals that express aggregating human proteins associated with molecular pathogenic progression of NGDs in five genetic backgrounds.

Here, starting with the original transgenic strain expressing the human synaptic protein alpha-synuclein in an N2 genetic background, we have introgressed the PD transgene pkls2386 [unc-54p::alphasynuclein::YFP + unc-119(+)] into four different wild type genetic backgrounds. Analysis of these new transgenic introgressed lines indicates that transgene effects vary greatly depending on the genetic background. To understand the genetic bases of these phenotypic differences, we have sequenced...
these new lines to recognize confounder of the heterogeneity in transgenes, measured various aspects of the life history, and investigated gene expression differences by microarray. These analyses identified genes that are up- and down-regulated in all genotypes and genes that expressed at a specific stage to particular genetic backgrounds. For example, the differential developments of those lines have been also confirmed from microarray data that the gene vt-1 expressed at different levels between the lines. Functional enrichment links these genes to the aggregation of alpha-synuclein, which is causative of PD, to the associated developmental arrest, metabolic, and cellular repair mechanisms.

Our studies provide opportunities to observe alterations in traits, including global gene expression, associated with the toxicity of misfolded protein aggregation that could not be readily observed in the canonical N2 background. This is a necessary and important step to identify the alleles responsible for individual variation in the onset and progression of NGDs.

498B Genome-wide association study of natural variation in hitchhiking behavior of *C. elegans*. H. Yang¹, D. Lee¹,², H. Kim¹,², Y. Paik³, E. Andersen³, J. Lee¹ 1) Seoul National University, Seoul, Korea; 2) Yonsei University, Seoul, Korea; 3) Northwestern University, Evanston, Illinois, USA.

When *C. elegans* larvae encounter harsh conditions such as high temperature, starvation and high density, they develop into a survival stage called dauer. Nictation, a hitchhiking behavior, is a striking feature of dauers. Nictation is a three-dimensional standing and waving behavior, which helps dauers to get on to other animals such as isopods. By this hitchhiking behavior, dauers can escape from harsh conditions and move to other habitats. As wild *C. elegans* often meet bad conditions and are found most frequently as dauer larvae, nictation is thought to play an important role for their survival and evolution. Here, we try to identify genetic factors regulating this hitchhiking behavior, especially focusing on the worldwide natural variation. We measured nictation ratio of 137 single nucleotide variation(SNV)-genotyped wild isolates and identified the natural variation in nictation ratio. Next, we used genome-wide association(GWA) analysis to find specific genomic regions linked to nictation variation and found a significant peak-SNV in chromosome II. Strains that have alternative type SNV showed 1.7-fold higher nictation in comparison to reference(N2) SNV-typed strains. To find the causal genetic factor, we are currently narrowing down this quantitative trait locus(QTL) using near isogenic lines(NILs) made by crossing CB4856(reference SNV) and MY23(alternative SNV) strains. Our study will provide new insights on genetic basis and regulating mechanism of natural variation in the hitchhiking behavior.


Androdioecy—having male and hermaphrodite sexes within a population—is rare in animals but has evolved three times in *Caenorhabditis* nematodes. Outcrossing rate is a key trait in androdioecious species, with effects on the abundance and distribution of genetic variation. In the laboratory, outcrossing varies among isolates in each androdioecious species. To understand the genetic basis for this variation, we generated and sequenced the first panel of Recombinant Inbred Lines (RILs) and constructed the first genetic map for *C. tropicalis* and we used these resources to identify a region of the genome linked to outcrossing rate. We scored the RIL panel for hermaphrodite mating probability with common tester males. Quantitative trait locus analysis identified a region on the right arm of the X chromosome linked to the variation in hermaphrodite mating propensity. Further investigation of the parental strains reveals differences in attractiveness of the hermaphrodite pheromones and developmental time that may underlie the variation in outcrossing rate. Our results further illustrate that mating is a complex trait, attributed to a variety of factors. Our result highlight some of the potential contributions of hermaphrodites to the sex ratio in androdioecious species.

500A Natural variation in a single amino acid underlies cellular responses to topoisomerase II poisons. S. Zdraljevic¹,², C. Strand³, H.S. Siedel³, D.E. Cook¹,², J.G. Doench³, E.C. Andersen¹,²,⁵ 1) Interdisciplinary Biological Sciences Program, Northwestern University, Evanston, IL 60208, USA; 2) Department of Molecular Biosciences, Northwestern University, Evanston, IL 60208, USA; 3) Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA; 4) Biology Department, Eastern Michigan University, Ypsilanti, MI 48197, USA; 5) Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, IL 60611, USA.

Many medications, including chemotherapeutics, are differentially effective from one patient to the next. Understanding the causes of these population-wide differences is a critical step towards the development of personalized treatments and improvements to existing medications. Here, we investigate natural differences in sensitivity to anti-neoplastic drugs that target topoisomerase II, using *Caenorhabditis elegans*. We show that wild isolates of *C. elegans* vary in their sensitivity to these drugs, and we use an unbiased statistical and molecular genetics approach to demonstrate that this variation is explained by a methionine-to-glutamine substitution in topoisomerase II (TOP-2). Using molecular dynamic simulations, we show that the presence of a non-polar methionine at this residue increases hydrophobic interactions between TOP-2 and the poison etoposide, as compared to a polar glutamine. We hypothesize that this stabilizing interaction results in increased genomic instability in strains that contain a methionine residue. The residue affected by this substitution is conserved from yeast to humans and is one of the few differences between the two human topoisomerase II isoforms (methionine in hTOPIIα and glutamine in hTOPIIß). We go on to show that this substitution influences binding and cytotoxicity of etoposide and two additional topoisomerase II poisons in human cell lines. Evidence suggests that hTOPIIß poisoning by these drugs contributes to various side effects associated with therapeutic treatment regimens including the formation of secondary malignancies and cardiotoxicity. Our results demonstrate the utility of using natural genetic variation in *C. elegans* to understand the genetics of drug responses and inform the development of drugs that target the tumor-specific hTOPIIα enzyme.
found that mutations in plates seeded with bacteria for ~11 years while the LSJ2 was cultured in axenic liquid media ~50 years. We have previously strains, N2 and LSJ2, share a common ancestor following its isolation from the wild in 1951. The N2 strain was cultured on agar Many biological traits have a significant but complex genetic basis. Identification of causal genetic variants leads to a better understanding regarding the mechanisms of biological trait differences. As a model for complex traits, we are studying an unintentional metazoan experimental evolution experiment performed in Caenorhabditis elegans to identify genetic determinants underlying response to a broadly administered class of anti-neoplastic compounds that poison the activity of topoisomerase II (TOP-2) enzymes. We identified numerous quantitative trait loci (QTL) that explain phenotypic variation among a panel of recombinant inbred advanced intercross lines generated between the Bristol and Hawaiian strains in response to amsacrine, dactinomycin, and etoposide. Interestingly, multiple overlapping QTL located on the center of chromosome V explain phenotypic variation in response to these three poisons, suggesting a common mechanism underlying the responses to these compounds. We constructed reciprocal Bristol and Hawaiian near-isogenic lines (NILs) that encompass all six chrV QTL. Interestingly, these NILs only recapitulated the etoposide and dactinomycin QTL effects, but failed to recapitulate the amsacrine QTL effects. We used these NILs to generate 80 recombinant NILs with smaller introgressed regions to separate a possible amsacrine-specific multi-locus interaction within the NIL region and to narrow the QTL confidence intervals associated with responses to all three drugs. Phenotypic analysis of these sub-interval NILs isolated the two etoposide and dactinomycin-specific QTL within the original NIL region and uncoupled an amsacrine-specific incompatibility locus from an amsacrine-specific QTL. These results highlight the complexity of genetic factors that influence metazoan responses to anti-neoplastic compounds.

502C Quantitative fitness analysis of two laboratory strains of C. elegans. Yuehui Zhao, Richard Campbell, Patrick McGrath School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA.

Many biological traits have a significant but complex genetic basis. Identification of causal genetic variants leads to a better understanding regarding the mechanisms of biological trait differences. As a model for complex traits, we are studying an unintentional metazoan experimental evolution experiment performed in C. elegans. Two laboratory domesticated C. elegans strains, N2 and LSJ2, share a common ancestor following its isolation from the wild in 1951. The N2 strain was cultured on agar plates seeded with bacteria for ~11 years while the LSJ2 was cultured in axenic liquid media ~50 years. We have previously found that mutations in npr-1 and glb-5 were fixed in the N2 strain, resulting in the suppression of aggregation and bordering behavior to avoid high O2 levels on agar plate. Using a digital PCR-based competition assay, we quantify the relative fitness effects of npr-1 and glb-5 ancestry/derived alleles. We find epistatic interaction between these two derived alleles: while the npr-1 derived allele shows fitness advantages in agar plate conditions regardless of genetic background, the glb-5 derived allele enhances the npr-1 fitness effect only in the derived npr-1 background. Unexpectedly, the fitness benefits of these two alleles seems to occur independently of their effects on behavior as the N2 strain showed increased fitness at 3% and 10% O2 levels where aggregation and bordering is suppressed. To systematically study the genetic G x G and G x E in these two strains, we performed fitness QTL mapping using inbred lines (RILs) from N2 x LSJ2 on agar plates under two culture conditions: non-starving and starving. We found one QTL with a strong effect on fitness centered over the chromatin remodeling factor nurf-1. Additionally, we found two QTLs showing a G x E interaction effect. Interestingly, despite lacking the derived alleles of npr-1 or glb-5, we found one of the RILs had a high relative fitness equal to the N2 strain. This RIL strain showed changes to bordering and aggregation behaviors distinguishable from the N2 strain. The potential implications for this high fitness outlier could be due to the fixation of a de novo mutation or higher ordered epistasis of specific combinations of N2 and LSJ2 alleles. We will report the result of our mapping experiments to distinguish between these two possibilities. This study implies experimental evolution approach could be a good method to understand metazoan complex traits and evolution.

Ecology and Evolution - Experimental Evolution and Ecology

503A Quantifying paternal mitochondrial DNA transmission in Caenorhabditis briggsae hybrids. S. adineh, J Ross Biology, California State University of Fresno, Fresno, CA.

In most species, mitochondria are maternally inherited. Work in Caenorhabditis elegans has shown that a cellular surveillance system eliminates sperm-borne mitochondria that enter an oocyte. Such a system might have evolved to prevent heteroplasmy (the presence of multiple mitochondrial genotypes in a cell), which can result in mitochondrial disorders. However, in C. briggsae hybrids, repeatedly backcrossing female hybrids to parental males has been suggested to result in paternal mitochondrial transmission. Observations in other taxa have also shown that hybridization increases paternal mitochondrial transmission, raising the hypothesis that hybrid genotypes cause the surveillance system to malfunction and thus facilitate the increase in paternal transmission. This hybrid dysfunction might occur because of recombination of unknown strain-specific molecular signals of paternal mitochondria and their unknown maternal receptor molecules. My objective was to compare the frequencies of paternal transmission in C. briggsae hybrid and control crosses. In twelve novel hybrid lines, all showed preliminary evidence of paternal mitochondrial DNA, although no firm conclusion can be reached as to whether this occurred via paternal mitochondrial transmission. Future work will be needed to unequivocally demonstrate whether paternal mitochondrial transmission occurs, and then to identify the genetic architecture of the quality control system responsible for paternal mitochondrial elimination.
504B  Genomic analysis of chronic heat stress resistance in the nematode *Caenorhabditis remanei*.  Christine O’Connor, Sally Claridge, Patrick Phillips  Institute of Ecology and Evolution, University of Oregon, Eugene, OR.

Selection can drive sub-populations to become differentiated, both in phenotype and genotype, but gene flow between them can affect the evolutionary trajectories of the phenotypes in question, potentially by mitigating the effects of selection and slowing the rate of adaptation to novel environments. Migration-selection dynamics are one of the fundamental aspects of speciation and population divergence, but they have not been rigorously investigated in an experimental context. Stress resistance is a heritable complex trait involving interactions between numerous genes and pathways. Previous studies in *Caenorhabditis elegans* have identified correlations between single genes and stress responses, but few have investigated genome-wide, causative relationships. Multiple questions remain in understanding how complex, polygenic traits evolve on the whole-genome scale under the opposing influences of strong selection and gene flow. The goals of this project are to dissect the genetic basis of chronic heat stress, a model complex trait, in *C. remanei*; to elucidate how gene flow affects rates of adaptation to a novel environment; and to investigate migration-selection dynamics.

We utilized an evolve-and-resequence framework, which allowed for selection on standing genetic variation in the ancestral strain of *C. remanei* that was derived from a wild isolate and lab-adapted for 75 generations. Populations of *C. remanei* derived from this ancestral population were evolved in pairs to either a control (20°C) or heat stress (31°C) environment for forty generations. We tested the effect of migration between sub-populations with three migration rates: 0 (no migration), 5, and 20 percent. Female fecundity was measured to estimate the strength of selection in the heat stress environment. We observe a significant effect of selection and a significant interaction between selection and migration.

The no-migration 31°C-evolved, 5-percent migration 31°C-evolved, and ancestor populations were sequenced via whole genome pooled population sequencing. The ancestral and descendant populations were compared on a locus-by-locus basis, allowing us to identify the number and location of putative loci under selection in the heat stress environment. We find that the 5-percent 31°C-evolved populations show fewer divergent regions than the no migration 31°C-evolved populations, as expected due to the homogenizing effects of migration. Preliminary analysis shows that there are more sites with significant divergence in the no migration populations.

505C  An investigation of the effects of artificial light at night on *C. elegans* offspring production and lifespan.  Hardik Naryya, Rosa Zhushma, Sharon Wise, Bryant Buchanan, Jessica Shinn-Thomas  Utica College, Utica, NY.

Artificial light at night (ALAN) has many broad-scale and global implications for ecosystems and wildlife that have evolved under a 24-h circadian cycle. With increased urbanization, artificial light at night has directly altered natural photoperiods and nocturnal light intensity. Artificial light at night can disrupt behavioral patterns such as foraging activity and mating in animals. Disturbances in natural light and dark cycles also affect melatonin-regulated circadian and seasonal rhythms in *Drosophila*. We investigated the impact of ecologically relevant levels of light pollution on an important invertebrate model, *Caenorhabditis elegans*, as the impact of night lighting at these light levels is currently unknown. In this study, we exposed worms to artificial light at four intensities: $10^{-4}$ lx (control, comparable to natural nocturnal darkness), $10^{-2}$ lx (comparable to full-moon lighting and a low level of light pollution), 1 lx (comparable to dawn/dusk or intense light pollution), and 100 lx (dim daylight level comparable to extreme light pollution) on a 12L:12D photoperiod (100 lx treatments experienced constant light). We measured the impact of these light treatments on offspring production in hermaphroditic *C. elegans*. We grew worms for 2 generations in each light treatment, and then recorded the lifespan and counted the number of hatched offspring produced in the F3 generation. Our data show no significant differences among light levels for lifespan or offspring production suggesting that at least for these life history traits, ALAN does not affect these soil nematodes. Future directions include measuring additional life history traits and circadian gene expression for worms exposed to ALAN.

506A  Mechanisms and evolution of agrochemical resistance-associated natural variation in wild populations of *C. elegans*.  Liisa Parts, Anthony Flemming, Alison Woollard  1) Department of Biochemistry, University of Oxford, Oxford, Oxfordshire, GB; 2) Syngenta, Jealott’s Hill International Research Centre, Bracknell, Berkshire, GB.

Resistance to pesticides is a global food security problem and a rising issue for the agrochemical sector, analogous to the global health concerns caused by widespread antibacterial resistance. A number of compound families, both wide spectrum pesticides and more targeted nematicides, are available for agricultural use to combat the damage caused by pests that results in about 15% of global crop loss annually. Most of these compounds were introduced decades ago and reports of resistance exist for each class, yet little is known about the molecular mechanism and evolutionary biology of resistance. Sometimes resistance takes decades to evolve, sometimes just a few years and some species evolve resistance more commonly than others.

We are evaluating the use of *C. elegans* as a model for understanding the mechanisms and evolution of pesticide resistance. Selection for resistance will act on natural variation in susceptibility in wild populations and such variation has been observed in *C. elegans*: for example the Hawaiian strain CB4856[1] is resistant to the nematicide avermectin through variation at the target protein. We have assessed natural variation in pesticide resistance by investigating the development of 25 highly divergent *C. elegans* wild isolates upon exposure to 29 bioactive insecticides, fungicides and nematicides and demonstrated both increased sensitivity as well as increased resistance to particular chemicals in different wild strains. We plan to identify the genetic basis of particular variation in resistance as well as to model the emergence of agrochemical resistance in an experimental evolution approach.

References:

Aging research primarily concentrates on how individuals age in order to develop strategies to prolong life or retard age-related changes. Another dimension of aging that has received less attention is the effects of individual aging on whole populations and the emergent property of population dynamics. In this context, aging is one life-history trait and has the potential to affect the number of individuals and the age-structure in a population.

To analyze the role of aging in the context of population dynamics, we developed an artificial ecosystem with the nematode C. elegans so that we can directly measure population dynamics. Worms are cultured in liquid medium with a controlled nutrient influx of E. coli, a predefined predation-rate, and automated worm counters are used to monitor population size. In parallel we developed a computational simulation that mirrors the laboratory ecosystem and allows us to systematically analyze the relationship of aging and other life history traits on population dynamics in high throughput.

Using this approach, we observed that wild type populations grew to a maximum population size of 1 million animals and declined rapidly to 300,000 animals in the first month. Finally, the population recovered and fluctuated constantly between 300,000 and 600,000 animals over the next 2 months. The first high inflection point followed by a crash of the population is typical for starting populations and is often observed during repopulations in the wild. In the starting population the number of animals is low and, therefore, food or prey accumulates until the population reaches a maximum. Food or prey is now the limiting capacity and the population decreases until it reaches an equilibrium between size of the population and availability of food. While this population is stable, the number of animals fluctuates over time. The specific population dynamics can be characterized by measuring the mean number of animals, the average minimum and maximum, the time between population peaks, and thence by calculating the amplitude and period of the fluctuations.

Next, we are exploring the population dynamics of long- and short-lived mutants in the laboratory and with the computational simulation to systematically analyze how aging in combination with other life history traits such as reproduction, metabolism, or dauer formation affects the stability of populations. We compare the respective population dynamic characteristics to wild type worm populations and estimate the relative risk of each population to become extinct. The presented data will address the impact of aging in the context of systems biology.

508C An investigation of nontarget insecticide effects of erythritol on C. elegans and two common agricultural crop plants. Bilal Hasanspahic, Edin Zvornicanin, Jasmina Samardzic, Andrew Rahme, Sara Scanga, Jessica Shinn-Thomas Utica College, Utica, NY.

Sugar substitutes, such as Truvia®, are increasingly gaining popularity in cooking and processed foods. Erythritol, a non-nutritive polyol, is the main component of Truvia. Previous studies showed that erythritol consumption does not affect plasma and urine osmolarity and electrolyte levels and it is not toxic or carcinogenic to rats. In contrast, erythritol was shown to significantly decrease longevity in Drosophila melanogaster compared to sucrose and other polyols with the exception of D-mannitol. Researchers have suggested that erythritol has potential use as an insecticide given its apparent safety for mammals but harmful effects on arthropods such as Drosophila. However, for erythritol to have practical use as an environmentally-friendly insecticide in agricultural settings, it must have neutral to positive effects on crop plants and other nontarget organisms, including soil and compost-dwelling animals such as C. elegans. The purpose of our research is to examine the dose-dependent, nontarget effects of erythritol on C. elegans lifespan and larval survival, and tomato (Solanum lycopersicum) and corn (Zea mays) germination and growth.

509A Transcriptomic analysis of the interaction between Caenorhabditis elegans and microbiome members. W. Yang, K. Dierking, H. Schulenburg Evolutionary Ecology and Genetics, Zoological Institute, Kiel, SH, DE.

The nematode C. elegans is continuously exposed to microbes in nature. Therefore, the associated microbial community has shaped the evolution of C. elegans life-history and its consideration is thus important for in-depth understanding of the nematode’s biology. Here we report of a high-throughput transcriptomics analysis across different life stages to explore the characteristics underlying the interaction between C. elegans and members of its natural microbiome. The tested microbiome members induce dramatic differences on gene expression compared to the lab food of E. coli OP50. Meta-analysis from our taxon-specific tool—WormExp suggests that multiple biological processes are influenced by the microbiome members, including as examples: 1) regulation of larval development; 2) stress resistance and longevity during adulthood; and 3) a diet response mediated by an AMPK pathway. A fine-scale analysis of the first point suggests that the microbiome members affect tissue specific development of C. elegans, including sexual differentiation, nervous system development and reproduction. Our findings provide a new perspective on C. elegans life history as shaped by its natural microbiome.

Ecology and Evolution - Phylogeny and Diversification

510B Nuclear fossils of Wolbachia reveal the complex history of nematode-Wolbachia evolution. M.L. Blaxter, D.R. Laetsch, G. Koutsovoulos Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, GB.

Much of the natural world exists in symbiosis. Wolbachia are common, intracellular, maternally transmitted, parasitic symbionts of terrestrial arthropods, causing a variety of reproductive manipulations to assure their own survival. Arthropod Wolbachia can also be beneficial, protecting their hosts from viral and bacterial infections. Filarial nematodes in the Onchocercidae (including human parasites Brugia malayi, Wuchereria bancrofti and Onchocerca volvulus) also carry Wolbachia symbionts, and these are
targets for anti-filarial chemotherapeutic interventions. While it was initially believed that onchocercid Wolbachia had a single origin and diversified with their nematode hosts, it is now becoming clear that the history of association between filarial nematodes and their symbionts is more complex. The symbionts of Onchocercidae derive from four Wolbachia supergroups (C, D F and J). Using 15 whole genome sequences of filarial nematodes and their C and D strain Wolbachia, we explored the evolutionary history of this symbiosis. Nematode phylogeny based on nuclear genes places Setaria labiopapillosa basal to other sequenced species. Within the other Onchocercidae, four species (Onchocerca flexuosa, Acanthochelionema viteae, Loa loa and Eleaphora elaphi) must have independently lost their infections. We screened the nematode genome sequences for nuclear Wolbachia transfers (NUWTs) – fragments of Wolbachia genomes integrated into the nuclear genome. S. labiopapillosa had no NUWTs and is therefore primitively uninfected. NUWTs in O. flexuosa, A. viteae, L. loa and E. elaphi confirm these species as being aposymbiotic. We identified the supergroup membership of the Wolbachias from which the NUWTs originated, and found that aposymbiotic species carried NUWTs derived from C type genomes, even if their most closely related symbiotic species carried D Wolbachia. Thus the history of Wolbachia in onchocercid nematodes includes not only cospeciation (as observed in the Onchocerca- Dirofilaria group in association with group C Wolbachia) and loss (in the aposymbiotic species), but also symbiont replacement. The supergroup D lineage of Wolbachia, found in the human parasites W. bancrofti and B. malayi, derives from a replacement event. We suggest that the biology of symbiosis may differ strongly in stably symbiotic species compared to those in which symbiont replacement has occurred.

511C What is different about the cells comprising the C. elegans stoma and pharynx? A.H.Jay Burr 1,2 1) Retired: Biological Sciences, Simon Fraser University, Burnaby, BC, CA; 2) Visiting Scientist: Nematology, University of California, Riverside, California.

In a recent article it was shown that the architecture of the tube of epithelial cells that comprise the stoma and pharynx of C. elegans is basically the same as for other nematodes: the sequential order and characteristics of the cell classes are conserved (Burr and Baldwin 2016). The row of radial cell classes pm1–pm5 are paired within each interradial sector and in this way are distinct from the single, unpaired radial cells e1, e3, pm6 and pm7. Immunofluorescence labeling (MH27) showed that marginal cells e2 and mc1 are not separated by the encircling pm1 syncytium, but share a common circumferential apical junction. Here I will add TEM evidence that the encircling pm1 cell syncytium passes peripheral to the adjoining e2 and mc1. Marginal cells e2, mc1, mc2 and mc3 form a continuous row and border with the same classes of radial cells in most nematodes.

In this paper I will compare certain features of the pharyngeal cells in C. elegans and closely related taxa that are different from other nematodes. 1) The unpaired radial cells e1 and e3 are not muscles in C. elegans and other taxa within Rhabditina, but do express muscle cytoskeleton in most other nematode taxa. To provide a terminology consistent across Nematoda, the pharyngeal ‘epithelial’ and ‘muscle’ cells of the C. elegans pharynx would better be distinguished by topological terms such as ‘unpaired radial’ cells (e1, e3, pm6–7) or ‘paired radial’ cells (pm1–5), and ‘marginal’ cells (e2, mc1–3), rather than by the confusing functional terms. 2) In C. elegans the paired radial cells of the pharynx become fused shortly after hatching or molting - a segment of the apposed plasma membranes that normally separate the pairs is dissolved (Shemer et al. 2004). However the muscle cytoskeleton remains paired. Fusion of these cells occurs only in two clades within the subborder Rhabditina: in Ehrhabditis, which includes C. elegans, and in a clade within Diplolastomorpha. 3) In C. elegans the six pm1 cells are fused within and between sectors to form a syncytium that encircles the stoma (Albertson and Thomson, 1976). Circumferentially fused radial cells have rarely been reported in other taxa.

An interesting question: What are the functional advantages of these special features?


512A Evolutionary patterns and developmental consequences of a GATA-type transcription factor radiation within the Caenorhabditis genus. A. Darragh, S.A. Rifkin University of California, San Diego, San Diego, CA.

GATA factors are a family of zinc finger transcription factors characterized by their GATA-binding domain and involved in essential and conserved developmental processes. Phylogenetic analyses predict a bilaterian ancestor with two GATA factors suggesting prevalent gene duplications and losses leading to the divergent number of GATA factors found in present day organisms. Outside of the nematodes, the maximum number of annotated GATA factors in a diploid animal species is six and their evolutionary history has been worked out to some degree. Caenorhabditis elegans has eleven GATA factors whose phylogenetic relationships remain unresolved. We are investigating the evolutionary history of Caenorhabditis GATA factors to model their functional evolution in hopes to elucidate constrains on protein family evolution. We searched the genomes of twenty Caenorhabditis species and four outgroup nematodes from Wormbase and the Caenorhabditis Genomes Project for proteins matching GATA domain profiles from PROSITE. Using Bayesian and maximum likelihood analyses, we estimated the evolutionary history of these GATA factors. We found that some subclades of the gene tree exhibit a very high turnover rate with rapid duplication and deletion. Moreover, the GATA factors have radiated within the Elegans supergroup. For example, the core of the endodermal cell specification network – med-1, med-2, end-3, end-1, and elt-7 – are unique to the Elegans supergroup, suggesting that this expansion engendered a rewiring of Caenorhabditis development. To gain functional insights into the evolution of these proteins we are quantifying their embryonic expression patterns in non-Elegans supergroup species using single molecule fluorescent in situ hybridization and are testing whether there is evidence that selection on the DNA binding domains accompanied the expansion.
513B Tempo, mode, and fitness effects of mutation in Caenorhabditis elegans over 400 generations of minimal selection. Ayush Saxena1, Matthew Salomon1,2, Chikako Matsuba1,2, Shu-Dan Yeh1,3, Charles Baer1 1) Dept Biol, University of Florida, Gainesville, FL; 2) John Wayne Cancer Inst., Santa Monica, CA; 3) National Central University, Taiwan.

The rate and molecular spectrum of mutations varies within and among genomes, species and higher taxa. The understanding of the mechanistic causes of variation is far from complete; the understanding of evolutionary causes is even more rudimentary. It has been posited that individuals in poor physiological condition will experience higher rates of mutation than those in good condition. Since deleterious mutations lead to poor condition, the possibility exists that individuals carrying a high mutation load will experience an elevated mutation rate. We tested that hypothesis with a set of "second-order mutation accumulation" (O2MA) lines of the nematode C. elegans. MA lines that had accumulated mutations under minimal selection for ∼250 generations ("first-order MA lines", O1MA) were sorted into high-fitness and low-fitness groups, replicated into new sets of O2MA lines, and allowed to accumulate mutations for another ∼150 generations of minimal selection. Whole-genome sequencing of 48 O2MA lines and their O1MA ancestors revealed significant variation in base-substitution rate and in total mutation rate both among the O1MA lines measured at generation 250 and in the O2MA lines measured at G400. However, O2MA rate does not depend on ancestral fitness, nor does the specific O1MA ancestry explain a significant fraction of the variance among O2MA lines. Thus, the signal of variation in mutation rate decays on the order of a hundred generations.

Multiple logistic regression of mutability on a set of predictor variables revealed that local three-base nucleotide context is the most important predictor of mutability, but that GC content of the 1 Kb surrounding a site and – importantly – local recombination rate are also significant predictors. Mutability explains a large fraction of the variance in standing nucleotide diversity. The deletion rate of low-fitness O2MA lines is less than that of high-fitness O2 lines. However, low-fitness O1MA lines carry more indels of putatively large effect than do high-fitness O1MA lines, whereas low-fitness O2MA lines carry fewer indels of putatively large effect than the high-fitness O2MA lines. Consistent with those findings, the mean mutational effect on experimentally-measured fitness is twice as great in the low-fitness O2MA lines as in the high-fitness lines. Taken together, these results strongly imply that, on average, epistasis is synergistic among new deleterious mutations.

514C Wild-isolates of C. elegans exhibit variable attraction to the sex-pheromone mimicry compound produced by the nematode-trapping fungus A. oligospora. Y. Chiang, Y. Hsueh Institute of Molecular Biology, Academia Sinica, Taipei, TW.

The nematode-trapping fungus A. oligospora produces odors that mimic sex and food cues to attract many nematode species (Hsueh et al. 2017). One of the compounds (MMB) that likely mimics male pheromone in Canoeabiditis nematodes is highly attractive to two lab strains of C. elegans (N2 and Hawaiian). To investigate whether this attraction is highly conserved among the wild-isolates, we analyzed the MMB chemotaxis behavior among the C. elegans wild isolates from the CeNDR collection. We found that MMB-attraction is highly polymorphic trait. Six out of forty wild-isolates tested exhibited weak attraction to MMB. Genetic analysis showed that this trait is controlled by QTL in some strains but a single locus in others. The high incidence of MMB-insensitive strains among the wild-isolates suggests that lost of MMB attraction might offer benefit to C. elegans in the natural environments.


A functional metabolic network is key for an organism’s survival, and thus for its fitness. Spontaneous mutations reduce fitness, so it stands to reason they affect metabolism in some way. However, not all metabolites are equally important in maintaining organisinal function, and the relationship between mutation and an organism’s metabolic network has rarely been investigated. By investigating a set of mutation accumulation(MA) lines of Caenorhabditis elegans, we aim to better understand the effect mutations have on the metabolic network. Using a set of 43 MA lines and their ancestor, a pool of 29 metabolites was measured for changes in mean metabolite concentration (M) and mutational variance (VM). We constructed a metabolic network map of these metabolites for C. elegans from the KEGG database, with the aim of characterizing the relationship between VM, M, and heritability found for these 29 metabolites and features of the metabolic network. Initial analysis shows a positive association between the largest k-core grouping of a metabolite and M and VM.

516B Let nature do the screen for you: Naturally occurring null and suppressor mutations in C. elegans wild isolates. D. Leighton1, J. Bloom1, L. Kruglyak1,2 1) Human Genetics, UCLA, Los Angeles, CA; 2) Howard Hughes Medical Institute.

A common method of discovering geneting interactions is to perform a mutant screen on a strain that already carries a strongly phenotypic mutation, in order to identify suppressors of that phenotype. We reasoned that deleterious mutations and their suppressors may appear naturally in wild populations of Caenorhabditis elegans, and could be identified by the existence of a wild isolate that bears a putative null mutation but not its expected phenotype. To this end, we searched WormBase for potential null mutations in sequenced wild isolates representing 90 C. elegans isotypes. Over 4000 potential null mutations were identified in over 2800 genes, including 130 genes whose nulls are expected to produce visible phenotypes. After applying quality control tests to enrich our dataset for those mutations most likely to be true nulls, we chose to focus on natural mutations in the essential genes abu-14, del-1 and elt-2. In addition to searching for naturally occuring null and suppressor mutations, we
have also developed tools to automate the search and validation of putative null mutations in wild genomes. It is our goal to publish a catalogue of such mutations that can be quickly updated as new genomes are published. This could prove a valuable resource to any researcher whose favorite gene is listed, as they need only order the strain, and confirm the absence of a null phenotype, to begin mapping suppressors.

Ecology and Evolution - Speciation

517C Genome wide screening of hybrid incompatibility identifies multiple \textit{C. briggsae} loci that rescue male sterility in F1 hybrid between \textit{C. briggsae} and \textit{C. nigoni}. Y. Bi, X. Ren, R. Li, Q. Ding, Z. Zhao  Biology, Hong Kong Baptist University, Hong Kong, HK.

Hybrid incompatibility (HI) between related species plays a key role in speciation. The genetic basis of HI has been intensively studied in yeast, \textit{Drosophila}, and mouse species, but remains largely unexplored in nematodes until recent isolation of a \textit{C. briggsae} sister species, \textit{C. nigoni}, which can mate with each other and produce a few viable progeny. We have recently generated a genome-wide HI map between the two species by phenotyping 111 hybrid strains, each carrying a defined introgression fragment derived from GFP-labeled \textit{C. briggsae} chromosome. Notably, the HI phenotypes were scored essentially in the \textit{C. nigoni} background produced by GFP-aided backcrossing for at least 15 generations. However, many HI phenotypes were manifested in the F1 generation, often in a crossing direction-dependent manner. Unfortunately, few HI loci have been mapped in F1 hybrid between the two species. We address this issue using the large collection of introgression strains. This is because the crossings between individual \textit{C. nigoni} strains carrying a defined \textit{C. briggsae} fragment and \textit{C. briggsae} wild isolate AF16 will produce selective homozygosity or hemizygosity of the \textit{C. briggsae} genomic fragment in the F1 hybrid, whereas the remaining genomic contents would be comparable to those of the F1 hybrids between the wild isolates of the two species. Contrasting the hybrid F1 phenotypes in the crossings of AF16 with \textit{C. nigoni} wild isolate or with an introgression strain permits isolation of HI loci in the F1 generation. We perform the crossings in both directions between AF16 and the introgression strains that are prioritized based on the even distribution of introgressions over the \textit{C. briggsae} genome. Altogether, the introgression fragments cover approximately 80% of the \textit{C. briggsae} genome. Surprisingly, we identified numerous \textit{C. briggsae} loci that produced an opposite HI phenotype in F1 hybrid compared to that in the introgression lines, including rescue of male sterility and killing of female, which is consistent with the BDM model of speciation. In addition, some autosomal HI loci demonstrate crossing direction-dependent HI phenotypes, for example, selective male killing or global killing of all progeny, while others show crossing direction-independent killing of all progeny. In summary, we are able to identify numerous \textit{C. briggsae} loci that produce crossing direction-dependent or independent incompatible phenotypes in F1 hybrids, which provide a foundation for molecular characterization of HI between the two nematodes.

518A Mapping of a Hybrid Lethal Gene of the X Chromosome of \textit{Caenorhabditis briggsae}. Blaime Bittorf, Rachel Slater, John Dougherty, Scott Baird  Biological Sciences, Wright State University, Dayton, OH.

In the cross of \textit{C. nigoni} males to \textit{C. briggsae} hermaphrodites, all F1 males arrest during embryogenesis. In the reciprocal cross there are some viable F1 male progeny. This unidirectional male-specific lethality in the F1 hybrids has been attributed to a hybrid lethal gene in a 500 Kb region of the X chromosome of \textit{C. briggsae}. \textit{Cbr-him-8} is a recessive maternal-effect suppressor of the male-specific lethal phenotype. \textit{Cbr-him-8} is required for X chromosome pairing. Without proper pairing, the X chromosomes are expected to be transcriptionally silenced (meiotic silencing of unpaired chromosomes, MSUC). It has been proposed that MSUC-based silencing of the X-linked hybrid lethal gene is the mechanism by which the male-specific lethality is suppressed. Based on this model, a co-suppression assay was used to map the hybrid lethal gene. Transgenic strains were constructed via microinjection of bacterial artificial chromosomes (BACs). The BACs were mixed with pCFJ909, a plasmid containing a functional \textit{cbr-unc-119} gene. This mixture was microinjected into the gonad of \textit{cbr-unc-119} mutant hermaphrodites. Transgenic offspring were selected based on the rescue of the \textit{cbr-unc-119} phenotype. \textit{C. nigoni} males were mated to transgenic hermaphrodites. These crosses were scored for viable F1 male progeny. A single BAC has been identified that rescues the male-specific hybrid lethal phenotype. Multiple other BACs have failed to rescue, including two overlap the rescuing BAC. The non-overlapping region of the rescuing BAC is 71.1 kb long. The corresponding region of the \textit{C. briggsae} X chromosome contains five gene predictions. Co-suppression assays will continue until a single gene can be definitively identified as the male-specific hybrid lethal gene.

519B Cytoplasmic-nuclear incompatibility in \textit{Caenorhabditis nouraguensis}. P. Lamelza\textsuperscript{1,2}, J. Young\textsuperscript{3}, H. Malik\textsuperscript{1,3}, M. Ailion\textsuperscript{1,2} 1) Molecular and Cellular Biology Program, University of Washington, Seattle, WA; 2) Department of Biochemistry, University of Washington, Seattle, WA; 3) Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA.

Understanding the cause of reproductive barriers that demarcate species (e.g. hybrid lethality and sterility) is essential for understanding the process of speciation. We have discovered a genetic incompatibility between two wild-isolates (JU1825 and NIC59) of \textit{Caenorhabditis nouraguensis} that results in F2 embryonic and larval lethality. Inviability seems to be the result of a maternally inherited cytoplasmic factor from each strain being incompatible with recessive nuclear loci from the other. Furthermore, cytoplasmic-nuclear incompatibility commonly occurs between other wild isolates, indicating that this is a significant reproductive barrier within \textit{C. nouraguensis}. We hypothesize that the maternally inherited factor is the mitochondrial genome and that mitochondrial dysfunction underlies hybrid death. Multi-generation backcross experiments suggest that JU1825 mtDNA is heteroplasmic for both JU1825-like and NIC59-like incompatibility loci.

We have generated genome assemblies of NIC59, JU1825 and JU2079 (an inbred strain derived from JU1825), and have mapped the nuclear incompatibility loci to single chromosomes by bulk sequencing of viable F2 hybrids. The nuclear incompatibility loci map to different chromosomes in reciprocal crosses, indicating genetically distinct cytoplasmic-nuclear
incompatibilities. We have more finely mapped one of the nuclear incompatibility loci by generating recombinant introgression lines, which we are currently sequencing.

**Gene Regulation and Genomics - Databases and Programs**

520C  **WormBase ParaSite: a portal for helminth genomics.** Bruce J. Bolt, The WormBase Consortium EMBL European Bioinformatics Institute, Wellcome Genome Campus, Cambridge, CB10 1SD, United Kingdom.

In recent years, the amount of publicly available genomic and transcriptomic sequence for parasitic helminths has vastly increased, partly due to a number of large-scale sequencing projects. To address the needs of the parasitology community and the challenges involved in the organisation and display of this data, we have developed WormBase ParaSite (http://parasite.wormbase.org) as a sub-portal of WormBase for helminth genomics. Since the first release in 2014, the database and website has undergone a period of rapid growth and development to become the home for the genomes of more than one hundred nematode and platyhelminth species. All genomes undergo automatic functional annotation and are made available through a web interface offering a number of tools including genome browsers, sequence search, a data export tool, variant effect predictor, and programmatic access.

521A  **Improvements to the JBrowse Genome Browse at WormBase.** S. Cain, T. Harris, S. Gao, A. Wright, P. Nuin, The WormBase Consortium Ontario Institute for Cancer Research, Toronto, Canada.

WormBase currently provides two graphical interfaces for genome browsing: the long-running and popular GBrowse software, and its successor, JBrowse. JBrowse is designed for improved speed and scalability, as well as enhanced customizability. We have been testing JBrowse side-by-side with GBrowse on the WormBase site for the past year. Functionally similar to GBrowse, JBrowse provides a faster and smoother user interface. Here we present three additions to JBrowse that are improvements of existing GBrowse functionality or entirely new features altogether. First, we have added the ability to create high resolution screenshots in JBrowse, a long-standing and popular feature of GBrowse. Second, we’ve added a new position weight matrix search that mimics similar functionality in GBrowse. Finally, we have introduced combination tracks that facilitate set operations like union, intersection and subtraction to yield a new track with the result. These enhancements demonstrate the utility of JBrowse; we encourage users to test the new system and submit comments and new feature requests.

522B  **EPICViz: An interactive visualization of *C. elegans* embryogenesis and gene expression.** T.J. Durham, A.J. Hill, R.H. Waterston Genome Sciences, University of Washington, Seattle, WA.

*Caenorhabditis elegans* is a widely used model organism for studying how different tissue types develop from a single fertilized cell. Gene expression plays an important role in this developmental process, and the Expression Patterns In *C. elegans* (EPIC, http://epic.gs.washington.edu/) study in our lab has generated measurements of the expression patterns for 227 transcription factor genes in each cell of developing *C. elegans* embryos at about 1 min intervals for the first ~350 min of development, along with positional coordinates for each nucleus, its diameter, and its cell type and lineage. Currently these data are available as movies or lineage diagrams that show the activity of just one gene at a time, making it difficult to compare expression patterns of multiple genes in time and space. To address this issue, we developed a browser-based interactive visualization tool that allows users in the *C. elegans* community to explore complex gene expression patterns in the anatomical context of the developing embryo. With this tool, users can highlight subpopulations of cells defined by gene expression patterns, cross-reference those subpopulations with particular embryonic lineages and tissue progenitors of interest, and map gene expression values onto a three-dimensional, rotatable model of the developing embryo that shows all cell divisions and migrations from the four cell to the comma stage. In addition, by inferring the developmental stage and tissue of origin of samples based on known expression patterns of marker genes, our visualization can also display RNA-seq expression measurements from tissue-specific or single cell data sets. Given the important role that signals from neighboring cells play in directing tissue differentiation, we believe that interpreting gene expression data with more positional context can provide important insight into the *C. elegans* developmental program and generate new hypotheses for pursuing at the bench.

523C  **A framework for automated, complete RNA-seq analysis.** D. Angeles-Albores$^{1,2}$, K.H. Min$^{1,2}$, P.W. Sternberg$^{1,2}$ 1) Division of Biology and Biological Engineering, Caltech, Pasadena, CA; 2) Howard Hughes Medical Institute.

Transcriptome profiling via RNA-seq is an increasingly important method because of its ability to quantify gene expression levels easily and accurately. Oftentimes groups possess the expertise to design transcriptome profiling experiments and to successfully extract high quality RNA, but encounter challenges with the analysis of the data because of the complexity of the bioinformatics and its technical requirements. RNA-seq experimental designs are relatively rigid and typically involve only a small number of genotypes. The stereotyped nature of these experimental designs affords us the capacity to create software that can automate the RNA-seq computational process from beginning to end. We are currently writing a software package that, when opened, will ask the user a small number of questions about the experimental specifications, then perform read pseudo-alignment using Kallisto and differential expression analysis using Sleuth. For *C. elegans* users, our software will include automated enrichment analyses. It will generate a small number of publication-grade graphs and output a summary of the RNA-seq analysis, including important diagnostics. Finally, we will output a summary of the results and a written methods section for users.

524A  **An interactive multipurpose genome browser with data integration capability: Rainbow Genome Browser.** A.R. Ozturk$^1$, C.C. Mello$^{1,2}$ 1) RNA Therapeutics Institute, University of Massachusetts Medical School, Worcester, MA; 2) Howard
Glycosyltransferases in human. We are updating the online OrthoList, and enhancing functionality by streamlining the user interface and adding the "snapshot" in time, or a single program, for determining the most complete list of genes conserved between OrthoList 1.0 and 511, or ~6.7%). These results suggest that querying new methods does not greatly change the number of genes introduced 70 of the 581 that were lost due to updates to the four original programs (bringing the total loss of genes from OrthoInspector7). Updates to the four original programs added 383 new genes (~5.0%), and, surprisingly, removed 581 genes (~7.6%) from OrthoList 1.0. Results from the two new programs only added 115 (~1.5%) new genes to OrthoList, and re-introduced 70 of the 581 that were lost due to updates to the four original programs (bringing the total loss of genes from OrthoList 1.0 to 511, or ~6.7%). These results suggest that querying new methods does not greatly change the number of genes predicted to be conserved between humans and C. elegans. Moreover, our analysis of the changes (additions and losses) between OrthoList 1.0 and 2.0 highlights the importance of regularly assessing orthology, rather than relying on a single "snapshot" in time, or a single program, for determining the most complete list of genes conserved between C. elegans and human. We are updating the online OrthoList, and enhancing functionality by streamlining the user interface and adding the ability to search based on protein domains.

To update OrthoList, we analyzed the most recent results from the four original orthology-prediction programs, which are based on newer sequence data and updates to the prediction algorithms, as well as from two additional programs (OMA6 and OrthoInspector7). Updates to the four original programs added 383 new genes (~5.0%), and, surprisingly, removed 581 genes (~7.6%) from OrthoList 1.0. Results from the two new programs only added 115 (~1.5%) new genes to OrthoList, and re-introduced 70 of the 581 that were lost due to updates to the four original programs (bringing the total loss of genes from OrthoList 1.0 to 511, or ~6.7%). These results suggest that querying new methods does not greatly change the number of genes predicted to be conserved between humans and C. elegans. Moreover, our analysis of the changes (additions and losses) between OrthoList 1.0 and 2.0 highlights the importance of regularly assessing orthology, rather than relying on a single "snapshot" in time, or a single program, for determining the most complete list of genes conserved between C. elegans and human. We are updating the online OrthoList, and enhancing functionality by streamlining the user interface and adding the ability to search based on protein domains.


525B OrthoList 2.0: an update and further analysis of human and C. elegans gene conservation. Woojin Kim1, Iva Greenwald1, Daniel Shaye2  1) Dept. of Biological Sciences, Columbia University, New York, NY; 2) Dept. of Physiology and Biophysics, University of Illinois-Chicago (UIC), Chicago, IL.

C. elegans is an important model for genetic studies relevant to human biology and disease. Therefore, it is critical to have a complete and up-to-date reference of genes whose functions are most likely to be conserved between C. elegans and humans. We previously assessed homology between C. elegans and humans by performing a meta-analysis of results from four orthology-prediction methods (Ensembl Compara1, OrthoMCL2, InParanoid3 and Homologene4), and defined a compendium of 7,663 C. elegans genes (~40% of the protein-coding genome) with clear human orthologs, which we called "OrthoList5. We subsequently released an online version (Shaye, et. al, WBG 2013) to enhance the accessibility and utility of OrthoList.

An evolutionary systems approach to an integrative study of the proteome and metabolome of Glycosyltransferases in C. elegans. R. Taujale1,2, O.O. Bifarín1, N. Kannan2, A.S. Edison1,2  1) CCRC, University of Georgia, Athens, GA; 2) Institute of Bioinformatics, University of Georgia, Athens, GA.

Glycosyltransferases (GTs) are a broad class of proteins involved in the transfer of a glycosyl group from a donor molecule to an acceptor. As such, they are involved in a wide range of biological functions through their roles in glycosylation modifications, synthesis of cellular receptors, biosynthesis of polysaccharides, glycolipids and glycoproteins. In C. elegans, GTs have been implicated to play roles in development, cuticle formation, detoxification, signaling and other pathways. However, only around 20% of the more than 260 GTs have been characterized. The large expansion of some of the GT families, presence of unique sugars and pathways in worms and the unknown specific roles of GTs in the implicated pathways pose specific questions and challenges that require a deeper understanding of the functional units of these GTs in C. elegans. We used a Bayesian statistical approach to align and classify more than 250,000 GT sequences across all taxonomic groups into functional categories based on the patterns of conservation and variation in large multiple sequence alignments. We use patterns unique to each GT family as a conceptual starting point for investigating their sequence-structure-function relationships. Implementing these methods, we can further pinpoint contrasting, similar and co-evolved features that differentiate, associate and functionally relate the GT families respectively.

We will present an initial analysis that highlights the presence of co-conserved features that distinguish multiple GT families, highlighting GTs in worms. We have generated a phylogenetic classification based on these features and mapped phenotypic associations for these families based on literature. Our analysis reveals several expanded and unique GT families in C. elegans with limited information that we hypothesize might be involved in detoxification, signaling and other pathways unique to worms. Using the co-conserved features as a starting point, we further investigate structural data to pinpoint targets for mutational and metabolomic studies. An initial metabolomic analysis by others in the lab of select GT mutants in C. elegans is revealing specific features that are statistically different, suggesting family specific phenotypic changes. Informatic analysis layered with multiple

Hughes Medical Institute, University of Massachusetts Medical School, Worcester, MA.

Current genome browsers are designed to focus in one specific region. However, in some instances it is desirable to visualize and compare the distribution of several distinct types of genomic elements such as piRNA genes or transposons within whole chromosome regions or even over the entire genome. For such tasks there is a need for a browser with a more versatile user interface, and with capacity for simultaneously displaying multiple abstractions. Here we describe a new browser that accomplishes these goals, supporting both traditional, region-specific browsing, while also supporting the rapid investigation of sequence features at much broader scales, revealing whole genome-level patterns. The browser, which we call the "Rainbow Genome Browser" is web based and developed in JavaScript using D3js library. The genomic and experimental data as well as data abstractions underlying the browser were generated using Python 3. The software is compatible with Google Chrome browsers. Recently we have been using the Rainbow Browser to visualize the genomic origins of RNA-dependent RNA polymerase (RdRP) derived small RNAs. As expected, the distribution of these small RNAs reveals the exons of nearly all germline-expressed mRNAs, as well as the genomic distribution of ncRNAs, transposons and pseudogenes. Interestingly, this analysis also reveals thousands of genomic regions that although not currently annotated as transcribed, nevertheless produce abundant small RNA reads. These findings underscore the need for a more comprehensive annotation of RNA-producing genomic features, and suggest that further functional-genetic investigations will be necessary to more thoroughly explore the worm’s amazing genetic repertoire.

525C An evolutionary systems approach to an integrative study of the proteome and metabolome of Glycosyltransferases in C. elegans. R. Taujale1,2, O.O. Bifarín1, N. Kannan2, A.S. Edison1,2  1) CCRC, University of Georgia, Athens, GA; 2) Institute of Bioinformatics, University of Georgia, Athens, GA.

Glycosyltransferases (GTs) are a broad class of proteins involved in the transfer of a glycosyl group from a donor molecule to an acceptor. As such, they are involved in a wide range of biological functions through their roles in glycosylation modifications, synthesis of cellular receptors, biosynthesis of polysaccharides, glycolipids and glycoproteins. In C. elegans, GTs have been implicated to play roles in development, cuticle formation, detoxification, signaling and other pathways. However, only around 20% of the more than 260 GTs have been characterized. The large expansion of some of the GT families, presence of unique sugars and pathways in worms and the unknown specific roles of GTs in the implicated pathways pose specific questions and challenges that require a deeper understanding of the functional units of these GTs in C. elegans. We used a Bayesian statistical approach to align and classify more than 250,000 GT sequences across all taxonomic groups into functional categories based on the patterns of conservation and variation in large multiple sequence alignments. We use patterns unique to each GT family as a conceptual starting point for investigating their sequence-structure-function relationships. Implementing these methods, we can further pinpoint contrasting, similar and co-evolved features that differentiate, associate and functionally relate the GT families respectively.

We will present an initial analysis that highlights the presence of co-conserved features that distinguish multiple GT families, highlighting GTs in worms. We have generated a phylogenetic classification based on these features and mapped phenotypic associations for these families based on literature. Our analysis reveals several expanded and unique GT families in C. elegans with limited information that we hypothesize might be involved in detoxification, signaling and other pathways unique to worms. Using the co-conserved features as a starting point, we further investigate structural data to pinpoint targets for mutational and metabolomic studies. An initial metabolomic analysis by others in the lab of select GT mutants in C. elegans is revealing specific features that are statistically different, suggesting family specific phenotypic changes. Informatic analysis layered with multiple
data sources from literature serve as a tool to identify targets and derive hypotheses to conduct deeper metabolomic studies that can help elucidate the functional changes and biological activity of the associated GT families.

**Gene Regulation and Genomics - Epigenetics**

**527A** FACT depletion-mediated reprogramming requires Histone H3 phosphorylation by the Aurora Kinase B. E. Koulundzic1,2, G. Baytek1, B. Uyar1, A. Akalin1, S. Lacadie1, B. Tursun1 1) Berlin Institute for Medical Systems Biology, Max Delbrück Center, Berlin, Germany; 2) Department of Biology, Humboldt University, 10115 Berlin, Germany.

Direct reprogramming of cellular identities by mis-expressing transcription factors (TFs) is limited in different tissue contexts. We previously identified the histone chaperone LIN-53 as a reprogramming barrier in C. elegans preventing the direct conversion of germ cells to specific neuron types (Tursun et al., Science 2011). We conducted genetic screens using transgenic animals expressing CHE-1, the ASE neuron fate-inducing TF, under the heat shock promoter together with ASE-neuron-specific reporter gcy-5:gfp to identify factors contributing to safeguarding cells against ectopic fates. Our screen for such safeguarding factors revealed that the histone chaperone FACT (Facilitates Chromatin Transcription) is a novel reprogramming barrier. FACT protects different tissues such as the germline and gut cells from being directly reprogrammed into neuron-like cells. In order to further dissect the mechanism of FACT depletion-mediated cell fate conversion we performed a suppressor screen and found that FACT requires the presence of Histone H3 phosphorylation by the Aurora Kinase B/AIR-2. RNAi against air-2 or temperature-sensitive air-2 mutants suppress cellular conversion and ectopic gene expression upon FACT depletion. Aurora Kinase B is predominantly known for its role in cell cycle progression. Nevertheless, we find that Aurora kinase B might have another role, independent of mitosis, as a gatekeeper of specific histone modifications such as H3Ser10ph in order to maintain a refractory epigenetic signature toward induction of ectopic cell fates. Currently, we are performing a thorough biochemical study in order to reveal the molecular interplay of FACT and AIR-2. Ongoing assays to interrogate protein-protein interactions by Mass Spectrometry identified novel interacting proteins that might further explain how FACT acts as a chromatin gatekeeper by counteracting AIR-2-mediated chromatin activation.

**528B** piChIP: A novel tool to dissect the role of chromatin factors during transgenerational epigenetic inheritance (TEI) in the C. elegans germline. A.C. Berkurek1, P. Dimaggio2, A. Akay1, E. Miska1 1) Genetics, Welcome Trust/Cancer Research UK Gurdon Institute, Cambridge, Cambridge, GB; 2) Department of Chemical Engineering, Imperial College, UK.

Non DNA-sequence based inheritance has been observed in many organisms from microbes to men and likely impacts public health. The mechanisms of non DNA-sequence based inheritance remain largely unknown. However, some examples of non DNA-sequence based inheritance in mammals, plants and invertebrates are linked to the control of selfish, transposable elements in the genome. However, current knowledge in this field is mostly restricted to molecular genetics experiments. Here we propose to discover the mechanistic basis of multi-generational non DNA-sequence based inheritance, also referred to as transgenerational epigenetic inheritance (TEI), using the laboratory animal model Caenorhabditis elegans.

21U Piwi-interacting RNAs (piRNAs) target transposable element mRNAs and induce a secondary siRNA response in the germline by recruiting histone methyltransferases SET-25 and SET-32. In this pathway, our goal is to identify chromatin and we have established a germline-specific single locus system that we named piRNA-related insertional chromatin non-coding RNA "epigenetic" marks that transfer information from generation to generation in the germline. Towards this goal sensor which encodes for a GFP-H2A construct with a piRNA target site in the 3' end. In order to immunoprecipitate (IP) the immunoprecipitation (piChIP). To create a locus-specific ChIP system, we used the LacI-lacO system by targeting the piRNA elements in the genome. However, current knowledge in this field is mostly restricted to molecular genetics experiments. Here we propose to discover the mechanistic basis of multi-generational non DNA-sequence based inheritance, also referred to as transgenerational epigenetic inheritance (TEI), using the laboratory animal model Caenorhabditis elegans.

**529C** The SET-2/SET1 complex physically interacts with multiple chromatin-modifying complexes to influence embryonic transcription and development. F. BEURTON1, C. BEDET1, M. CARON1, J. GOVIN2, Y. COUTE2, D. CLUET1, M. SPICHTY1, F. PALLADINO1 1) Laboratoire de Biologie et Modélisation de la Cellule, ENS de Lyon, LYON, LYON, FR; 2) Exploring the Dynamics of Proteomes, CEA Grenoble, GRENOBLE, FR.

Methylation of histone H3 Lys4 (H3K4me) is associated with promoters of actively transcribed genes in all species, and is catalyzed by the highly conserved SET1/MLL family methyltransferases acting in large multisubunit complexes known as Compass in yeast and SET1/MLL in mammals. Compass related complexes from yeast to mammals contain a SET1 or MLL-related catalytic subunit, and the common subunits ASH2, RbBP5, WDR5 and DPY30. In addition, the CFP1/CXXC1 zinc finger protein is unique to SET1 complexes. C. elegans contains a single homologue of SET1, named SET-2, and single homologues of all additional subunits, including CFP-1. All subunits contribute to global H3K4 methylation and share common phenotypes, but the biochemical composition of the complex has not been described.

In order to purify the SET-2 complex from C. elegans, and identify protein interactors that contribute to the implementation and regulation of H3K4 methylation, we undertook a proteomic approach. In immunoprecipitation experiments using tagged components of COMPASS, followed by tandem MS/MS, we identified all common as well as unique subunits of COMPASS and
MLL-related complexes. In addition, this approach allowed us to identify novel interactors. I will present evidence based on biochemical, molecular and genetic approaches, as well as transcriptional profiling, showing that SET-2/SET1 physically and functionally interacts with a complex network of epigenetic factors to influence the outcome of H3K4 methylation and gene expression during development.

530A H3K27me3 epigenetic mark is responsible for sensitivity of Caenorhabditis elegans exposed to elevated temperature.  
Nivedita Chatterjee, Jaeseong Jeong, Jinhee Choi  University of Seoul, Seoul, Korea.

Epigenetics, phenotypic characters without modification of gene sequence, possess reversibility as well as the heritable transgenerational transfer of epigenetic marks which argues for it inclusion in environmental health risk assessment. The aim of the present study was to evaluate the role of histone methylation in inflated temperature conditions. To this end, we used C.elegans as model systems and two temperature conditions (20°C, optimum temperature for C.elegans and 23°C, as higher temperature condition). We found that the first two generations (F0 & F1) lifespan was markedly decreased which were eventually adapted until sixth generations (F6). Likewise lifespan, the reproduction and brood size also exhibited adaptation in F5-F6 generation after significant alterations in earlier generations of exposures (23°C). In parallel, we found that histone modification as major mechanism of temperature sensitivity by evaluating let-558:gfp expression in the germline of the elevated (23°C) temperature exposed worms. Next, we found that in mes-2 (H3K27 di-/trimethyl transferase) marked up-regulation of reproduction which was maintained in following generation while significant decrease in reproduction in utx-1 (H3K27 demethylase) mutant due to higher (23°C) temperature exposure. These results were further supported with the gene expressions results. On the contrary, other histone methylation related genes and mutants’ (met-1, H3K36 trimethylase; set-2, H3K9 trimethylase; set-2 H3K4 methyltransferase; spr-6, H3K4me2 demethylase) reproduction did not show significant alterations due to exposure to inflated (23°C) temperature. Among the mon-di-tri methylation of H3K27 mark, only H3K27me3 protein mark exhibited as differently expressed (demethylated) due inflated temperature exposure. Taken together, our results clearly showed that exposure to higher temperature caused H3K27me3 demethylation which plays the critical role in elevated temperature sensitivity of Caenorhabditis elegans.

531B Transmission of an epigenetic “MEssage” for germline development in C. elegans.  
Chad Cockrum, Jeremy Kreher, Susan Strome  MCD Biology, University of California at Santa Cruz, Santa Cruz, CA.

During C. elegans development, two primordial germ cells (PGCs) must acquire germ fate and proliferate into hundreds of germ cells to form a complete and fertile adult germline. How PGCs acquire and maintain germ cell fate is poorly understood. Two important contributors are the chromatin regulators MES-4, a histone methyltransferase, and MRG-1, a chromodomain-containing protein. Without maternal delivery of these proteins to embryos, germ cells in early larvae fail to proliferate and die, resulting in sterile adults. We hypothesize that MES-4 and MRG-1 transmit the memory of a germline gene expression program from parent germ cells to progeny germ cells to ensure that PGCs express the appropriate genes for their survival and proliferation. Our working model is that 1) during embryogenesis, MES-4 maintains a memory of which genes were previously expressed in the parental germline by maintaining H3K36me3 on nucleosomes that package those genes, and 2) MRG-1 effects the memory by binding to H3K36me3 generated by MES-4 and activating an appropriate germline transcription program in PGCs. To test our model, I am determining whether PGCs from L1s that lack MES-4 or MRG-1 fail to launch an appropriate germline gene expression program. Due to the previous limitation in gathering enough PGCs for mRNA profiling, I first profiled mRNAs from mutant adult germlines that inherited maternally provided MES-4 and MRG-1, but cannot express one of the gene products (M+Z-). mes-4 M+Z- and mrg-1 M+Z- mutant adult germlines misexpress a similar set of genes that are normally repressed in germ cells: somatic genes and genes on the X chromosome. Thus, MES-4 and MRG-1 are required for a proper germline gene expression program and they regulate similar genes in adult germlines. We predict that PGCs in L1 progeny of these mutant adults, which completely lack MES-4 or MRG-1 (M-Z-), fail to acquire germ cell fate because they fail to launch a proper germline transcription program and more severely misexpress somatic genes and genes on the X chromosome. To test this prediction, I developed a method to isolate PGC pairs from wild-type and mutant L1 larvae to profile their mRNAs. I showed that this process can successfully profile the mRNA population from single sister pairs of wild-type PGCs. This dissection approach offers advantages over FACS sorting large populations of PGCs: I can analyze mutant PGCs instead of RNAi PGCs, PGCs are of a uniform age, and PGCs spend minimal time in buffer before RNA preparation. I am currently analyzing the transcriptome of PGCs isolated from mes-4 and mrg-1 M-Z- mutant larvae, to learn how maternal MES-4 and MRG-1 ensure PGC survival and proliferation.

532C Identity and function of the variant histone H3.3 in C. elegans.  
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Nucleosomes are the basic elements of chromatin architecture and are instrumental for its structure and function. Modulation of nucleosome function is in part achieved by the incorporation of histone replacement variants. H3.3 is a major variant of histone H3 and is highly conserved among eukaryotes. Genomic studies revealed that H3.3 is incorporated into transcriptionally active as well as telomeric regions. To date, H3.3 has been shown to be involved in a diversity of processes including neuron plasticity, tumorigenesis, DNA damage response and ES cells differentiation. It was also shown to be crucial for fertility and embryonic development - mice or flies lacking H3.3 show severe sterility and lethality phenotypes. The C. elegans genome encodes five H3.3 homologues, only two of which, his-71 and his-72 have been analyzed in detail. These genes are expressed ubiquitously, incorporate into actively transcribed regions and promote longevity in the context of insulin signaling mutants. However, no major developmental phenotypes have been observed, presumably due to redundancy.
We have generated deletion alleles for the three remaining genes with H3.3 homology - his-69, his-70 and his-74, and combined the alleles to generate an H3.3 null strain. Surprisingly, both hermaphrodite and male H3.3 null animals appear to be fully viable and fertile at 20°C. A reduction of the brood size was detected when growing the worms at a higher temperature, indicating that at least some of the H3.3 genes play a role in response to stress experienced in these conditions. Consistent with this observation, we found that the deletion of H3.3 leads to changes in the expression of genes involved in stress response, based on RNA-seq experiments.

We have generated gfp-fusion alleles with all five genes encoding H3.3 homologues and found that the expression was strikingly stage- and tissue specific. Nevertheless we found that despite some divergence in amino acid sequence, chromatin incorporation of all five H3.3 homologues depends on the conserved histone chaperone HIRA. We are currently characterizing the genomic distribution of H3.3 under different stress conditions to gain more insight into the function of this histone variant.

533A The C. elegans DOT1L and c-Myc homologues cooperate in the nucleus to co-regulate target genes. R. Esse, A. Ceballos, E. Gushchanskaia, A. Mora Martin, A. Grishok  Department of Biochemistry, Cell Biology & Genomics, Boston University School of Medicine, MA.

Transcriptional control is mediated by interactions of transcription factors with their cognate DNA elements, as well as by epigenetic modifications to chromatin catalyzed by a variety of enzymes. Thus, understanding the crosstalk between transcription factors and epigenetic modifiers is of prime importance. The Dot1-like protein (DOT1L) is an evolutionary conserved methyltransferase with catalytic specificity towards histone 3 lysine 79 (H3K79). Owing to its crucial involvement in some aggressive leukemias, it has been gaining prominence in cancer research, with chemical inhibitors of DOT1L currently in clinical trials. Recent observations suggest that the role of DOT1L in malignant transformation can be generalized to contexts beyond leukemia. For instance, DOT1L has been implicated in breast cancer progression, and this has been attributed to its cooperation with c-Myc. However, the mechanistic details underlying this association are unknown. Previous work in our lab has shown that DOT-1.1, the C. elegans DOT1L homologue, is recruited to chromatin by ZFP-1 (similarly to DOT1L recruitment by AF10 in mammals), and this complex negatively modulates transcription. Interestingly, promoters of the ZFP-1/DOT-1.1 target genes are enriched in E-boxes, the consensus binding motif for c-Myc. Prompted by the exciting hypothesis that DOT-1.1 and MML-1, the C. elegans c-Myc homologue, cooperate genome-wide, we performed ChiP-seq for MML-1 and defined target genes as those with peak(s) close to their transcription start sites. Interestingly, the majority of MML-1 targets are also ZFP-1/DOT-1.1 targets. In order to understand the relevance of such co-localization for gene expression, we profiled mRNA and miRNA in wild-type worms and dot-1.1, zfp-1, and mml-1 lof mutants by microarrays. We found significant overlaps between genes upregulated in the three mutants, and the same was observed for downregulated genes. A significant global increase of non-coding transcripts was observed in either mutant compared with wild-type. Therefore, ZFP-1/DOT-1.1 and MML-1 co-regulate both coding and non-coding genes and globally inhibit non-coding transcription, including that of pri-miRNA. Recently, C. elegans enhancers were annotated based on their characteristic histone modifications signature, ATAC-seq profile and functional experiments. Notably, ZFP-1 localizes to ~50% of intronic enhancers. We have selected two of the enhancers for ChiP-qPCR and MML-1 was enriched at both. Thus, ZFP-1/DOT-1.1 and MML-1 co-regulate both promoters and enhancers. This is consistent with the developmental phenotypes (e.g. HSN migration defects) shared by the zfp-1, dot-1.1 and mml-1 lof mutants. Further investigation is underway to uncover the mechanism of cooperation of ZFP-1/DOT-1.1 and MML-1.

534B Transgenerational effects of chronic ethanol treatment in Caenorhabditis elegans. D.M. Guzman, J.T. Pierce, V.R. Iyer  University of Texas at Austin, Austin, TX.

Alcoholism is a prevalent and often devastating disease, characterized by a psychological and physical dependence on alcohol consumption. Familial patterns of alcohol dependence, as well as human twin studies, suggest a heritable component to the susceptibility of this disease. However, simple genetic inheritance alone does not predict development of alcoholism. There is growing evidence that suggests that exposure to some drugs of addiction can lead to heritable changes in gene expression in the form of epigenetic alterations. In order to determine if chronic ethanol exposure alters alcohol response behaviors in progeny, we used the model nematode C. elegans for its unique advantages in transgenerational epigenetic studies. We treated parental generation (F0) animals with either ethanol or control buffer, to generate the EtOH-line and Control-line, respectively. Three subsequent generations of these lines (F1-F3) were tested for sensitivity to acute intoxication displayed by these naïve animals. For each of the three generations, we found that the EtOH-line showed resistance to ethanol-induced locomotor inhibition relative to the Control-line. These results suggest that chronic exposure to EtOH confers resistance to intoxication in subsequent generations. This is particularly interesting because inherent resistance to the negative effects of alcohol consumption is thought to contribute to alcohol abuse susceptibility in humans. Further studies will focus on elucidating the neuronal and genetic mechanisms that may confer this apparent heritable resistance to alcohol.

535C Biochemical Characterization of Caenorhabditis elegans Protein Arginine Methyltransferase Orthologs PRMT-7 and PRMT-9. A. Hadjikyriacou1, S. Clarke2  1) Department of Chemistry and Biochemistry, University of California Los Angeles, Los Angeles, CA; 2) Molecular Biology Institute, University of California Los Angeles, Los Angeles, CA.

Caenorhabditis elegans contains orthologs of protein arginine methyltransferases (PRMT) 1, 5, 7 and 9. PRMT-7 and PRMT-9 are two evolutionarily conserved enzymes, with distinct orthologs in plants, invertebrates, and vertebrates. Biochemical characterization of these two enzymes reveals that they share much in common with their mammalian orthologs. In this study, we identify the methyltransferase activities of C. elegans PRMT-7 and probe for its substrate specificity, looking at methylation of a broad collection of substrates in a preferential motif. In addition, our work shows that C. elegans PRMT-9, is an important
enzyme in *C. elegans* that seems to have a conserved substrate and methylation activity close to its mammalian counterpart; we show that it methylates a previously uncharacterized substrate, that we named SFTB-2. SFTB-2 is the conserved *C. elegans* ortholog of the human RNA splicing factor SF3B2. This can indicate a potential role of PRMT-9 in the regulation of *C. elegans* splicing. This work shows that in contrast to PRMT-7, *C. elegans* PRMT-9 appears to be biochemically similar to its human ortholog. Biochemical characterization of these PRMT enzymes in *C. elegans* can tell us a lot about the function of PRMTs in the nematode and add another layer of regulation.

536A Early experiences mediate distinct adult gene expression and reproductive programs in *Caenorhabditis elegans*. M.C. Ow1, K. Borziak2, S. Dorus3, S.E. Hall4 1) Dept Biol, Syracuse University, Syracuse, NY; 2) Center for Reproductive Evolution, Dept Biol, Syracuse University, Syracuse, NY.

Environmental stress during early development in animals can have profound effects on adult phenotypes via programmed changes in gene expression. Using the nematode, *C. elegans*, we demonstrated previously that adults retain a cellular memory of their developmental experience that is manifested by differences in gene expression and life history traits; however, the sophistication of this system in response to different environmental stresses, and how it dictates phenotypic plasticity in adults that contribute to increased fitness in response to distinct environmental challenges, was unknown. Using transcriptional profiling, we show here that *C. elegans* adults indeed retain distinct cellular memories of different environmental conditions. We identified approximately 500 genes that exhibit highly significant and opposite (“seesaw”) transcriptional phenotypes in adults that entered dauer due to starvation (PD*stv*) compared to adults that entered dauer due to crowding (PD*phb*), and are distinct from animals that bypassed dauer (CON). Moreover, we show that two-thirds of the genes in the genome experience a 2-fold or greater seesaw trend in gene expression, and based upon the direction of change, are enriched in large, tightly linked regions on different chromosomes. Importantly, these transcriptional programs correspond to significant changes in brood size depending on the experienced stress, such that we observe a decrease or increase in PD/CON brood size for Stv or Phe conditions, respectively. In addition, we demonstrate that the observed seesaw gene expression and brood size differences require different signaling mechanisms for the Stv- and Phe-induced changes. The starvation program requires endocrine signaling and the germline, while the pheromone program is dependent on systemic RNAi signals from the soma. Interestingly, both transcriptional programs require the AGO CSR-1. Thus, signaling between the soma and the germline can generate phenotypic plasticity as a result of early environmental experience, and likely contribute to increased fitness in adverse conditions and the evolution of the *C. elegans* genome.

537B Mechanism and function of genome organization in muscle development and integrity. Anna Mattout, Jennifer C. Harr, Veronique Kalck, Adriana Gonzalez-Sandoval, Susan M. Gasser Quantitative biology, Friedrich Miescher Institute for Biomedical Research, Basel, CH.

The loss of nuclear structural integrity leads to tissue-specific pathologies in a set of human diseases called laminopathies. These diseases, such as Emery Dreifuss Muscular Dystrophy (EDMD), are late-onset, tissue-specific, and degenerative. A *C. elegans* mutant that recreates the EDMD phenotypes arises from the introduction of a specific gain-of-function mutation into lamin (Y59C), which affects the proper release of a muscle-specific heterochromatic reporter from the nuclear periphery. We found that this muscle-specific misorganization of heterochromatin correlates with transcriptional defects and with perturbed locomotion and muscle integrity in *C. elegans*. To identify if chromatin misorganization is a cause or an effect of these physiological defects or if the alteration of gene expression is the primary cause, we took advantage of a cec-4 deletion mutant. The perinuclear *C. elegans* chromodomain protein-4 (CEC-4) anchors heterochromatin by binding H3K9 methylation. The mutant releases H3K9me containing heterochromatin from the nuclear periphery in embryos and does not alter transcription. In a strain that contains the cec-4 deletion, expresses the lamin Y59C mutant and harbors a muscle-specific heterochromatic reporter, there is a recovery of the proper positioning of the muscle-specific heterochromatic array in muscle. Additionally, we found that the cec-4 mutation rescues the impaired locomotion phenotype of the lamin mutant. This argues that the lamin-induced sequestration or retention of tissue specific genes in a heterochromatic compartment drives defects that can be overcome by reversing perinuclear sequestration. To identify the mechanism behind this phenomenon we are using in vivo biochemical tagging in the LNM-1 Y59C mutant and wild-type counterpart to identify perinuclear components that mediate sequestration of muscle-specific gene promoters. By identifying the proteins involved in this process, we can determine the mechanism in which misorganization of chromatin can lead to a loss of tissue integrity.

538C Epigenetic screening of RNAi library with transgenic worms *pkls1582 (let-858::GFP)* strain. Jaeseong Jeong, Nivedita Chatterjee, Youngho Kim, Soo young Bae, Jinhee Choi University of Seoul, Seoul, Korea.

*Caenorhabditis elegans* is an emerging epigenetic model system because detailed mechanisms of epigenetic process including ncRNAs and chromatin modifications are being studied, although it is considered that DNA methylation is absent. The RNA-mediated interference (RNAi) is a valuable tool to investigate specific function of the genes in *C. elegans*. The feeding of the RNAi to *C. elegans* results in gene inactivation through the specific degradation of the targeted endogenous mRNA. To identify the genes which possess relation with the histone modification (methylation), we performed GFP florescence expression in a high-copy transgene array containing strain (NL2507) *pkls1582[let-858::GFP + rol-6(su1006)]* with RNAi library. The strain *pkls1582 (let-858::GFP)* is a model of transcriptionally repressed locus bearing the heterochromatic histone modifications of H3K9me3 and H3K27me3. We use hpl-2 (RNAi), de-silencing of inactive transgene arrays in germ cells and cause higher GFP fluorescence in gonad arms of *pkls1582 (let-858::GFP)*, as a positive control and 384 transcription factor RNAi were fed until young adult stage. As a result, a number of RNAi such as irx-1, ceh-10, *fhk-10* showed epigenetic changes on the germ cells’ histone modification status. For further study, we planning to do transgenerational study to identify whether this epigenetic changes possess heritable transferability to the next generations or not.
Methylation of both histone H3K4 and H3K9 is required to promote odor adaptation. T. Hsu1, N. L’Etoile2, B. Juang2 1) Department of Biological Science and Technology, National Chiao Tung University, Taiwan; 2) Department of Cell and Tissue Biology, University of California San Francisco, CA.

Methylation of histone H3 lysine 4 (H3K4me) usually activates gene expression, whereas methylation of histone H3 lysine 9 (H3K9me) is associated with gene repression. Such antagonistically regulation between H3K4me and H3K9me plays an important role in development. Here we present evidence how coordination of histone methylation controls gene expression and protein association within sensory neurons in response to environmental change. In C. elegans, the primary activity in the AWC sensory neuron is to seek for attractive volatile chemical compounds such as butanone. Prolonged exposure to this odor decreases the seeking behavior, termed adaptation. The process of the modulation is termed neuronal plasticity. In previous work, we found that the olfactory plasticity process requires the involvement of histone methylation. Prolonged odor-exposure induced endogenous small interfering RNA pathway directed the chromatin assembly factor HPL-2 via association with H3K9 to promote odor adaptation in adult animals. We show here that overexpression of an H3K9 demethylase JMU-2 in the AWC neuron blocks adaptation. Moreover, deletion of an H3K4 methyltransferase SET-2 leads to inhibition of adaptation. Genetic analysis reveals that HPL-2 and SET-2 seem to affect adaptation by acting in the same pathway. We also find that the AWC neuron-specific inactivation of an anchor protein WDR-5, which guides SET-2 to interact on H3K4me, in the presence of HPL-2 and SET-2 abolishes adaptation. Further, we identify a novel histone-binding protein which interacts with H3K4me to promote adaptation by using analysis of bimolecular fluorescence complementation. These findings indicate that both H3K4me and H3K9me not only are required for promoting adaptation but also coordinate in the same genetic pathway to establish the long-term olfactory memory.

Analyzing of comparative transcriptomics, SIN-3/HDAC complex is related to epigenome in early embryogenesis of Caenorhabditis elegans. Y. Ohnishi, T. Nagano, H. Kojima, M. Ito Bioinformatics, Ritsumeikan University, Kusatsu, Shiga, JP.

Epigenetic regulation by histone acetylation, methylation, and long non-coding genes is proven to have crucial roles during embryogenesis. Since DNA is wrapped around histones forming a specific chromatin structure, several histone modifications provide tight regulation of transcription by changing this arrangement. Different histone modifications compete at the same residues and subsequently become the markers that facilitate induction of other histone modifications. Histone acetylation is catalyzed by histone acetyltransferases (HATs) and histone deacetylases (HDACs); the latter regulate various gene groups and are known to form different complexes centered on transcription corepressors, SIN-3, NuRD, and CoREST.

To elucidate the functions of SIN-3/HDAC complex in early embryogenesis, we analyzed SIN-3 encoded by the gene sin-3. Interestingly, embryonic lethality was detected in more than 20% of the sin-3 (tm1276) mutants. qRT-PCR was then used to measure sin-3 mRNA levels at three developmental stages of the N2 strain, including early embryo, larva first (L1), and adult stage. Our results showed that mRNA levels of sin-3 in early embryo stage were higher than that of in L1 stage and adult stage (7.8-fold and 6.5-fold, respectively). Comparative transcriptomics in early embryo stage of the N2 strain and sin-3 (tm1276) mutant was also performed. Many essential genes in embryogenesis including polarity mediators (mex-1, mex-3, mex-5, mex-6, pos-1, spn-4) and cell fate regulators (axp-1, mes-1, mom-1, nos-2, pal-1, pie-1, skn-1) were found to be transcriptionally upregulated by SIN-3. These results suggest that SIN-3 has essential roles in regulation of epigenome in embryogenesis and cell fate determination of C. elegans. Therefore, it is our future plan to functionally identify other HDACs complexes involving NuRD and CoREST by comparative transcriptomics.

Characterising C. elegans COMPASS complex in gene expression. Bharat Pokhrel1, Alex Appert2, Przemyslaw Stempork2, Yan Dong2, Rosamund Clifford1,2, Yannic Chen1, Julie Ahringer2, Ron Chen1,2 1) Faculty of Biological Sciences, University of Leeds, Leeds, GB; 2) The Gurdon Institute, University of Cambridge, Cambridge, United Kingdom.

Trimethylation of histone lysine 4 (H3K4me3) is regarded as an active promoter mark across all eukaryotes. H3K4me3 is mainly deposited by an evolutionarily conserved COMPASS complex that contains two key subunits: SET-2, H3K4me3/2 methyltransferase, and CFP-1, a conserved CpG binding protein. Perturbation of this mark has been found to be associated with different diseases, development defects, and incorrect cell fate specification. However, it is unclear whether H3K4me3 play an instructive role in transcription or just a consequence of gene expression. To address this question, we phenotypically characterised and compared cfp-1 (tm6369) mutants with a reported loss-of-function set-2 (bn129) mutant allele. We observed dramatic loss of H3K4me3, poor fertility, slow growth and ectopic gene expression in both cfp-1 and set-2 mutants. We found that the role of H3K4me3 is to fine tune gene induction, likely by modulating other histone modifications that play an essential role in gene activation. Our results shed light on the role of H3K4me3 in chromatin regulation and function.

Exploring the rules for piRNA::target recognition in C. elegans. Enzhi Shen, Craig mello RNA Therapeutic Institute, UMASS medical school, worcester, MA.

piRNAs play a central role in the silencing of foreign genetic elements in the C. elegans germline. piRNAs surveillance is thought to integrate information from two other Argonaute pathways, the CSR-1 and WAGO pathways. The CSR-1 and WAGO Argonautes recruit RNA-dependent RNA polymerase (RdRP) to their targets to guide the re-amplification of their pathway-specific small RNAs (sRNAs). CSR-1 sRNAs are antisense to most expressed mRNAs, while WAGO Argonautes target silent mRNAs. piRNAs engage the Piwi Argonautes (PRG-1) and are thought to scan mRNAs via micro-RNA, seed-like, base-pairing interactions. "Self" mRNAs appear to be protected from piRNA recognition by CSR-1 targeting. Upon recognition of a foreign sequence PRG-1 then recruits RdRP to produce de novo WAGO-associated sRNAs. Once established, WAGO sRNAs maintain
silencing by recruiting RdRP back to their targets each generation. This system is thought to underlie transposon defense and transgene silencing and may also have a role in regulating endogenous germline gene expression. piRNAs, also known as 21U-RNAs, consist of over 15,000 distinct genomically encoded small RNA species that are transcribed by pol-II. piRNAs are known to tolerate mismatched pairing during target recognition, however, the rules for targeting are not known. To explore piRNA base pairing we used CRISPR to modify an endogenous piRNA to target gfp. We monitored GFP silencing and WAGO sRNA accumulation for perfectly matched piRNA/target RNA pairing and for a series of 20 single-point-mutant mismatches extending from nucleotide 2 to 21. We show that a seed region from nt 2-8 is important for efficient silencing, and that additional 3’ pairing, including pairing with the terminal nt, nt 21, also contribute to silencing. These findings help explain the role of piRNAs in transcriptome surveillance and will also help inform our search for endogenous regulatory targets of piRNAs.

543B Investigating the role of the SWI/SNF chromatin remodeling complex in the differentiation of the invasive phenotype. J.J. Smith, A.Q. Kohrman, D.Q. Matus Biochemistry & Cell Biology, Stony Brook University, Stony Brook, NY.

The success of many metazoan developmental programs relies on the ability of specialized cells to transgress basement membranes (BM). Cancer progression also relies on cellular invasion. Though the developmental and clinical importance of cell invasion is evident, studying its dynamics in vivo has proven to be challenging. Using high-resolution microscopy, as well as genetic and cell biological techniques we study the process of anchor cell (AC) invasion during C. elegans development. We have recently demonstrated that the conserved nuclear hormone receptor transcription factor, NHR-67, is required to maintain the AC in G1/G0 cell cycle arrest, a requirement for invasive behavior. Independent of cell cycle arrest, the AC utilizes the histone deacetylase, HDA-1, for the generation of invadopodia, and the expression of pro-invasive genes. These results suggest that invasion is a differentiated cellular behavior requiring cell cycle arrest and epigenetic modification of the genome. To identify additional chromatin modifiers that mediate invasion, we are conducting a tissue-specific RNAi screen. To date, we have identified several new pro-invasive genes which encode subunits of the SWI/SNF chromatin remodeling complex. The complex exhibits pleiotropy, and in C. elegans it contributes to cell fate specification in the somatic gonad, and cell-cycle exit and differentiation of muscle precursor cells. Here, we show a conserved role for the SWI/SNF complex in coordinating cell cycle arrest, as loss of swsn-1 results in a mitotic AC. Specifically, we are examining the role of the SWI/SNF complex in maintenance of the post-mitotic state and the regulation of pro-invasive gene expression, through potential interactions with NHR-67, HDA-1, other chromatin modifiers and the cell cycle machinery. Together, these results will provide new insight into the role of the SWI/SNF complex in orchestrating invasive activity.

544C Investigating the role of chromatin modifiers in transgenerational epigenetic inheritance. R. Woodhouse, A. Ashe School of Life and Environmental Sciences, University of Sydney, Sydney, NSW, AU.

Mounting evidence in a number of organisms suggests that some epigenetic modifications acquired by an individual during its lifetime can be inherited for multiple future generations. This phenomenon is termed transgenerational epigenetic inheritance, and may provide a mechanism for the inheritance of environmentally acquired traits. We are studying transgenerational epigenetic inheritance using the nematode C. elegans.

We have developed a system in which RNAi-induced silencing of a GFP transgene is robustly inherited for multiple generations in the absence of the initial RNAi trigger. We show that two chromatin modifiers are required for effective transmission of transgene silencing. We also present data on the broader functions of these proteins in the germline. We show that hermaphrodites with compromised chromatin modifying function have reduced fertility, producing fewer live offspring, fewer total eggs and more unfertilized eggs in comparison to wild type animals. This indicates decreased self-sperm production and/or fertilization, suggesting defects in the male germline. These animals also display extended lifespan. There is a known inverse relationship between fertility and lifespan, but we have preliminary evidence from different deletion mutants that the brood size and lifespan effects that we observe may be independent. We are currently investigating whether these phenotypes can be decoupled, and will report these results.

Gene Regulation and Genomics - Gene Regulation

545A Adaptation of the nematode C. elegans to hypoxia and reoxygenation stress reveals an unexpected function of the neuroglobin GLB-5 in innate immunity. Z. Abergel1, B. Zuckerman1, V. Zelmanovich1, L. Romero1, R. Abergel1, L. Livshits1, Y. Smith2, E. Gross1 1) Dept. of Biochemistry and Molecular Biology, IMRIC, Faculty of Medicine, The Hebrew University of Jerusalem, Ein Kerem; 2) Genomic Data Analysis Unit, The Hebrew University - Hadassah Medical School, The Hebrew University of Jerusalem.

Deprivation of oxygen (hypoxia) followed by reoxygenation (H/R stress) is a major component in several pathological conditions such as vascular inflammation, myocardial ischemia, and stroke. However how animals adapt and recover from H/R stress remains an open question. Previous studies showed that the neuroglobin GLB-5(Haw) is essential for the fast recovery of the nematode Caenorhabditis elegans (C. elegans) from H/R stress. Here, we characterize the changes in neuronal gene expression during the adaptation of worms to hypoxia and recovery from H/R stress. Our analysis shows that innate immunity genes are differentially expressed during both adaptation to hypoxia and recovery from reoxygenation stress. Moreover, we reveal that the prolyl hydroxylase EGL-9, a known regulator of both adaptation to hypoxia and the innate immune response, inhibits the fast recovery from H/R stress through its activity in the O2-sensing neurons AQR, PQR, and URX. Finally, we show that GLB-5(Haw) acts in AQR, PQR, and URX to increase the tolerance of worms to bacterial pathogenesis. Together, our studies suggest that innate immunity and recovery from H/R stress are regulated by overlapping signaling pathways.
Finally, we will screen for new interactions playing a role in regulating regulatory networks. To gain insight into this communication, we used a set of 19 intestinal reporter strains we used previously to perturbations. Together, these findings suggest that there may be extensive communication between metabolic and gene regulatory networks. Gene regulatory networks play important roles in every process of life, including cell differentiation, metabolism, development and stress response. Our group previously delineated a tissue-specific gene regulatory network for the C. elegans intestine. In this network, we found a modest overlap between regulatory & physical interactions, indicating that other factors regulate gene expression indirectly. In other studies, we discovered multiple transcription factors that respond to metabolic network perturbations. Together, these findings suggest that there may be extensive communication between metabolic and gene regulatory networks. To gain insight into this communication, we used a set of 19 intestinal reporter strains we used previously with transcription factor RNAi, and screened a novel RNAi library of ~1,600 metabolic genes. For every screen, we fed L1 synchronized animals, E. coli HT115 RNAi cultures, incubated for 48hrs at 20°C and visually scored increased or decreased fluorescence in the intestine in triplicate. Interactions that were found two or three times were retested, and only interactions that were retested were kept in the final dataset.
549B Alternative polyadenylation directs tissue-specific miRNA targeting in C. elegans.  S. Blazé1,2, H.C. Geissen1,2, H Wilky1,2, R. Joshi1, J. Newbern1,3, M. Mangone1,2,3,4 1) Molecular and Cellular Biology Graduate Program, Arizona State University, Tempe, AZ, USA 85281; 2) The Biodesign Institute at Arizona State University 1001 S McAllister Ave, Tempe, AZ 85287-5601; 3) Barrett Honors College, Arizona State University, 751 E Lemon Mall, Tempe, AZ 85281; 4) College of Letters and Sciences, Interdisciplinary Studies: Biological Sciences & Informatics, Arizona State University, Tempe, AZ, USA 85281.

The ENCODE consortium revealed an incredible diversity in the non-coding sequence regions located between the stop codon and poly(A)-tail of C. elegans mRNAs. These regions, named 3 ’Untranslated Regions (3’UTRs) are primarily targeted by microRNAs (miRNAs) that recognize tiny sequence elements in 3’UTRs, which guide Argonaute proteins and their associating complexes to induce post-transcriptional gene silencing.

We have previously shown that almost half of worm protein coding genes are expressed with multiple 3’UTR isoforms through a mechanism known as alternative polyadenylation (APA), but its biological role in individual tissues and especially its impact on post-transcriptional gene regulation at this level has not been properly characterized.

Here, we have systematically isolated and sequenced mRNAs and APA events from eight C. elegans somatic tissues: GABAergic and NMDA neurons, arcade and intestinal valve cells, seam cells, epidermis, intestine, pharynx, and body muscle tissues.

Mapping their transcriptomes revealed extensive heterogeneity in 3’UTR isoform usage that appears to drive the tissue-specific expression of miRNA targets. Nearly all ubiquitously transcribed genes use APA and harbor miRNA targets in their 3’UTRs, which are commonly lost in a tissue specific manner, suggesting widespread usage of post-transcriptional regulation modulated through APA to fine-tune gene expression.

Using a unique tool to detect post-transcriptional regulation in vivo, we show that the human disease gene orthologs rack-1 and tct-1 evade miRNA regulation through a tissue-specific APA event in the body muscle, driving protein expression needed for proper tissue function.

We conclude that APA carries important biological implications for establishment and maintenance of tissue identity in C. elegans.

In summary, through extensive transcriptome analysis, we now provide evidence that APA is widely operative among C. elegans tissues and rearranges the expressed landscape of miRNA targets to promote unique patterns of tissue-specific protein expression. Our results also provide an important resource for the worm community and are available through our C. elegans APA-centric database, www.UTRome.org.

550C An nhr-67 promoter element mediates expression in AC and VU uterine cells.  S. Bodofsky, S. Birnbaum, L. Thompson, S. Clever, B. Wightman  Biology Department, Muhlenberg College, Allentown.

The tailless family of nuclear receptors is highly conserved among animals. In humans, it functions in regulating neuronal stem cell differentiation. The C. elegans tailless ortholog, nhr-67, is expressed in a dynamic pattern in pre-uterine cells: initially in the 4 pre-VU cells during the L2, then upregulated in the anchor cell (AC) in response to the lin-12/lag-2 Notch reciprocal signaling system. During the L3 stage, nhr-67 expression is maintained at high levels in the AC and at low levels in VU descendants that produce the adult ventral uterus. nhr-67 is required for expression of the lin-12/Notch receptor in pre-VU and VU cells and for multiple markers of AC identity, indicating that it functions in differentiation of both uterine cell types. Loss of nhr-67 in the AC and pre-VU cells leads to a loss of lag-2 expression in the AC and a failure of the AC to exit the cell cycle, indicating that differentiation of the AC is compromised.

Deletion of a 276bp region of the nhr-67 promoter results in a loss of nhr-67 expression in pre-VU, AC, and VU cells. A 160bp enhancer that includes eight conserved candidate cis elements is necessary and sufficient for nhr-67 expression during ventral uterine development. The region includes two E box sequences that we propose bind the HLH-2 transcription factor, which functions in AC and pre-VU development. Deletion analysis and site-directed mutagenesis experiments indicate that homodimeric HLH-2 binding sites are required for AC and pre-VU expression, but loss of hhl-2 compromises reporter gene expression in the AC, but not pre-VU cells. Our data demonstrate the primary role of the E box sequences in regulating nhr-67 in the AC and pre-VU cells; the former apparently via HLH-2 homodimers and the latter via either another HLH protein or HLH-2 heterodimer.

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551A The role of chromosome condensation on RNA Pol II and transcription.  L Breimann1,2, K Kolyvanov1, S Ercan2, S Preibisch1  1) Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine, Berlin, Germany; 2) Center for Genomics and Systems Biology, New York University, New York, NY.

Chromosome condensation facilitated by condensin complexes is a prerequisite for mitotic segregation of DNA and impaired condensin function has been linked to genome instability, a hallmark of cancer. Recent data suggest that condensins not only function during mitosis but also affect gene regulation during interphase by condensing chromosomes. However, the mechanistic foundation for this gene regulatory function of condensin remains poorly understood.

Studying condensin separate from its function in mitosis is required to understand the role of condensin-mediated chromatin condensation on gene regulation. Most metazoans express two condensin isoforms, while Caenorhabditis elegans additionally expresses condensin DC (dosage compensation) that binds to and represses transcription from the X chromosome in
hermaphrodites to equalise X chromosome transcript levels in both sexes. Therefore, studying condensin dynamics using condensin DC allows quantification of transcription and RNA Pol II dynamics in interphase without affecting mitotic condensin functions.

In our study, we aim to provide a quantitative and dynamic understanding of how chromosome condensation regulates transcription on a single-cell and single-molecule level. Therefore, we are using high-resolution lightsheet microscopy and single-molecule transcription imaging to elucidate the connection between regulation of chromosome structure and transcription during development. To measure the effects of condensin DC-mediated condensation on transcription, we quantify expression of individual genes by single molecule RNA fluorescence in situ hybridization (smFISH) and live transcription imaging and correlate these data with Pol II and condensin DC signals. We combine the high spatio-temporal resolution of lightsheet acquisitions with the high degree of stereotypicity of C. elegans to map fixed smFISH acquisitions to the precise developmental stage of each fixed sample.

Taken together, this approach will allow us to investigate condensin-mediated transcriptional regulation with an unprecedented level of detail and reveal how chromosome condensation directly affects transcription.

552B Genomic analyses of interactions that govern an RNA regulatory network in stem cells. Brian Carrick¹, Aman Prasad¹, Douglas Porter¹, Peggy Kroll-Conner², Timothy Guthrie¹, Marvin Wickens¹, Judith Kimble¹,² 1) Department of Biochemistry, University of Wisconsin-Madison, Madison, WI; 2) Howard Hughes Medical Institute, University of Wisconsin-Madison, Madison, WI.

mRNA regulation governs when, where, and how much protein an mRNA produces and thereby determines cell identity, function and behavior. A single mRNA regulatory protein can bind to and control hundreds to thousands of mRNAs, forming a “network” that enables control of a biological outcome. This work focuses on C. elegans FBF-1 and FBF-2 (collectively termed FBF), which maintain germline stem cells (GSCs). FBF is a member of the PUF (Pumilio and FBF) family of RNA-binding proteins (RBPs), which bind mRNAs via sequence-specific elements in the 3’ untranslated region (3’UTR) and regulate expression. We have now identified the RNAs bound by FBF in vivo in both oogenic and spermatogenic germlines using the technique of iCLIP (individual-nucleotide resolution CrossLinking and ImmunoPrecipitation). The goal of this work is to learn FBF targets that function independently of gender, as well as gamete-specific targets and to thereby gain insight into FBF sub-networks. Like other PUF proteins, FBF acts with protein partners which can modulate binding specificity in vivo. The challenge now is to understand how these partners affect FBF regulatory networks in vivo. Currently, we are delineating protein – protein interfaces of FBF with its partners and generating CRISPR-induced mutants that abrogate those partnerships. Ultimately we will analyze how the FBF sub-networks fluctuate in response to changing FBF partnerships.

553C Real time observation of DNA unwinding mode of Caenorhabditis elegans HIM-6 helicase. S. Choi¹, H. Cho², H. Kim², B. Ahn¹ 1) Life science, University of ulsan, Ulsan, KR; 2) Life science, Ulsan National Institute of Science and Technology, Ulsan, KR.

Bloom’s syndrome (BS) is caused by mutations in the BLM gene encoding BLM helicase. BLM helicase contains conserved RecQ helicase domain which includes 3’-5’ helicase activity. BLM unwinds various DNA helicase substrates including replication forks. Cells from BS patients, that lack the BLM, show defects in the response to replicative stress and contain a multitude of chromosomal aberrations. A BLM homolog, him-6, has been identified in C. elegans based on its close homology with human BLM. Here, we used single-molecule FRET to measure DNA unwinding in real time. Our results showed that the HIM-6 repetitively unwound a long forked DNA depending on ATP concentrations and resolved flap and D-loop DNA, all of which are intermediates in DNA repair. Also, we found that HIM-6 unwound at most 25 base pairs of duplex DNA in a speed of 28 nucleotides per second before moving back. Besides, repetitive unwinding mode of HIM-6 was changed to unidirectional unwinding in the presence of RPA, indicating that its unwinding mode can be modulated by proteins in vivo. This study can be used to search proteins which would modulated HIM-6 unwinding mode on different DNA structures, such as Holliday junction and G4-quadruplex.

554A A new Caenorhabditis elegans 3’-5’ exonuclease. S. Choi, B. Ahn  Life science, University of ulsan, Ulsan, KR.

There are a few RNase D family members in Caenorhabditis elegans, ZK1098.3, mut-7 and C14AA4. The RNase D domain is itself similar to the proofreading 3’-5’ nuclease domain found in many DNA polymerases. In particular, ZK1098.3 is homologous to 3’-5’ exonuclease domain of Werner syndrome protein or n ortholog of human EXD3 (exonuclease 3’-5’ domain containing 3an ortholog of human EXD3 (exonuclease 3’-5’ domain containing 3). Here, we characterized the nuclease activity of ZK1098.3 which encodes a 784 amino acid protein. ZK1098.3 efficiently degraded the 3’ recessed strand of double-stranded DNA, but had little or no activity on blunt-ended DNA, the 5’ recessed strand of dsDNA or single-stranded DNA. ZK1098.3 mutants or its RNAi worms were sensitive to UV irradiation and ZK1098.3 foci appeared in response to UV irradiation. These results suggest that this nuclease is concerned with a various DNA repair process, such as nucleotide excision repair (NER) or base excision repair (BER).

We are going to further present the RNase activity of ZK1098.3 and developmental roles of this nuclease.

555B Homeostatic response to zinc deficiency in C. elegans is mediated by the LZA enhancer and the zinc transporter ZIP-2.3. N. Dietrich, C. Tan, D. Schneider, K. Kornfeld  Department of Developmental Biology, Washington University, Saint Louis, MO.

The essential element zinc plays an important structural and functional role in proteins in all living organisms. Zinc homeostasis is critical, because both zinc deficiency and excess are deleterious; in humans, defective zinc homeostasis leads to several disease states. The mechanisms utilized by animals to respond to excess zinc have been extensively characterized, but
much less is known about how animals sense and respond to zinc deficiency. A primary method animals use to increase zinc content is through the zinc transport family known as the Zrt, Irt-like proteins (ZIPs). *Caenorhabditis elegans* is a powerful experimental system to study the mechanisms of zinc deficiency based on sophisticated genetic and cell biological approaches, and studies of *C. elegans* are likely to be relevant to humans, since both worms and humans have 14 ZIP family members. To characterize the response to zinc deficiency, we measured the transcriptional response of all 14 *C. elegans* ZIP genes and identified three that are activated in response to low levels of dietary zinc. By analyzing the promoters of these genes, we identified an evolutionarily conserved cis-regulatory element, which we named the low zinc activating (LZA) element. The LZA is both necessary and sufficient for the induction of mRNA in response to low dietary zinc. To determine factors that function in trans to mediate the activity of the LZA, we used RNAi of candidate genes and showed the LZA requires the function of the GATA transcription factor ELT-2 and the mediator complex member MDT-15 to cause an increase in mRNA expression in low zinc conditions. The function of this element is also conserved in human cells, as cells transfected with the LZA in a vertebrate promoter displayed a sequence dependent induction of a reporter gene. These results identify a novel and conserved enhancer that responds to low zinc in animals. One of the genes induced by low zinc is zipt-2.3. To analyze the function of this gene, we identified a deletion allele and showed that mutant animals display hypersensitivity to zinc deficiency. Thus, zipt-2.3 is necessary to survive a low zinc challenge. To analyze the biochemical activity of ZIPT-2.3, we expressed the protein in vertebrate cells and identified a deletion allele and showed that mutant animals display hypersensitivity to zinc deficiency. Thus, zipt-2.3 is necessary to survive a low zinc challenge. To analyze the biochemical activity of ZIPT-2.3, we expressed the protein in vertebrate cells and measured zinc uptake. ZIPT-2.3 mediated zinc uptake, demonstrating that it functions as a zinc transporter. These results elucidate low zinc homeostasis by identifying a zinc transporter that is induced by low zinc and is necessary to respond to zinc deficient conditions.

**556C Quantitative proteomics and interactomics in Caenorhabditis elegans.** S.F. Dietz¹, M. Vasconcelos Almeida², R.F. Ketting², F. Butter¹ ¹) Quantitative Proteomics, Institute of Molecular Biology, Mainz, Rhineland Palatinate, DE; 2) Biology of non-coding RNA, Institute of Molecular Biology, Mainz, Rhineland Palatinate, DE.

Quantitative proteomics by mass spectrometry is a powerful tool to investigate the interactions and expression of proteins. We applied different molecular biology and quantitative proteomics methods to define interacting proteins, the composition of complexes and differences in protein expression levels in the model organism *Caenorhabditis elegans*, demonstrating that the combination of these techniques can be routinely used to identify and characterize endogenous proteins in the absence of suitable antibodies. We here compare several approaches for quantitative proteomics: 1) introducing a mass label metabolically via feeding with *E. coli*, 2) reductive demethylation, which introduces a mass tag at the peptide level during the sample preparation and 3) label free quantitation, which compares peptide intensities across different mass spectrometry measurements. To benchmark these three techniques for their applicability and benefits, we performed DNA pull-down experiments with nuclear extracts using the the telomeric sequence of *C. elegans* (TTAGGCAₙ) or a scrambled control (AGGTCAn). In all three approaches, we were able to determine proteins binding to the telomeric repeat sequence including the known binders POT-1, POT-2 and MRT-1 alongside proteins not previously implicated in telomere biology that are currently further investigated. Moreover, in a pilot experiment, we coupled native size exclusion chromatography with label-free quantitation to obtain a global overview of *C. elegans* protein complexes. Proteins belonging to a complex should show co-elution. This could be validated by several members of known complexes, like the CUL-4/DDB-1 ubiquitin ligase complex, the NuRD/CHD chromatin remodeling complex and the DCR-1/RDE-4 complex which co-occurred in same fractions, suggesting that protein-protein interactions can be recovered using this approach. Additionally, this extended fractionation allowed us to measure an in-depth proteome of >5000 proteins. Besides interactomics, mass-spectrometry based quantitative proteomics can be used to study differences in protein levels similar to next generation sequencing for transcriptomes. We determined significant protein abundance differences between the wildtype and the *pot-2(tm1400)* knockout strain by label free proteomics. Overall, we show that diverse classical molecular biology techniques can be combined with quantitative proteomics approaches to readily uncover protein functionality in *C. elegans*.

**557A hizr-1 mediates a subset of the cadmium-activated stress response.** B.J. Earley, C. Cubillas, M.D. Lyons, K. Kornfeld Washington University in Saint Louis, Saint Louis, MO.

Exposure to the toxic metal cadmium is a global health problem. There is an urgent need to develop therapeutic strategies that target cadmium injury, but the mechanisms responsible for cadmium toxicity and the organismal response to cadmium remain poorly understood. Cadmium is chemically similar to zinc, since they are in the same column of the periodic table. By analyzing the transcriptional response to high dietary zinc, we discovered the High Zinc Activation (HZA) enhancer that mediates transcriptional activation. Furthermore, we demonstrated that the HZA also responds to cadmium. HIZR-1 is a nuclear receptor transcription factor that directly binds the HZA enhancer, thereby activating multiple zinc homeostasis genes in response to high dietary zinc. Loss-of-function mutants in *hizr-1* fail to activate zinc homeostasis genes, and *hizr-1* mutants are hypersensitive to high zinc toxicity. We hypothesized that HIZR-1 might also function as a cadmium receptor. Consistent with this model, we demonstrated that cadmium binds the ligand binding domain of HIZR-1 with comparable affinity to that of zinc. Using transgenic animals expressing a HIZR-1::GFP fusion protein, we showed that HIZR-1 accumulates in the intestinal nuclei following exposure to both zinc and cadmium but not a control metal, manganese. To characterize the global transcriptional response to cadmium and the role of *hizr-1*, we are currently identifying cadmium activated transcripts. We have identified multiple cadmium induced genes that are *hizr-1* dependent as well as many that are *hizr-1* independent. To determine the function of these genes, we are currently performing genetic studies of cadmium sensitivity in mutant animals. Surprisingly, *hizr-1* mutants are not hypersensitive to cadmium toxicity, suggesting that targets of *hizr-1* promote high zinc resistance but not cadmium resistance.
Thus, cadmium may hijack the high zinc homeostasis response, and this response may not be protective in the face of cadmium toxicity.

558B  The Nuclear Receptor HIZR-1 Uses Zinc as a Ligand to Mediate Homeostasis in Response to High Zinc.  B.J. Earley 1, C. Cubillas 1, K. Warnhoff 2, K. Kornfeld 1  1) Development Biology, Washington University School of Medicine, Saint Louis, MO; 2) Department of Molecular Biology, Massachusetts General Hospital, Boston, MA.

Nuclear receptors were originally defined as endocrine sensors in humans, leading to the identification of the nuclear receptor superfamily. Despite intensive efforts, most nuclear receptors have no known ligand, suggesting new ligand classes remain to be discovered. Furthermore, nuclear receptors are encoded in the genomes of primitive organisms that lack endocrine signaling, suggesting the primordial function may have been environmental sensing. We identified a novel Caenorhabditis elegans nuclear receptor, HIZR-1, and showed that it is a high zinc sensor in an animal and the master regulator of high zinc homeostasis. To analyze the transcriptional response to high zinc, we characterized four genes activated by high dietary zinc: the zinc-binding metallothionein genes mtl-1 and mtl-2, and the cation diffusion facilitator (CDF) zinc transporter genes cdf-2 and ttm-1b. We showed that transcriptional activation of these genes is mediated by the high zinc activation (HZA) element, a DNA enhancer. We performed a forward genetic screen to identify mediators of high zinc activated transcription that resulted in the discovery of the high zinc activated nuclear receptor (HIZR-1). hizr-1 encodes a nuclear receptor transcription factor that has an evolutionarily conserved DNA-binding domain (DBD) and ligand-binding domain (LBD). We demonstrated that HIZR-1 is both necessary and sufficient to activate transcription of endogenous zinc-homeostasis genes in response to high dietary zinc. We used genetic and biochemical approaches to analyze HIZR-1 function. The LBD directly bound zinc, which promoted nuclear accumulation and activation of the protein, indicating the LBD regulates protein activity and zinc is a physiological ligand; the DBD directly bound the HZA enhancer, which mediates transcriptional activation of multiple genes involved in zinc homeostasis. These findings advance the understanding of zinc biology by identifying a sensor for high zinc in animals and elucidate homeostatic systems by defining a positive feedback loop embedded in a negative feedback circuit. To develop a comprehensive understanding of the homeostatic response mediated by hizr-1, we are currently using multiple techniques to identify the full set of hizr-1 target genes. Thus far, we have identified more than 100 genes transcriptionally activated by high zinc conditions, including multiple genes involved in autophagy, lipophagy, insulin production/processing and carotenoid metabolism. The analysis of HIZR-1 expands the understanding of the NR superfamily by (1) identifying transition metals as a physiological ligand that is distinct from previously described classes such as steroids and lipids and (2) identifying direct nutrient sensing as a new function that may represent a primordial role of NRs.

559C  Direct and Positive Regulation of bed-3 by BLMP-1.  H.T Fong, T Inoue  Biochemistry, National University of Singapore, Singapore, SG.

In the Caenorhabditis elegans, blmp-1 encodes the ortholog of the human zinc finger and SET domain-containing transcriptional repressor PRDM1/BLIMP1. blmp-1 is a heterochronic gene regulating several developmental pathways such as dauer formation and cell migration. Previously, we reported that BLMP-1 positively and directly regulates bed-3, a gene required for molting and vulval development. This provides the first evidence that BLMP-1 can act as a transcriptional activator in C. elegans. To identify interacting partners of BLMP-1 in the regulation of bed-3, we tested 59 candidate genes using a GFP reporter and qPCR and identified three positive regulators, F58A3.1/ldb-1, F25H8.2 and ZK1128.5/ham-3. Moreover, knock-down of these genes do not reduce bed-3 expression level in the blmp-1 mutant background, suggesting that these genes may act with BLMP-1. F25H8.2 is a homolog of the human NAF1 required for H/ACA ribonucleoprotein complex assembly and ham-3 encodes a component of the SWI/SNF complex. Knock-down of other components of these complexes also reduce bed-3 expression. Thus, these results suggest that ldb-1, H/ACA ribonucleoprotein complex and SWI/SNF complex regulate bed-3 and may contribute to positive regulation of bed-3 by BLMP-1.

560A  The role of specificity in parallel networks of post-transcriptional control in the germ line.  D. Fonseca 1, V. D. Bhat 2, T. Lo 2, Z. T. Campbell 2  1) Biology, Ithaca College, Ithaca, NY; 2) Biological Sciences, University of Texas-Dallas, Richardson, TX.

Proper eukaryotic development requires the precise regulation of post-translational modifiers such as RNA-binding proteins. Defects in RNA-binding proteins or their target sites can disrupt the cell-cycle, resulting in tumor formation, a hallmark of cancer. In C. elegans, members of the conserved PUF (Pumilio and FBF) protein family regulate germline proliferation. PUF-8 and FBF (two paralogs fbf-1 and fbf-2) function in the mitotic region of the germ line yet have distinct biological functions. Both recognize RNA via eight modular repeat units. However, the length of the consensus binding motif differs. PUF-8 recognizes an eight nucleotide element (PBE) while FBF-2 binds to a nine nucleotide consensus motif (FBE). Previous work has shown that the fifth repeat of FBF-2 is essential for flipping out the sixth base of the mRNA strand. We hypothesized that alterations to this repeat may switch its binding preference from FBE to PBE. This mutant enables us to examine if specificity is a driving force for establishment of parallel regulatory functions. To test this, we will examine if this mutation in FBF-2 rescues the mitotic tumor phenotype associated with loss of puf-8 function in the distal region of the germ line.

We used an in vitro molecular genetics screen to identify mutations in the fifth repeat of FBF-2 that resulted in a switch for binding from FBE to PBE. After screening ~5,000 unique transformants, one provided the key molecular phenotype. Mutations with confirmed FBE to PBE binding switches, are being introduced in the endogenous C. elegans fbf-2 locus using CRISPR/Cas9. Phenotypic and genetic characterization of these mutants will reveal key mechanistic features required for PUF regulatory function.
Regulation of RNA polymerase II recruitment during X-chromosome dosage compensation. N. J. Fuda1, B. J. Meyer1,2 1) Molecular and Cell Biology, UC Berkeley, Berkeley, CA; 2) Howard Hughes Medical Institute.

The dosage compensation process offsets the difference in X-chromosome copy number between the sexes through an X-chromosome-wide mechanism that equalizes RNA polymerase II (Pol II) transcription. A specialized condensin complex, the dosage compensation complex (DCC), regulates a diverse group of genes that have wide-ranging expression levels and distinct regulatory pathways, and the mechanisms underlying the global control are not understood. Our lab used Global Run-On sequencing (GRO-seq) to quantify the levels of transcribing Pol II on genes in wild type and DCC-mutant embryos. We found that the level of transcribing Pol II increased uniformly across X-chromosome genes in DCC-mutant embryos, indicating that dosage compensation regulates an early step in transcription, prior to transcription elongation. Before Pol II can transcribe across genes during the elongation phase, several steps in the transcription cycle need to occur in the promoter region: chromatin opening to allow Pol II access to the promoter, general transcription factor (GTF) binding and Pol II recruitment to form the pre-initiation complex (PIC), and initiation of transcription. We are using two approaches to investigate the regulation of Pol II recruitment during dosage compensation. First, we are examining the dynamics of Pol II behavior using high-resolution microscopy of fluorescently-tagged Pol II in live wild-type and DCC-mutant embryos to characterize Pol II recruitment to X chromosomes and autosomes. Second, we are performing ChiP-seq of GTFs in wild-type and DCC-mutant embryos to determine whether dosage compensation alters PIC formation to reduce Pol II recruitment. We will present results from both approaches, which clarify the role of Pol II recruitment in controlling gene expression during dosage compensation and the mechanism underlying the regulation of Pol II recruitment.

Examining the interplay between cell cycle regulators and chromatin factors in coordinating cell cycle exit and differentiation. M. Godfrey, A. van der Vaart, S.J.L. van den Heuvel Developmental Biology, Utrecht University, Padualaan 8, 3584CH Utrecht, The Netherlands.

Combining cell-division arrest with terminal differentiation is critical for normal development and tumor suppression, yet it remains poorly understood how these processes are jointly regulated. Recently, we used conditional gene inactivation in the C. elegans mesoblast lineage to better understand the coordination between cell cycle exit and differentiation (Ruijtenberg and van den Heuvel, Cell 2015). Mesoblast daughter cells lacking certain (combinations of) negative regulators of the G1/S transition failed to properly exit the cell cycle and showed mild overproliferation. Interestingly, inactivation of subunits of the SWI/SNF chromatin remodeling complex created a similar phenotype. Strikingly, simultaneous inactivation of SWI/SNF subunits and cell cycle inhibitors dramatically interfered with cell cycle exit and caused massive overproliferation. These data support that SWI/SNF-mediated chromatin remodeling acts redundantly with cell cycle inhibitors to promote cell cycle exit during terminal differentiation.

Sarcopenia and Calsequestrin, Junctin, and the ryanodine receptor. Brittany Graham1, Ian A. Hope1, Marie-Anne Shaw2 1) School of Biology, FBS, University of Leeds, LS2 9JT, UK; 2) Leeds Institute of Biomedical and Clinical Sciences, St James’s University Hospital, Leeds, LS9 7TF, UK.

Sarcopenia is the degeneration of skeletal muscle with age. One factor thought to contribute to ageing is the reduced force of natural selection with age, which means gene function in later life is under less selective pressure. We suggest that gene products required stochiometrically may become progressively more out of balance with age, and this might contribute to sarcopenia. The ryanodine receptor, RyR1, is the Ca^{2+} channel of the skeletal muscle sarcoplasmic reticulum (SR) and a strong candidate for such age-related deterioration as this protein is subject to elaborate control systems within an extensive protein complex and cells are sensitive to cytoplasmic Ca^{2+} levels.

C. elegans provides a good model for studying ageing. Assessment of the C. elegans RYR1 orthologue, unc-68, and other genes relevant to calcium homeostasis revealed age-related divergence in expression profile for two genes. Prior transcriptomic RNA-seq data1,2 was analysed to assess transcript levels across the lifespan through an online pipeline called Galaxy. While the expression levels of most of the selected genes appeared consistent in comparison to unc-68, csq-1 showed a large decrease in relative gene expression with age. Calsequestrin, CSQ-1, is responsible for Ca^{2+} binding within the SR. At low luminal Ca^{2+}, CSQ-1 inhibits RyR1 opening, and it has been suggested that absence of CSQ-1 may increase Ca^{2+} leak from the SR. The second gene identified, K09A9.6, encoding aspartate beta-hydroxylase, also showed a marked, if smaller, decrease in transcript levels across the lifespan, relative to unc-68. One of the products of the mammalian orthologue of K09A9.6, ASPH, is Junctin, a CSQ-1 binding protein in the SR membrane that localises with RyR1. Interactions between RyR1 and CSQ-1 are facilitated by Junctin. To validate the RNA-seq studies, a quantitative PCR analysis is being conducted on N2 C. elegans at 0, 5, 10 and 20 days of adult life.
This analysis has provided a basis for further investigation into potential contributions of CSQ-1, Junctin and RyR1 to sarcopenia. Strains with modifications of these genes could reveal more about their role in muscle ageing and lead to these genes becoming targets through which to delay human muscle ageing.

We thank the *C. elegans* researchers who made their transcriptome data freely available for our analysis.


**564B**  *clk-2* is a novel player in the *C. elegans* nonsense-mediated mRNA decay. Yanwu Guo, Rafal Ciosk Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, 4058 Basel, Switzerland.

Surveillance of aberrant RNAs is important for development and disease. To identify novel factors monitoring mRNA processing, we created a strain expressing a GFP reporter under the control of an aberrant 3'UTR. The reporter mRNA is degraded in wild-type animals. Following mutagenesis, we isolated mutants permitting expression of this reporter and found that most of them correspond to known components of the *C. elegans* nonsense-mediated mRNA decay pathway (NMD; encoded by the *smg* genes). The SMG proteins are conserved players in the NMD pathway, which is used to degrade aberrant mRNAs containing premature termination codons but also affects the stability many "normal" mRNAs. In worms, a previous study by Quinn et al reported NMD-mediated processing of pseudogenes. However, based on RNA sequencing datasets from different eukaryotes, the connection between NMD and pseudogenes appears to be nematode-specific. In addition to known NMD factors, our screen uncovered *clk-2*, previously implicated in DNA damage, as a novel player in the nematode NMD. CLK-2/TEL2 is a conserved protein, best known in humans for a function in telomere biology. Nevertheless, TEL2 was previously linked to NMD and our results suggest that CLK-2/TEL2 is a conserved player in the NMD pathway. The molecular mechanism of CLK-2 in NMD is currently under investigation.

**565C**  Identification of transcription factors binding to the conserved PD motif in the osm-9 TRPV channel gene promoter in Caenorhabditis elegans. E. Pujadas1, J. Cartella2, M. Nishiguchi1, S.E. Hall2 1) Dept Biol, Syracuse University, Syracuse, NY; 2) Monroe Community College, Rochester, NY.

Environmental stress experienced during early development can affect adult phenotypes through stable changes in gene expression. In *C. elegans*, stress experienced during the first larval stage, including starvation, crowding, and high temperature, promotes entry into the non-aging, stress-resistant dauer stage. Larva will exit dauer and resume continuous development when conditions improve. We have shown previously that postdauer (PD) adults retain a cellular memory of their developmental history that is reflected in changes of gene expression and behavior compared to continuously developing adults (CON).

Recently, we have shown that the *osm-9* TRPV channel gene is down-regulated in ADL neurons of PD adults, while remains expressed in CON adults, resulting in altered olfactory behavior in response to high concentration of ascr#3 pheromone component. We identified a *cis*-acting promoter element called the PD motif that is required for the down-regulation of *osm-9* in PD ADL neurons, which is composed of a DAF-3 SMAD binding site adjacent to a 29 bp conserved sequence region. The PD motif is present in the upstream regulatory regions of 977 *C. elegans* genes and is bound by DAF-3 and ZFP-1 in PD animals. However, our mutational analysis of the PD motif indicates that additional transcription factors bind to the PD motif to both positively and negatively regulate *osm-9* expression. We have used a yeast one-hybrid approach performed by undergraduates in the laboratory and classroom to determine what other transcription factors are binding to the sequence components of the PD motif to regulate gene expression based on developmental history. Our preliminary results suggest that additional SMAD proteins acting in the TGF-β pathway, DAF-8 and SMA-4, are also directly interacting with the PD motif. We are currently verifying and characterizing the role of transcription factors identified in our screen in the regulation of *osm-9* in *C. elegans* animals. Given that the PD motif is present in numerous genes, our results will contribute to the understanding of their regulation due to a TGF-β sensitive PD motif.

**566A**  Modeling NGLY1 deficiency in Caenorhabditis elegans. T. Hansen, A. Golden National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD.

To date, there are approximately 7,000 rare diseases worldwide, 80% of which are thought to be monogenic in origin. In the United States alone, it is estimated that 1 in 13 people suffer from a monogenic rare disease. Unfortunately, the development of treatments for these patients has been difficult as disease populations are inherently small and treatment development is very expensive. As a result of this dilemma, rare disease patients face a major medical disparity, as only 5% of all rare diseases have current treatment. To ameliorate this, we suggest modeling monogenic rare diseases in *C. elegans*, a well-characterized model system. *C. elegans* are inexpensive to work with, have a fast, three-day generation time, and have a simple anatomy yielding comprehensible phenotypes. Importantly, the *C. elegans* genome has conserved orthologous genes to roughly 60% of all human genes, permitting its use to model thousands of diseases via CRISPR-based genome editing.

In this project, we modeled a rare monogenic disease known as NGLY1 deficiency. Patients with this disease are homozygous or compound heterozygous for mutations in *NGLY1*, a peptide-N-glycanase, and suffer with developmental delay/intellectual disability, hyperkinetic movement disorder, and hypolacrma (absence of tears). Studies in other organisms, and *C. elegans* (Lehrbach & Ruvkun. *eLife* 2016), suggest that NGLY1 may be involved in the degradation of misfolded proteins in the ER. Using CRISPR/Cas9, we modeled one patient allele, R542X, by introducing a nonsense mutation at the conserved Arg residue.
in the *C. elegans* ortholog, *png-1*. We also made a complete gene deletion of *png-1*, serving as a null mutant. Both mutants do not yield an obvious morphological or developmental phenotype under normal growth conditions. However, when treated with the proteasome inhibitor, bortezomib, both mutants arrest at an early larval stage. Taking advantage of this phenotype, we are currently conducting a forward-based, chemical mutagenesis suppressor screen on our patient R542X model. This experiment could provide genes that, when mutated, revert the phenotype to normal growth in the presence of inhibitor. Such genes would be great drug targets as treatment for NGLY1 deficiency. This may also identify novel factors involved in NGLY1-associated pathways, like the ER-associated degradation pathway, and help to elucidate the unknown disease mechanism. We also plan to model additional patient alleles.

567B The BLMP-1 transcription factor affects rhythmic mRNA accumulation during *C. elegans* larval development. Y. Hauser, M. Meeuse, G. Hendriks, D. Gaidatzis, S. Carl, H. Grosshans Friedrich Miescher Institute, Basel, CH.

Development of an organism requires proper timing of events such as cell proliferation and cell fate choices. Biological ‘clocks’ are thought to orchestrate such timekeeping but their molecular nature and properties have remained elusive. Previously, we reported oscillating transcript levels for roughly 20% of the *C. elegans* transcriptome. Different transcripts peak at distinct time points but always once per larval stage (e.g., every ~8 hrs at 25°C), and fold changes are large at > 2.1-fold and often > 10-fold. We propose that these massive oscillations are the output of a developmental clock, thus providing a robust phenotype to dissect a developmental clock in a genetically tractable system. Here, we report that periodic transcription drives the rhythmic mRNA level changes. We identify BLMP-1, orthologue of the mammalian lineage specifier Blimp1/Prdm1, as a relevant transcription factor: Loss of BLMP-1 activity causes arrhythmic animal development, molting defects, death, and impairs oscillatory expression of its target gene *daf-6*. Hence, we consider *blmp-1* a candidate core clock gene, and I will present results from our ongoing analysis aimed at understanding the extent and physiological role(s) of *blmp-1* controlled gene expression oscillations.

568C Genome-wide mapping and characterization of regulatory elements across development. Jürgen Jänes, Yan Dong, Alex Appert, Julie Ahringer The Gurdon Institute, University of Cambridge, Cambridge, United Kingdom.

The genome-wide identification of regulatory regions is necessary for understanding developmental gene regulation. Regulatory elements such as promoters and enhancers are usually nucleosome depleted, and so can be identified by their accessibility to digestion by nucleases such as DNAse I or to transposition by Tn5 (ATASeq). Using ATAC-seq, we have generated genome-wide maps of regulatory elements across the six *C. elegans* developmental stages and characterized their properties. We define more than 30,000 sites, with most regions showing developmental changes in accessibility.

To annotate and characterize the regulatory regions, we further mapped transcription initiation, transcription elongation, and four histone modifications (H3K4me3, H3K4me1, H3K36me3 and H3K27me3) at the six developmental stages. Consistent with previous work showing that transcription initiation occurs at both promoters and enhancers, we find that more than 80% of the ATAC-seq sites have transcription initiation signal. In contrast, only a subset has clear transcription elongation signal linked to genes, indicative of promoter activity. Using these metrics, we annotated over 10,000 accessible regions as promoters; the remainder are annotated as putative enhancer elements. Examining histone modifications at promoters, we found that the classical promoter-associated signature of high H3K4me3 and low H3K4me1 is associated with broadly expressed genes but does not reliably identify promoters of developmentally regulated genes. Genes are associated with a range of patterns of regulatory elements, ranging from a single promoter to having multiple promoters and up to dozens of putative enhancers scattered upstream as well as within intronic regions.

Characterizing accessibility of the 30,000 sites during development, we found that a quarter of sites show no changes. These are associated with genes broadly expressed across development and are enriched for H3K36me3. We also observed that a third of regions decrease and a third of regions increase in accessibility over development. Sites with increasing accessibility have lower GC content whereas sites with decreasing accessibility have higher GC content, and are enriched for H3K27me3. We also identified sequence features that are enriched at regulatory regions associated with transcription elongation. We identify and characterise features of *C. elegans* regulatory elements across development, providing an important new resource for studying transcription regulation.

569A The *C. elegans* condensin-like dosage compensation complex spreads linearly and bi-directionally from its recruitment sites. D. Jimenez, S. E. Albritton, L. Street, S. Ercan Department of Biology, NYU, New York, NY.

The evolutionarily conserved Structural Maintenance of Chromosomes (SMC) protein complexes include condensins and cohesins, which are critical regulators of chromosome structure in many nuclear processes. In many metazoans, there are two types of condensins that bind to distinct regions of chromosomes and serve distinct functions, yet it remains unclear how condensins target large chromosomal regions in the genome. In *C. elegans* a third complex called condensin DC is specifically targeted to the X chromosomes to accomplish dosage compensation. Condensin DC interacts with several other proteins as part of the genetically defined Dosage Compensation Complex (DCC). The DCC represses X gene expression two fold to compensate for unbalanced expression between XX hermaphrodite animals and XO male animals. Our previous work suggests that condensin DC binding to the X chromosomes is accomplished in two steps: recruitment and spreading. The recruitment initiates at a small number of primary recruitment sites that contain clusters of a 12-bp motif that coincide with high occupancy transcription factor target (HOT) regions. Initial recruitment is followed by cooperative recruitment at a set of secondary sites and spreading of the complex to the X chromosome. The mechanism by which condensin DC spreads along the X remains
unknown. Our published work using X;A chromosome fusions showed that the DCC spreads linearly from the X into the autosomal region up to ~1-3 Mb, with the DCC binding tapering off the further it spreads from the X, suggesting that as the DCC spreads further, it unloads and is unable to load again without a primary recruitment site in proximity. Supporting the ability of the DCC to spread large distances, deletion of a single recruitment site resulted in depletion of DCC binding within a 1-2 Mb window on the X. Here, we show that addition of multiple recruitment sites on an autosomal results in DCC recruitment and spreading, however the amount of both recruitment and spreading is lower than that occur on the X chromosome and the X;A fusions. When two primary recruitment sites are inserted at a distance (>30kb) from a secondary recruitment site on chromosome II, we observed that the DCC is recruited and spreads bi-directionally to a ~800 Kb domain. Our future work aims to determine the mechanisms of this linear bi-directional spreading, focusing on two possibilities: sliding of the condensin ring or hopping from one site to another site in physical proximity. Work from several species indicate that the molecular activities of SMC complexes are conserved, thus X-specific binding of condensin DC is a clear system to address how SMC complexes target large chromosomal domains in the genome.

570B Characterization of the transcriptional network that promotes gonadal development in Caenorhabditis elegans.

Menesha Lake, Caleb J. Braun, Naden Kreitz, Sellers Swann, Kayman Strickland, Mary B. Kroetz. Biology, University of South Alabama, Mobile, AL.

During embryogenesis, the C. elegans gonad originates as a primordium of cells consisting of two somatic gonadal cells that flank two germ line precursor cells. The gonadal primordium is morphologically identical in the two sexes. Midway through the first larval stage, the somatic gonadal cells divide and differentiate, and during the course of development they will ultimately form one of two dramatically different organs: either a single-armed testis in the male or two bilaterally symmetric ovolesis connected to a central uterus in the hermaphrodite. To help define genes responsible for promoting gonadal development, we identified transcripts enriched for expression in the developing somatic gonad compared to the whole animal using cell-specific RNA-seq (Kroetz and Zarkower, 2015). Additionally, transcripts that were expressed in a sex-biased manner in the gonad have also been identified. Sex-biased transcripts were defined as transcripts that were enriched in one sex of gonadal cells compared to the expression in the gonadal cells of the other sex. Of the more than 900 gonad-enriched transcripts, approximately 80 encode for proteins that are transcriptional regulators. Seventeen transcription factors are expressed in a male-biased manner, and nine transcription factors are expressed in a hermaphrodite-biased manner in the developing gonad. To better understand if and how these transcription factors are controlling expression of the gonad-enriched transcripts, we are characterizing the expression patterns of proteins whose transcripts show the strongest gonadal enrichment. We are using CRISPR-Cas to fuse GFP to the C-terminus of the endogenously expressed gonadal proteins. We then use loss-of-function mutations or RNAi against the sex-biased or gonad-enriched transcription factors to determine if the transcription factors are important for expression of the GFP-tagged genes. This work will better define the transcriptional networks controlling gonad development and give insights into how key genetic regulators promote sex-specific organogenesis.

571C A novel multi-domain protein CCAR-1 regulates transcription and co-transcriptional splicing.

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Accumulating evidence indicates that pre-mRNAs are co-transcriptionally spliced. However, mechanisms or factors affecting the fidelity of co-transcriptional splicing in living organisms are yet to be determined. We have previously reported use of fluorescence alternative splicing reporter worms for elucidating mechanisms of alternative splicing regulation in C. elegans. Here, we report a novel protein CCAR-1, which regulates transcription and alternative splicing. Fourteen ccar-1 alleles were isolated in a screen for mutants defective in neuron-specific selection of exon 7a of an unc-32 splicing reporter. With the same reporter, we had already proposed a model for the neuron-specific regulation of exon 7a by a neuron-specific CELF family splicing factor UNC-75 and RBFOX family splicing factors ASD-1 and FOX-1 (NAR, 2013). Like in the unc-75 and asd-1, fox-1 mutants, exon 7b was selected instead of exon 7a in the nervous system of the ccar-1 mutants. Unlike in the other mutants, however, double- and triple-inclusion of mutually exclusive exons unc-32 exon 4a/4b/4c occurred in the ccar-1 mutant. mRNAseq analysis revealed tens of alternative exons regulated by CCAR-1. Unlike in other splicing factor mutants, inclusion levels of all the affected exons were elevated in the ccar-1 mutant except for unc-32 exon 7a. Among such target exons was ccar-1 exon 5, indicating that CCAR-1 negatively regulates its own expression at the pre-mRNA splicing level like many other typical splicing factors. Unexpectedly, the intron downstream from ccar-1 exon 5 begins with evolutionarily conserved GA sequence instead of GT, and exon 5 was no longer skipped even in the wild-type worms when the GA site was changed into GT or GC in the splicing reporters. Nuclear run-on analysis revealed that co-transcriptional excision of ccar-1 intron 4 and intron 5 was facilitated in the ccar-1 mutant. These results indicate that CCAR-1 represses co-transcriptional splicing of exons with weak splice sites, which is critical for proper regulation of alternative splicing.

mRNAseq also suggested that CCAR-1 represses abundance of its own mRNA, which was confirmed by upregulation of a ccar-1 transcriptional fusion reporter in the ccar-1 mutants. Chromatin immunoprecipitation (ChIP)-qPCR analyses of the wild-type and ccar-1 mutant strains suggested that CCAR-1 is associated with its own promoter region to pause RNA polymerase II (Pol II) in the wild type. ChIP-seq analysis revealed that CCAR-1 is also associated with gene bodies of expressed genes like CTD-phosphorylated RNA Pol II, which led us to find that CCAR-1 and Pol II can be co-immunoprecipitated. These results indicate that CCAR-1 is a novel class of factors that associates with Pol II and represses transcription of its own pre-mRNA as well as co-transcriptional splicing of exons with weak splice sites to affect alternative splicing events in a variety of genes.
572A  How transcription factor literacy influences target gene transcription.  B. Lancaster, B. Goszczynski, J. McGhee  Alberta Children's Hospital Research Institute, University of Calgary, Calgary, Alberta, CA.

Transcriptional activation is a complex multifactorial process that centres on two fundamental properties of transcription factor proteins: sequence specific DNA binding and the subsequent recruitment of RNA polymerase to a target gene promoter. We have established an experimental system to investigate, inside the living animal, how transcription factor binding affinity at a promoter quantitatively influences transcriptional activity of a target gene. In C. elegans, the major intestinal transcription factor ELT-2 binds to a core TGATAA motif. The intestine-specific asp-1 protease gene is a direct target of ELT-2 and is controlled by two TGATAA sites. We have created two versions of asp-1 by introducing a novel KpnI restriction site at different locations in the coding region, thereby producing two reporters distinguishable by restriction digestion. The two reporters are combined into multicopy extrachromosomal transgenic arrays in the same animal, one reporter controlled by a wildtype promoter, the other by a promoter with altered ELT-2 binding sites. RNA isolation followed by RT-PCR, restriction digestion, and electrophoresis, allows the levels of both reporter transcripts to be measured simultaneously. Promoter swaps have confirmed that there is little reporter bias. We have measured relative affinities of ELT-2 for TGATAA variants in vitro by competitive band shifts, by both direct competition of two target sequences and by high-throughput competition of a library of target sequences (Spec-Seq). As an example, the in vitro ELT-2 binding affinity to the core sequence CATGATAATC is ten-fold lower than to ACTGATAAGA. When this low affinity sequence is introduced into both of two TGATAA sites of the asp-1 promoter, the level of asp-1 transcripts measured in vivo are reduced five-fold. Overall, the relation between transcription factor binding affinity at the promoter and mRNA production by the target gene appears to be roughly linear, with a plateau at the maximum transcriptional output. We have measured the production of nascent transcripts by nuclear run-on and confirm that the reduction in transcripts seen with variant promoters is the effect of decreased transcriptional initiation/elongation as opposed to mRNA processing and/or stability. We now aim to use this experimental system to investigate how chromatin factors might influence the relation between ELT-2 binding and target gene transcription.

572B  pals-22, a member of an expanded C. elegans gene family, is involved in repetitive transgene silencing.  Eduardo Leyva-Diaz1, Inés Carrera1,2, Nikolaos Stefanakis1,3, Oliver Hobert1  1) Department of Biological Sciences, Howard Hughes Medical Institute, Columbia University, New York, NY 10027, USA; 2) Worm Biology Laboratory, Institut Pasteur de Montevideo, Matoajo 2020, 11400 Montevideo, Uruguay; 3) The Rockefeller University, New York, NY 10065, USA.

Repetitive DNA sequences are subject to chromatin-based gene silencing in various animal species. Under specific circumstances repetitive DNA sequences can escape such silencing. For example, while exogenously added, extrachromosomal DNA sequences that are stably inherited in multicopy, repetitive arrays in the nematode C. elegans are often silenced in the germline, such silencing often does not occur in the soma. This indicates that somatic cells may utilize factors that prevent repetitive DNA silencing. Such “anti-silencing” factors have been revealed through genetic screens that identified mutant loci in which repetitive transgenic arrays are aberrantly silenced in the soma (Fischer et al. 2008, Grishok et al. 2005, Hsieh et al. 1999, Knight and Bass 2002). We describe here a novel mutant locus, pals-22 (for protein containing ALS2CR12 domain) with a transgene silencing phenotype. We find that pals-22 is a member of a large family of divergent genes, defined by the presence of an ALS2CR12 domain (39 members, including 2 pseudogenes). While family members are highly divergent they show striking patterns of genomic clustering. The family expansion appears C. elegans specific and has not occurred to the same extent in other nematode species (e.g. C. briggsae only codes for 6 pals genes). A PALS-22::GFP fusion protein that is fully functional in rescue assays displays a cytoplasmic subcellular localization, which rule out any direct chromatin-mediated silencing mechanism. This fusion protein is expressed across different tissues: the nervous system (pan-neuronally), muscle (pharyngeal, vulval, anal and body wall muscle), gut and seam cells. Lastly, the transgene silencing phenotype of pals-22 depends on the biogenesis of small RNAs, since silencing is abolished in the RNAi defective mutant rde-4, suggesting that pals-22 might regulate RNAI dependent silencing in the cytoplasm of neurons and other tissues.

573C  Identification of transcription factors regulating pan-neuronal gene expression.  Eduardo Leyva-Diaz1, Nikolaos Stefanakis1,2, Inés Carrera1,3, Oliver Hobert1  1) Department of Biological Sciences, Howard Hughes Medical Institute, Columbia University, New York, NY 10027, USA; 2) The Rockefeller University, New York, NY 10065, USA; 3) Worm Biology Laboratory, Institut Pasteur de Montevideo, Matoajo 2020, 11400 Montevideo, Uruguay.

While neuronal cell types in a nervous system display an astounding degree of diversity in their molecular composition, all neuron types share a panel of common features defined by the expression of pan-neuronal genes. Though a great deal is known about how the expression of neuron-type specific identity features is controlled in the nervous system, there is a lack of knowledge into which factors are controlling pan-neuronal gene expression programs. Through an extensive analysis of the cis-regulatory control region of a battery of pan-neuronal genes, we addressed several distinct scenarios for how pan-neuronal gene expression could be achieved. We found that pan-neuronal gene expression is controlled through a surprisingly complex array of redundantly acting cis-regulatory modules that direct expression to broad, overlapping domains through the nervous system. These redundantly acting cis-regulatory modules are responsive to a host of distinct trans-acting factors. We found that terminal selector factors and HOX genes as some examples of these factors, however the identity of most of them remains unknown. We are currently conducting multiple genetic screens to identify these trans-acting factors that bind to those cis-regulatory elements and control pan-neuronal gene expression. Our first mutants are presently being cloned and tested for involvement in the regulation of different pan-neuronal genes. In addition, we are performing a small-scale cis-regulatory analysis of a conserved vertebrate pan-neuronal gene to test whether this redundant modular model is conserved in vertebrates. Identifying factors involved in pan-neuronal gene regulation will provide insights into how the fully differentiated neuronal fate is developed, and might reveal key regulators of neuronal identity maintenance during normal development and neurodegeneration.
**575A Cis-regulatory changes in genes putatively under balancing selection.** L. Long, P. McGrath school of biology, Georgia Institute of Technology, Atlanta, GA.

Balancing selection is an evolutionary force that maintains two or more alleles in a population despite the actions of genetic drift. Genes under balancing selection offer a great resource to study the genetic basis of fitness advantages in natural environments. These fitness changes could be associated with different behavior response to salient stimuli. Previously, we have identified two pheromone receptors, srx-43 and srx-44, under balancing selection, putatively due to differences in expression in three sensory neurons (ASI, ADL, ASJ). Further, we showed that chemoreceptor genes are enriched in genomic regions of high Tajima’s D, suggesting that ~100 additional chemoreceptor genes are also under balancing selection. We aim to systematically study the cis regulatory differences between the two haplotypes in high Tajima’s D regions that can potentially explain their functional differences under selection. In order to achieve this, RNA-seq was conducted on the heterozygous progeny from the crossing of two C. elegans isolates, CB4856 and N2. We found massive allele specific expression between N2 and CB4856 alleles. In general, genes in high Tajima’s D region are expressed at a low level, which is consistent with our prediction that these genes are enriched for chemoreceptors whose expression is restricted to certain neurons. Overall, genes in high Tajima’s D region are more differentially expressed than other genes in the genome. To map these transcriptional differences back to the neuronal network and look into more details about the chemoreceptor genes, we used FACS to isolate specific types of neurons and studied the allele specific expression in sensory, inter and motor neurons. This study offers a new method to dissect the evolution and functional impact of cis-regulatory elements.


There is an increasing requirement for innovative toxicity test systems that are translational to humans yet allow reduction, refinement and replacement (3Rs) of animal use. It has been shown that “stand alone test models” are not sufficient for hazard assessment and combinatorial testing with multiple 3R models (especially for developmental and reproductive toxicology (DART)) is required to improve potential hazard identification. We introduce a combinatorial strategy using zebrafish (Danio rerio) larvae, nematodes (Caenorhabditis elegans) and social amoeba (Dictyostelium discoideum) as an innovative toxicity test system to enable identification of adverse outcome pathways (AOP) for human hazard identification and characterization. The selected test systems allow high-throughput screening and facilitate rapid, reproducible testing of compounds both on an organismal phenotypic level as well as on a molecular level (transcriptomics and activity profiling of kinetics). We have tested 42 DART positive compounds and each of the species showed high sensitivity and specificity levels indicating high predictive power. Of 42 DART positives only 1 compound was missed by all three test systems. Nine compounds were used in a detailed molecular proof of principle study to investigate molecular effects of DART chemicals in the test systems. Overlapping molecular responses of DART compounds could be identified within species amongst the selected set of DART compounds and also across species. Toxicogenomic profiling and hazard assessment revealed that the individual species are promising predictors for DART with clear added value.

**577C Identification of a novel trans-activation domain in the bZIP protein CEBP-1.** R. Malinow, K. Kim, Y. Jin.

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Transcription factors exert versatile molecular control of gene expression. Generally, transcription factors have both DNA binding domains and regulatory domains such as transactivation or signal sensing domains. The C. elegans bZIP protein CEBP-1 is a conserved transcription factor required for multiple processes including synapse development, neuronal stress response, and axon regeneration. CEBP-1 consists of 319 amino acids with the C-terminal bZip domain homologous to the CCAAT/enhancer binding family, and N-terminal shows no clear homology by sequences. Here, we took advantage of genetic interaction screening and CRISPR genome editing to uncover a putative trans-activation domain of CEBP-1. We previously reported that loss of function of cebp-1 fully suppresses the larval lethality in null animals of nipi-3 (Kim et al, 2016), which encodes a conserved Tribbles pseudokinase and is required for animal development and innate immunity. We designed a highly efficient scheme to select for suppressor mutations of nipi-3(0). We characterized a large number of mutations in cebp-1. We found many mutations affecting the canonical DNA binding domain of CEBP-1. Unexpectedly, we identified several missense mutations clustered in the N-terminal region of CEBP-1, and these mutations behave similarly as genetic null of cebp-1. To further explore the extent of the putative domain, we designed sgRNA-based targeted mutagenesis to this region. Our data reveal a stretch of 10-12 amino acids are absolutely essential for cebp-1 function. C/EBP proteins in higher vertebrates also contain transactivation domains in the N-terminal region to modulate the activity of the DNA binding bZip domain. We are currently testing the hypothesis that this N-terminal domain may act as a transactivation domain.

578A Comparing miRNAs across developmental stages of C. elegans and other nematodes. C.J. McGill, L. Serra, B. Rodriguez, M. Macchietto, J.L. Phan, A. Mortazavi 1) Developmental and Cell Biology, University of California Irvine, Irvine, CA; 2) Institution of Health and Bioinformatics, University of Minneapolis, Minneapolis, MN; 3) Department of Molecular Biology and Biochemistry, University of California Irvine, Irvine CA.

MicroRNAs (miRNAs) are small, 17-22bp, non-coding RNAs that have been shown to have a significant regulatory effect on gene expression. Measuring this regulation is vital to understanding how gene expression progresses through developmental programs. We are interested in studying this through the Caenorhabditis elegans developmental model system. Due to older technologies, previous studies had to be done on thousands of worms at a time, and thus, the resolution was poor. New technology has enabled us to cut that number to few worms, which has drastically improved the resolution. We are also profiling miRNAs at every stage of development to study the progression of miRNAs. We will compare our results to other nematodes such as Steinernema carpocapsae and Caenorhabditis angaria. This will be the first comparison of miRNAs expression at the single-worm level among distant nematode species with a focus on miRNAs involved in gene regulation during development.

579B Identification of novel kal-1 transcriptional regulators via bioinformatic approaches. Z. Mielko, L. Leitner, D. Carriker, E. Santorella, M. Hudson  Molecular and Cellular Biology, Kennesaw State University, Kennesaw, GA.

Kallmann Syndrome (KS) is a rare genetic condition that alters olfactory sensation and also hypothalamic neuron migration, which ultimately inhibits reproductive development. Over 20 KS genes have been identified to date, although only 35% of KS patients have identifiable mutations in those genes. This suggests that additional KS-related loci remain undiscovered. We hypothesize that transcription factors required for the expression of known KS genes may be KS loci in their own right. The human kal-1 gene is strongly conserved between vertebrates and invertebrates and when mutated, leads to X-linked KS. Curiously this gene is not found in rodents. As such, we are using a C. elegans model of X-linked KS to identify transcriptional regulators of the kal-1 gene.

The kal-1 promoter region contains multiple transcription factor binding sites identified via bioinformatic approaches and GFP-ChIP. Our reporter gene and loss-of-function analyses demonstrate the robustness of the kal-1 regulatory region to single transcription factor loss-of-function constructs from GFP-ChIP datasets. We performed a deletion analysis of the kal-1 promoter to narrow down which regions are required for kal-1 transcription at various stages of development and in what specific cell type. These data are being corroborated at single-cell level using kal-1-GFP and histone mCherry co-lineaging data.

580C Regulation of APL-1, the C. elegans ortholog of the Alzheimer’s Disease associated APP protein. A.B. Mir, A. Chait, C. Li 1) Biology, City College of New York, New York, NY; 2) Psychology, City College of New York, NY.

The neurodegenerative disorder Alzheimer’s disease (AD) can be characterized by the presence of amyloid plaques in the brain. These plaques consist chiefly of the β-amyloid peptide, a cleavage product of the poorly understood amyloid precursor protein (APP). The study of APP in mammalian systems is complicated by the presence of three separate genes encoding functionally overlapping proteins: APP, APLP1 and APLP2. We are using C. elegans, which has only a single APP-related gene apl-1, to examine the role of APP-related proteins.

APL-1 is a single-pass transmembrane protein; null alleles of apl-1 are lethal. This lethality can be rescued by expression of the APL-1 extracellular domain. Similarly, mutants in which the intracellular and transmembrane domains of APL-1 are deleted (apl-1(yn5)) are viable, demonstrating that the extracellular domain of APL-1 is necessary and sufficient for viability. In mammalian models, the extracellular domain of APP is released by the cleavage activity of the α-secretase ADAM complex. We investigated the cleavage of APL-1 in C. elegans by examining the ADAM mutants adm-4 and sup-17.

apl-1(yn5) mutants show a general developmental delay as well as a limited larval lethality at 25°C. This temperature sensitive lethality is greatly enhanced by knockdown of the IGF/insulin receptor ortholog daf-2; the double mutants show L1 arrest. In a forward screen to isolate suppressors of this L1 arrest, we isolated six suppressor mutants.

To further understand how apl-1 is regulated, we are comparing the promoter regions of Caenorhabditis strains to identify conserved regions. We have generated constructs that delete different regions and will assay whether they are able to rescue the apl-1 null phenotype.

581A Possible regulation of gene expression by RDE-4 and ADAR through dsRNA in introns. A. Mora-Martin, A. Grishok  Department of Biochemistry, Boston University School of Medicine, Boston, MA.

RDE-4 is a double-stranded RNA (dsRNA)-binding protein and a member of the Dicer complex required to initiate silencing in response to exogenous dsRNA. Research in our lab revealed that RDE-4 cooperates with a chromatin-binding protein ZFP-1, a C. elegans homolog of mammalian AF10, in regulation of hermaphrodite-specific neuron (HSN) migration. Supporting this fact, we reported that rde-4(null) and zfp-1(lof) mutants showed: 1) similar profiles of changes in gene expression (Grishok et al., 2008) and 2) HSN undermigration phenotypes (Kennedy and Grishok, 2014). However, the mechanistic connection between ZFP-1 and RDE-4 is not clear. Our research identified DOT-1.1, a histone methyltransferase, as an essential partner of ZFP-1 in transcription regulation (Cecere et al., 2013). Moreover, we found that dot-1.1 lof mutants exhibit HSN migration defects, similar to those observed in rde-4 and zfp-1 mutants. Therefore, we considered the possibility that RDE-4 directly or indirectly regulates dot-1.1 or zfp-1 genes. RDE-4 is well known to compete with ADAR proteins in binding with dsRNAs substrates.
ADARs are dsRNA-specific adenosine deaminase enzymes that convert adenosine to inosine, which is then read as G in sequencing. We found that the dot-1.1 gene contains dsRNA structures in its introns that are targeted by ADARs, according to the sites of deamination identified genome wide by the Bass (Whipple et al., 2015) and Rajewsky (Ivanov et al., 2015) labs. On the basis of these findings, we propose the possibility that RDE-4 and ADARs regulate dot-1.1 pre-mRNA splicing, which contributes to the HSN phenotype observed in the rde-4 mutant worms.

582B Modeling Rare Diseases in Caenorhabditis elegans. B. Nebenfuehr, A. Golden, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD.

Humans harbor approximately 7,000 rare diseases, ~80% of which are monogenic. A rare disease is defined as affecting less than one in 1,500 people. Combined, these rare diseases affect nearly 1 in 10 Americans (25 to 30 million people), and treatments only exist for around 5% of these diseases. Thanks to the advent of whole genome sequencing, the gene(s) responsible for many rare diseases are known, opening the door for more comprehensive studies. Around 60% of the more than 20,000 protein-coding genes in Caenorhabditis elegans are estimated to have human counterparts. We can therefore study worm phenologies of human disease, or the distinct phenotype in worms that, while different from the human phenotype, stems from mutations in a homologous gene. The CRISPR-Cas9 system also allows us to mutate individual nucleotides analogous to those implicit in the human diseases to mimic the patient alleles. Presented here are examples of three ongoing projects designed to uncover cellular interactors and potential drug targets for improving and expanding the treatments available to patients suffering from a rare disease.

Long-QT syndrome (LQT) is an autosomal dominant class of arrhythmia characterized by an extended time of repolarization of the heart after each beat (>440ms instead of the normal 350-440ms). This can lead to sudden cardiac arrest, particularly during exercise or other excitement. One of the genes most closely associated with LQT is the alpha-subunit of the potassium channel KvLQT1, or KCNQ1. The C. elegans ortholog of KCNQ1 is kqt-3 (87.9% homology) and deletion alleles of this gene display arrhythmic pumping in the pharynx (NemaMetrix, Eugene, OR). We are examining causative patient alleles in kqt-3 in worms to study their effects on pharyngeal pumping, and will attempt suppressor, enhancer, and drug screens to try to reverse, or enhance, these phenotypes.

Rare mutations in the Seipin gene BSCL2 are associated with the autosomal recessive congenital generalized lipodystrophy, or Berardinelli-Seip Syndrome (BSCL). This disease manifests primarily as a near complete loss of adipose tissue and severe insulin resistance. The yet-unnamed worm ortholog of seipin sits at the R01B10.6 locus and has 97.1% homology to the human isoform 1 of seipin. Preliminary evidence suggests no phenotype for C. elegans seipin mutants, but a full gene knockout has not yet been generated.

The iron-sulfur cluster scaffold protein NFU1 is associated with the autosomal recessive multiple mitochondrial dysfunctions syndrome, which is characterized by encephalopathy, hypotonia, and psychomotor delay, as well as a lactic acidosis and a failure to thrive. Mutants in the C. elegans ortholog ipd-8 (90.5% homology) are sterile. We are generating a new balancer for this region so that we can carry out suppressor screens. Updates on the progress of these three projects will be presented.

583C spn-4 gene cascade prediction in Caenorhabditis elegans by using multi-omics and big data analysis. Y. Nomoto, K Kojima, M Ito, Life Science, Ritsumeikan University, Kusatsu, Shiga, JP.

Early embryogenesis in Caenorhabditis elegans is regulated by maternal gene products called polarity mediators. The maternal gene product SPN-4 is a translational regulator with an RNP-type RNA-binding domain and is involved in centrosome rotation during asymmetric cell division in early embryos. In the previous study, multi-omics, transcriptomics, and proteomics revealed that 52 genes were regulated by SPN-4. Some of the identified genes encoded transcription factors. Therefore, in order to clarify the comprehensive functions of SPN-4, it is necessary to identify the SPN-4 gene cascade.

SPN-4 gene cascade was constructed by considering genes for which mRNA level fluctuation was caused by transcription factors, which are translationally regulated by SPN-4. We identified such genes by comparative transcriptome analysis between wild-type and ?spn-4 (tm291) mutant. SPN-4 downstream transcription factors were identified from the genes that were translationally regulated, i.e., genes for which mRNA levels were not affected by the absence of spn-4 function and by considering the corresponding gene ontology information (GO: 0006351 transcription, DNA-templated). Among them, the genes of transcription factor candidates, which were translationally regulated by SPN-4, were found by excluding those with an expression time different from that of SPN-4 and its products. For these transcription factors, the downstream genes listed in the nematode gene database (WormBase) and with mRNA level unaltered by the absence of spn-4 function were identified and defined as the genes being indirectly and transcriptionally regulated indirectly by SPN-4. Furthermore, these genes, which were regulated by the transcription factors included in downstream genes, which were extracted by interaction and expression timing. Then, we built the gene cascade of spn-4. Our results suggest that the spn-4 gene cascade may be responsible for the development of germline and feeding organs such as pharynx and intestine during embryo development of C. elegans.

584A Discovering molecules involved in dopamine neuron function. J. Quinde, C. Roach, J. Toms, B. Nelms, Biology, Fisk University, Nashville, TN.

The neurotransmitter dopamine (DA) is an important regulator of voluntary movement and cognition. Proper dopaminergic transmission controls various neurobiological functions with aberrant signaling often resulting in a number of psychomotor disorders, including Parkinson’s disease (PD). Identifying the essential components in the DA signaling pathway will allow us to better understand the normal function of dopaminergic neurons and gain insight into the mechanisms that could cause disease, and therefore lead to potential therapeutic strategies for treating DA-related disorders.

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To this end, we have generated a genetic profile for DA neurons using *Caenorhabditis elegans* (*C. elegans*). The *C. elegans* hermaphrodite has only 8 dopamine neurons, but many processes are conserved at the molecular level. To discover genes defining DA neuron identity in an unbiased manner, we employed next generation (NGS) sequencing technology, cell-specific RNA-Seq, to assess mRNA transcripts that are highly expressed in DA neurons as compared to the whole worm. I will highlight the genes we have found to be most abundantly expressed in DA neurons and results from assays for a phenotype associated with excess DA in the synapse known as swimming induced paralysis (SWIP). The identification of these genes, many of which have not been studied in dopamine neurons to date, may give us new insight into mechanisms of dopamine neuron function and disease.

585B Transcriptomic analysis of post-embryonic development across two distantly related nematode genera. **B.C. Rodriguez**1, L. Serra1, M. Macchietto2, A. Mortazavi1,2 1) University of California Irvine, Irvine, CA; 2) Center for Complex Biological Systems, University of California, Irvine, Irvine, CA 92697, USA; 3) Institute of Health and Bioinformatics, University of Minneapolis, Minneapolis, MN.

Entomopathogenic nematodes (EPNs) are parasitic nematodes that can efficiently kill insects and are primarily composed of two intensively studied genera: *Steinernema* and *Heterorhabditis*. EPNs and *Caenorhabditis elegans* last common ancestor was approximately 200 MYA. Observations comparing both nematodes affirm their morphological similarities. However, when examined closely, their reproduction, cellular structures, and living preferences are distinguished. *Steinernema* carpopcapssae are gonochoristic; they have a male and female. On the other hand, *Caenorhabditis elegans* is hermaphroditic and male. We are interested in characterizing sex determination in *Steinernema* carpopcapssae and comparing the conservation of sex determining genes to *C. elegans*. We are comparing the post-embryonic developmental stages larvae 1, larvae 2, larvae 3, larvae 4, infective juvenile, young adult male and female gene expression at the single-worm level to *Caenorhabditis elegans*. to assay gene expression at each stage by using the Smart-Seq2 protocol, which amplifies small quantities of mRNA from the nematodes into cDNA for sequencing. The *S. carpocapsae* samples were analyzed to examine correlation and quality of samples. *C. elegans* samples will be collected to give a complete picture of the gene expression profiles and how each stage compares to the other organism. We are particularly interested in the sexes of both nematode species since they have similarities (both have males) and differences (*C. elegans* has hermaphrodites and *S. carpocapsae* has females). We have sequenced single worms for L1, infective juvenile, adult male and female for *S. carpocapsae*. We are isolating mRNA for each of the listed stages to enrich the analysis and have a complete assessment of the pathways involved in determining male and females in *S. carpocapsae*. We will then compare at single-worm level the conservation of sex determining genes to *C. elegans*.

586C The Wnt pathway regulates multiple anterior sister lineage genes in the *C. elegans* embryo. **J.D. Rumley**, A. Zacharias, J. Murray Department of Genetics, University of Pennsylvania, Philadelphia, PA.

Embryonic patterning requires many signaling pathways to control cell fates. A fundamental step in development is patterning the body axes. Anterior-posterior axis patterning depends on the Wnt pathway, which acts through the transcription factor TCF and its coactivator β-catenin. Classic Wnt targets are transcriptionally activated when Wnt signaling is active through the TCF:β-catenin complex binding to cis regulatory regions and are repressed when Wnt signaling is inactive through TCF binding without β-catenin. In contrast, emerging evidence suggests the existence of direct “opposite” targets, activated by TCF without β-catenin and repressed by TCF:β-catenin. The relative importance, however, of these direct opposite targets compared to that of indirect opposite targets is not known.

In the *C. elegans* embryo, almost all cell divisions are patterned by the Wnt/β-catenin asymmetry pathway, in which the nuclear level of POP-1/TCF is higher in the unsignaled anterior sister cell and nuclear level of SYS-1/β-catenin is higher in the signaled posterior sister cell. Many genes expressed during embryogenesis are specifically expressed in either anterior or posterior sister lineages but not both. Several genes expressed in posterior lineages are classic Wnt targets, expressed when Wnt signaling is active. In contrast, the regulation of most anterior lineage genes, the expression of which suggests they could be “opposite” Wnt targets, is not well understood.

Currently I am testing whether these genes are direct opposite Wnt targets and how they are regulated. RNAi depletion of either POP-1 or SYS-1 shows that these factors are necessary for proper regulation of multiple anterior genes suggesting they are either direct or indirect opposite targets. Currently I am identifying anterior gene enhancers, examining the expression patterns they drive, and determining if these enhancers are bound by POP-1. Examining expression after mutating any POP-1-bound sites will help to determine whether these targets are direct opposite targets. This work will clarify mechanisms regulating expression of anterior sister lineage genes in the *C. elegans* embryo, and may have implications for Wnt pathway regulation of genes expressed in unsignaled cells in other species.

587A Oligonucleotide libraries in parallel reporter assays to probe *C. elegans* regulatory element functions. **Hillel Schwartz**1, Margaret Ho2, Paul Sternberg1 1) HHMI and Division of Biology and Biological Engineering, California Institute Of Technology, Pasadena, CA, USA; 2) Department of Neurobiology, Johns Hopkins University, Baltimore, MD, USA.

The *C. elegans* community has been amassing a large collection of information about candidate functional noncoding elements in the *C. elegans* genome, using a range of techniques including identifying evolutionary conservation, DNase hypersensitivity, and ChIP-seq. Our knowledge of the abilities of these elements to regulate gene expression has not been increasing to pace. To address this gap, we are adapting the “Massively Parallel Reporter Assay” (MPRA) to *C. elegans* transgenes. By synthesizing 200mer oligonucleotides in arrays and incorporating individual oligonucleotide sequences into transgene constructs, it is possible to affordably generate transgene libraries that pair candidate transcriptional regulatory
elements with unique bar codes, to establish lines containing mixtures of these transgenes, and to monitor the transgenes’ DNA representation and their reporter expression levels using high-throughput DNA and cDNA sequencing, respectively. In this way, pooled transgenic lines each incorporating hundreds of individual transgenes can be examined on a whole-animal basis for the ability of candidate elements, or designed mutant versions of those elements, to affect reporter gene expression, basally or in response to a genetic modification or to an environmental stimulus.

To establish this method, we have tested a modest 715-element library, including point mutants and controls. 120 bp candidate transcriptional control elements were selected by identifying sites of DNase hypersensitivity in proximity to genes known to be regulated by hypoxia, and incorporated in transgene libraries in a wild-type and a hif-1(fft) background. We are in this way able to identify elements that cause their reporter genes to express basally or to respond to hypoxia treatment in a hif-1-dependent or hif-1-independent fashion.

588B  Comparative genomics of endoderm cell fate specification in Steinernema carpocapsae. Lorryane Serra1, Marissa Macchietto 2, Bryan Rodriguez2, Cassandra McGill 1, Ali Mortazavi 1 1) University of California Irvine, Irvine, CA; 2) Institute of Health and Bioinformatics, University of Minnesota, MN.

We are interested in using entomopathogenic nematodes (EPNs) from the family Steinernematidae, which are nematodes that parasitize and efficiently kill insects and are used as satellite model organism, to study the conservation of endoderm development in nematodes. The Steinernema carpocapsae genome lacks the GATA transcription factors END-1 and END-3, which control endoderm development in the E-cell of the 8-cell stage in C. elegans while their downstream target genes are conserved and expressed abundantly during endoderm development. Therefore, there must be an alternative set of early-expressed Transcription Factors(TFs) that determine endoderm cell fate in S. carpocapsae. We are isolating single-cells from S. carpocapsae and have sequenced individual single-cells from early stages of S. carpocapsae to identify early zygotic TFS that could be cell lineage specific. The embryonic localization of these TFS will be verified using single molecule fluorescent in situ hybridization(smFISH). We will compare our results in S. carpocapsae to the matching single-cell data from C. elegans E-cells to perform the first comparison of gene expression at the single-cell level among homologous cells across distant nematode species with a focus on regulatory genes controlling early endoderm development.

589C  Mutation of a highly conserved, intronic sequence prompts temperature-dependent aberrant splicing of precursor mRNA. T.J. Sokolich1, G. Prajapati1, C.W. LaMunyon1, D. Shakes2 1) Biological Sciences Department, Cal Poly Pomona, Pomona, CA; 2) Department of Biology, College of William and Mary, Williamsburg, VA.

Traditionally, the study of essential and pleiotropic genes have posed unique challenges in the domain of reverse genetics. Knockout of essential genes can result in death or sterility, thus complicating phenotypic analysis. The complete inactivation of pleiotropic genes permits superficial investigation but may shroud the significance of independent expression events. Easily toggled between states, temperature sensitive mutations can abate some of these obstacles in gene function studies. The spe-6 gene of C. elegans is required for spermatid activation, major sperm protein assembly, and the proper execution of meiosis I. Two intronic mutations in spe-6 (zq18, and hc190) independently and in a temperature-dependent manner reduce the expression of this gene. At 25ºC, a majority of spe-6(zq18) precursor mRNA is aberrantly spliced, subsequently invoking a reduction of fertility. At 15ºC, fewer spe-6(zq18) transcripts are incorrectly spliced, and affected worms maintain normal fertility. spe-6(hc190) follows this same pattern of conditional fecundity. However, aberrant splicing of this allele has not been detected, possibly due to nonsense-mediated mRNA decay. The zq18 and hc190 alleles are characterized by guanine-to-adenine substitutions on the 5th and 1st bases, respectively, immediately flanking the 3’ end of the exon. Furthermore, the 5’ intronic sequence encompassing these two mutations are highly conserved amongst Eukaryotic genes and implicated in exon-intron junction recognition. Here, we report the effects of zq18- and hc190-like point mutations in other genes without a native restrictive temperature. MosSCI and the CRISPR/Cas9 system were used to recreate the aforementioned substitutions in Cbr-unc-119 and spe-44. It was found that Cbr-unc-119(zq18-like) does not have a restrictive temperature and is phenotypically comparable to the wild type allele. Similarly, zq18- and hc190-like substitutions in spe-44 do not result in substantial phenotypic variance from wild type worms. From this, we conclude that zq18- and hc190-like mutations do not universally elicit a temperature-sensitive ‘toggle’ in the gene that they reside. With further investigation, we hope to elucidate the relationship between highly-conserved intronic positions and temperature-sensitive gene expression.

590A  A conserved mitochondrial surveillance pathway is required for defense against Pseudomonas aeruginosa. E. Tjahjono, N. V. Kirienko  Department of BioSciences, Rice University, Houston, TX.

All organisms require surveillance systems to mitigate the damage done by insults (e.g., abiotic stressors or pathogens) that perturb their homeostasis. Recent work from a large number of labs has contributed to a model generally known as the cellular surveillance system. A remarkable variety of inputs have been linked to this system, including protein translation, mitochondrial activity, and other inputs associated with core cellular machinery. As a variety of stresses and pathogens are known to disrupt these processes, so hosts have developed elegant methods to monitor their normal activity. Here we report the involvement of such a surveillance system, monitoring mitochondrial homeostasis, in innate immune response to the multithost, opportunistic pathogen Pseudomonas aeruginosa.

We set out to characterize the host defense pathways used to promote survival in the face of P. aeruginosa using a well-established pathogenesis system called Liquid Killing. Unlike other C. elegans - P. aeruginosa pathosystems, Liquid Killing strongly depends on the activity of a small, soluble, iron-binding molecule (siderophore) produced by P. aeruginosa to acquire...
this key micronutrient. Pyoverdine, the siderophore in question, has been shown to cause mitochondrial damage, activate a hypoxic response, and ultimately kill the host. Importantly, this virulence factor is indispensable for pathogenesis in mammals. Using transcriptome profiling, genetic analysis, and pathogen and chemical exposure assays, we were able to show that the host defense against pyoverdine intoxication is specific and have little in common with innate immune defenses against intestinal colonization with \textit{P. aeruginosa} or other pathogens.

Promoter analysis of differentially expressed genes identified a subset containing a highly-conserved, 11-nucleotide motif termed ESRE for Ethanol and Stress Response Element. This motif was previously linked to differential gene expression in response to a variety of abiotic stresses, such as ethanol and hypoxia. Interestingly, we show that ESRE gene induction occurs after exposure to a variety of treatments that destabilize and disrupt mitochondria, including \textit{P. aeruginosa}. This indicates that the ESRE gene network plays some role in mitochondrial surveillance. Finally, we were able to identify a family of bZIP transcription factors required for full ESRE gene expression. This work, which identifies a key role for ESRE in recognizing mitochondrial dysfunction due to pathogen attack, links innate immunity and abiotic stress response in \textit{C. elegans}.

591B Stochastic gene expression of neuron-specific genes. J.J.H. Traets, J.S. van Zon AMOLF, Amsterdam, NL

Genetically identical animals under the same environmental conditions still show strong variability in their behavior, but the source of this variability is unknown. Every neuron expresses hundreds of genes that are essential for its neuronal functioning, such as receptors for sensing and ion-channels. Gene expression is an inherently stochastic process, and hence variability in gene expression of neuron-specific genes could be an important origin of variability in behavior. To address this question, we quantify variability in gene expression in neurons of the nematode \textit{C. elegans}. We focus on the well-characterized ASE neurons, which are responsible for salt sensing. The transcription factor \textit{che-1} controls ASE neuron specification and maintenance, by inducing hundreds of ASE-specific genes. Crucially, \textit{che-1} also upregulates its own expression, acting as a genetic switch. Hence, variability in \textit{che-1} levels could lead to coordinated variability in targets, and it could as well impact the state of the genetic switch. To measure variability in expression of \textit{che-1} and its targets, we use single molecule FISH to count individual mRNA molecules in the ASE neurons. So far, we have found significant variability in expression of \textit{che-1} and several target genes involved in salt sensing behavior; the ion-channels \textit{tax-2} and \textit{tax-4}, and the receptor-type guanylate cyclase \textit{gcy-22} and \textit{gcy-14}. Surprisingly, we found that \textit{che-1}, \textit{tax-2} and \textit{tax-4} are expressed in very low levels in young worms, ~6 mRNAs per cell. We used stochastic modeling to show that such low levels of \textit{che-1} drastically impact the ability to control expression of itself and its targets, raising the question how \textit{che-1} is still capable of inducing the expression of all target genes and what this implicates for the fluctuations on protein level. Finally, we observed a strong bimodal expression of \textit{gcy-22} in a number of cells around the pharynx, including neuronal support cells. We are currently examining whether this stochastic \textit{gcy-22} expression impacts behavior.

592C The period protein homolog LIN-42 negatively regulates microRNA biogenesis in \textit{C. elegans}. Priscilla Van Wynsbergh, Katherine Cascino, Rachel Weinstein, Christiane Olivero, Joseph Spina Biology Department, Colgate University, Hamilton, NY.

\textit{C. elegans} heterochronic pathway is composed of multiple proteins and microRNAs that regulate developmental timing. microRNAs are ~22 nucleotide RNAs that post-transcriptionally silence gene expression, and are thus essential in diverse biological processes. Previous work in our lab and others has shown that the heterochronic pathway member and circadian rhythm Period protein homolog, LIN-42, regulates microRNA biogenesis. Like other period proteins, levels of LIN-42 oscillate throughout development. In other organisms this cycling is controlled in part by phosphorylation and dephosphorylation events. KIN-20 is the \textit{C. elegans} homolog of the \textit{D. melanogaster} Period protein kinase doubletime. Worms containing a large deletion in \textit{kin-20} develop slower and have a significantly smaller brood size than wild type \textit{C. elegans}. Here we analyze the effect of KIN-20 on the expression of the canonical heterochronic microRNA let-7. We find that let-7 levels are significantly decreased and that mRNA levels of the let-7 target \textit{lin-41} are increased in \textit{kin-20} mutant worms relative to wild type. Additionally, we find that primary let-7 levels are unchanged in \textit{kin-20} mutant worms suggesting that KIN-20 impacts microRNA biogenesis downstream of microRNA transcription. Surprisingly, we find that mature let-7 levels are significantly decreased in \textit{lin-42} mutant worms grown in the presence of \textit{kin-20} RNAi compared to control RNAi treatment. To further explore the relationship of KIN-20 and LIN-42, we analyzed the effect of KIN-20 on endogenous LIN-42 expression. Based on the homologous system in \textit{D. melanogaster}, we hypothesized that KIN-20 phosphorylates LIN-42 to trigger LIN-42 degradation. Unexpectedly, we find that KIN-20 effects on LIN-42 differ depending on the isoform analyzed. Altogether these results suggest that though KIN-20 affects LIN-42 expression, KIN-20 acts independently of LIN-42 to regulate let-7 miRNA expression. These findings further our understanding of the mechanisms by which these conserved circadian rhythmic genes interact to ultimately regulate rhythmic processes, developmental timing and microRNA biogenesis in \textit{C. elegans}.

593A The role of pheromones in behavioral changes during development of \textit{Caenorhabditis elegans}. H. Wada1, R.A. Butcher2, M Fujiwara1, T Ishihara1 1) Molecular Genetics Laboratory, Department of Biology, Faculty of Science, Kyushu University, 2) University of Florida.

Many animal species change their behavior according to their stage of development. Although such behavioral changes are thought to be important for their fitness, neural modifications that underlie the changes are not fully understood. \textit{Caenorhabditis elegans} changes their olfactory preferences during development. Larvae exhibit a weak chemotactic response to a food-associated odor diacetyl, whereas adults exhibit a strong response. Previously, we showed that germline proliferation is required for the olfactory changes. The adult worms lacking germline cells failed to exhibit a strong response to diacetyl. In addition to that, we recently found that pheromone sensation is also required for the regulation. The adult worms which have defects either
in pheomone synthesis or in a pair of pheomone-sensing neurons, ASK, failed to exhibit strong chemotactic response to diacetyl at adult regardless of whether their germline cells proliferates normally.

Here, we have tested the possibility that germline proliferation might principally affect pheomone sensitivity, and the enhanced perception of pheomones subsequently cause the enhanced chemotaxis to diacetyl. By utilizing a Ca²⁺ indicator, YC3.60, we observed the neuronal responses of ASK sensory neurons to pheomone stimuli consisting of ascaroside C3, C6, C9. Contrary to our expectations, the responses to pheomones of adult worms lacking germline cells were indistinguishable from those of the adult animals with an intact germline. Thus, it seems that germline proliferation and pheomone sensation may act in parallel to regulate chemotaxis.

Now we are planning to observe the neuronal activities in the olfactory circuit of pheomone–synthesis defective animals under diacetyl stimuli. Further analyses will reveal how the animals change their behavior by integrating their internal states and environmental inputs.

594B Differential requirements for the methyl donor SAM in stress-induced gene expression. Wei Ding1, Daniel Higgins2, Read Pukkila-Worley1, Amy Walker1 1) Program in Molecular Medicine, UMass Medical School, Worcester, MA; 2) Rutland Informatics; 3) Program in Innate Immunity, UMASS Medical School, Worcester, MA.

Transcriptional processes are tightly linked to metabolic products. For example, the 1-carbon (1CC) cycle produces SAM (s-adenosylmethionine), the methyl donor used for histone and nucleic acid modification. While 1CC disruption has been proposed to broadly affect epigenetic processes, it is surprising that shifts in SAM levels affect specific methylation events, such as activating H3K4 methylation, suggesting that changes in SAM metabolism may affect specific genes or biological processes.

Because the mammalian H3K4 methyltransferase (HMT) family is complex, it has also been difficult to discern specific roles for individual HMTs. Using C. elegans, we found that reducing SAM caused defects in H3K4me3 accumulation on promoters of selected infection response genes during pathogen exposure, that induction of these genes failed and that these animals had reduced survival. Furthermore, set-16/MLL, appeared to have critical role in this process. This suggests SAM may be important for stress responsive transcription and that distinct H3K4 methyltransferases may have specific roles.

To test this model, we treated C. elegans with three stresses, bacterial, detoxification and heat, then examined survival and genome wide mRNA expression patterns in animals with low SAM (sams-1(RNAi)), or after knockdown of H3K4 HMTs set-2/SET1 or set-16/MLL. We found that transcriptional responses to bacterial or detoxification stress were blunted in sams-1(RNAi) animals. set-2/SET1 or set-16/MLL RNAi had negligible effects on in control animals, however, elicited distinct effects in response to bacterial, detoxification stress. set-2/SET1 appears less broadly required during bacterial or detoxification stress.

Many bacterial or detoxification stress genes were also strongly attenuated after set-16/MLL knockdown. Canonical heat shock gene expression, however, increased normally in response to heat after sams-1, set-2 and set-16 RNAi. Finally, our biological assays revealed two important effects. First, sams-1, set-2/SET1 and set-16/MLL RNAi animals all died rapidly from the pathogen, despite variations in transcriptional responses. Interestingly, other 1CC genes, such the SAM synthases sams-2 and sams-3, are normally induced in response to a pathogen, but fail in sams-1, set-2/SET1 and set-16/MLL RNAi animals, suggesting feedback loops maintaining 1CC function may be critical for pathogenic challenge. Finally, set-16/MLL was sensitive to all three stresses, whereas set-2/SET1 animals survived normally on the detoxification agent and after heat shock. Thus, set-16/MLL may have a more general role in stress responsive transcription. Our data suggests that depletion of SAM could reduce the ability of a tissue to respond to stress, thereby increasing potential for injury and exacerbating disease progression.

595C A network of transcription factors regulates lysosomal lipolysis in C. elegans. A. Way, V.K. Mony, E.J. O'Rourke Department of Biology, College of Arts and Sciences, University of Virginia, Charlottesville, VA, USA.

Adaptation to nutritional changes is a prominent example of plasticity in living organisms. Evolutionary conserved pathways like Insulin signaling, mechanistic Target of Rapamycin (mTOR), TGF-β, caloric restriction (CR) and Notch receptor pathways have been characterized for their effects in growth and reproduction along with changes in fat metabolism in response to nutritional stress. Within each of these pathways “canonical” transcription factors (TFs) control the expression of distinctive downstream effectors. On the other hand, many TFs have shared regulators and targets. Given the plasticity in nutrient responses, it is an open question whether different upstream nutrient sensors would canalize nutritional information through their canonical TFs to activate adaptive responses, or whether the adaptive downstream effectors would be controlled by a small subset of specialized TFs common to all nutrient sensing pathways modulating a given adaptive molecular response.

We have performed transcriptional analyses to dissect the regulation of genes encoding the C. elegans lysosomal lipases lipl-1, lipl-3 and lipl-4. Although known to have at least partially redundant roles, here we show that the genes encodes these lysosomal lipases are differentially controlled by a network of transcriptional activators and repressors that yield distinct expression patterns in animals with altered nutrient sensing or nutritional status. Surprisingly, HLH-30 (mammalian TFEB), a transcription factor we established to be required for activation of the lipl genes in fasting conditions, is found to be required for induction of lysosomal lipase upon mTOR inhibition but not in CR, insulin deficient, TGF-beta deficient, sterile worms, or other tested models of metabolic dysregulation. By contrast, DAF-16 (mammalian FOXO) is found to control lysosomal lipases downstream of multiple nutrient sensing pathways. Similarly surprising, PHA-4, a gene activating gene expression in CR animals, and NHR-49 a master activator of beta-oxidation upon fasting, act as repressors of the lipl genes, suggesting a dual role for PHA-4 and NHR-49 in adaptation to food scarcity. Additionally, DAF-3 (co-SMAD), NHR-80 (HNF4), SKN-1 (Nrf2), and HSF-1 contribute to regulation of the expression of the lysosomal lipases. All in all, we found that although the specificity of lipase expression depended on the same TFs downstream of multiple nutrient regulatory pathways, they are also under the promiscuous regulation of disparate TFs.

*Way A. and Mony V.K. contributed equally to the work presented in the abstract.
596A What regulates the regulators? The C. elegans endoderm, the ELT-2 GATA factor and the chromatin environment. Tobias Wiesenfahrt, Stephane Filibotte, Jingjie Duannu, Paul Mains, Don Moerman, James McGhee 1) Biochemistry and Molecular Biology, University of Calgary, Calgary, AB, CA; 2) Department of Zoology, University of British Columbia, Vancouver, BC, CA.

We are using the C. elegans intestine to understand the robustness and flexibility of transcription factor networks and how these are influenced by the chromatin environment. The C. elegans endoderm develops under control of a series of partly redundant GATA-type transcription factors: END-3 > END-1 > ELT-7 > ELT-2. ELT-2 is the predominant transcription factor driving intestine differentiation and function, but is not normally involved in endoderm specification. We have recently shown (to our surprise) that ELT-2 can replace all other intestine GATA factors, i.e. can actually specify the intestine, if it is expressed under the end-1 promoter in addition to its own promoter. However, multiple copies of transgenic end-1p::elt-2 are more efficient at specifying the endoderm (88% rescue) than a single copy MosSCI insertion (<1% rescue). In a genetic screen, we mutagenized approximately 14,000 genomes with EMS and identified 17 strains with enhanced ability of a single copy end-1p::elt-2 to specify endoderm. The starting end-1(−) end-3(−) strain, with a single copy insert of end-1p::elt-2, was rescued by an extra-chromosomal array carrying multiple copies of the end-1p::elt-2 together with a heat shock inducible peel-1 construct. Thus we were able to kill all worms that still relied on the extra-chromosomal array for their survival after the EMS treatment. Whole genomic sequences of these independent strains provide a short list of potential candidate genes. Two such candidate genes that were independently mutated in multiple strains have been verified by RNAi or CRISPR: K01G5.9 (4 independent mutations), encoding a Taspase 1 homologue and pqn-82 (2 independent mutations), encoding a homologue of a S. cervisiae SWI/SNF subunit. Taspase appears to be involved in the proteolytic processing of general transcription factors such as TFIIA and trithorax. The SWI/SNF complex appears to be involved in chromatin remodeling and transcriptional regulation. Disrupting the function of these two candidate genes shows a twofold enhancement of ELT-2 levels in the early endoderm relative to the unmutagenized strain, resulting in 50% rescue rate for pqn-82 and 7% rescue rate for K01G5.9. We are currently working on a method to identify the mutated genes in the remaining 11 candidate strains that were hit only once. Thus, this genetic screen could be revealing a set of general chromatin proteins that control the levels of specific transcription factors, such as ELT-2, in the early embryo.

597B The C. elegans anterior Hox gene, ceh-13, activates posterior Hox genes, nob-1 and php-3, to ensure robust posterior cell development. Amanda L. Zacharias, Ellicia Preston, Teddy D. Lavon, Shaili D. Patel, John Isaac Murray Department of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.

The C. elegans Hox cluster contains only six genes, which are orthologous to only four of the Drosophila Hox transcription factors, possibly because the worm lacks a segmented body plan. Unlike genes in other animal Hox clusters, these Hox genes are not expressed in the same order, anterior to posterior, as their order on the chromosome, suggesting that their regulatory mechanisms may have diverged as well. Furthermore, expression of the anterior Hox ortholog, ceh-13, extends to parts of the extreme posterior of the animal, raising the question of whether it regulates posterior fates.

We investigated the regulation and function of three C. elegans Hox genes: ceh-13, an anterior Hox1/labial homolog, and nob-1 and php-3, two posterior Hox13/Abdominal-B homologs with redundant functions. In most species, expression of these Hox genes does not overlap but we identified high overlap between rescuing nob-1 and ceh-13 transgenes, with ceh-13 expressed one cell cycle earlier than nob-1 in several posterior daughter lineages within the ABp and E lineages. We identified enhancers sufficient to drive part or all of the spatio-temporal expression pattern of both ceh-13 and nob-1, including two putative shadow enhancers. We tested whether ceh-13 might be an upstream regulator of nob-1 by assaying nob-1 reporter expression in ceh-13 mutants. We found that ceh-13 is required for nob-1 reporter expression in the ABpp and ABprpp sublineages.

 Arrested ceh-13 mutant embryos and L1 larvae have major defects in head morphology as well as occasional defects in tail morphology similar to those observed in nob-1/php-3 mutants. We tested for similarities in single-cell developmental phenotypes of these two mutants by automated lineage tracing. We found that nob-1/php-3 mutant embryos have widespread defects in cell division and migration in the posterior of the embryo, including non-cell autonomous migration defects, consistent with previous reports. ceh-13 mutants have a fewer defects, most of which are in CEH-13 expressing cells, including in posterior cells where nob-1 expression requires ceh-13.

Taken together, these results have several interesting implications. First, it suggests that along with input from the Wnt pathway through POP-1/TCF and SYS-1/6-catenin, nob-1 is regulated by different combinations of transcription factors in different sublineages, with only some dependent on ceh-13. Second, we observed that an anterior Hox gene positively regulates expression of a posterior Hox gene and plays an important role in the development of extreme posterior structures. This is consistent with the regulation and function of the Hox genes in C. elegans having diverged from the strict positional roles observed in other animals, suggesting that the evolutionary “rules” for animal body plans may be more flexible than previously appreciated.

Gene Regulation and Genomics - Genomics


Many late-onset neurodegenerative diseases, such as Huntington’s disease (HD), are caused by aggregation of misfolded proteins. Individuals stricken with these diseases succumb to the detrimental symptoms associated with them. Discovering the cellular and genetic pathways that are protective against protein aggregation will improve our understanding of potential treatments for these diseases.
Huntington’s disease is associated with aggregation of mutant huntingtin protein, containing an expanded polyglutamine tract. In HD patients, individual’s genetic backgrounds can modulate the severity of the disease. However, the variants responsible for modulating disease phenotypes are not known. To ask what natural variant(s) play a role in modifying protein aggregation, we use a C. elegans polyglutamine model expressing a fluorescent 40-glutamine expansion (Q40-YFP) in muscle cells. We have shown that introduction of Q40-YFP into genetically diverse wild strains of C. elegans results in a wide range of phenotypes, from suppression to strong enhancement of Q40-YFP aggregation and toxicity. Here, we have identified a 400kb genetic interval in the DR1350 strain, containing variants that increase polyglutamine aggregation. An RNAi survey of 24 candidate genes in that interval identified atg-5, an important component of autophagy that, when knocked down, rescued the increased aggregation. Interestingly, genome sequencing identified 2 variants within the 3’UTR of atg-5 in the DR1350-derived interval. Animals carrying this interval have increased ATG-5 expression and deficient autophagosome formation. Because autophagy is important for degradation of protein aggregates, we hypothesize that natural variants in the DR1350 genetic background increase polyglutamine aggregation by causing increased levels of ATG-5 expression and thus misregulating autophagosome formation.

599A Bioinformatic and genetic approaches to understanding the cnd-1 regulatory network. W. Aquino Nunez, Z. Mielko, D. McCalla, E. Christensen, C. Hosea, K. Bronson, V. Owens, M. Hudson Kennesaw State University, Kennesaw, GA.

NeuroD1, the vertebrate ortholog of cnd-1, is a basic-Helix-Loop-Helix protein involved in neuronal and pancreatic beta cell fate specification. NeuroD1 loss-of-function mutations have been implicated in human visual impairment, learning disabilities, deafness, and neonatal diabetes. In C. elegans, cnd-1 is expressed in many unidentified head neurons and also in D-class motor neurons. Only three genes are known to act downstream of cnd-1 in C. elegans; unc-3, unc-4, and unc-30. All three of these genes are transcription factors that are expressed in D-class motor neurons. However, the remaining downstream targets of cnd-1 have not been identified.

In order to investigate the regulatory role of cnd-1, we performed RNA-extractions from wildtype and cnd-1 loss-of-function embryos, followed by RNaseq. We assembled a transcriptome outlining differentially expressed genes during embryogenesis and are currently following up by validating candidate cnd-1 target genes using genetic approaches. In addition, NeuroD1 is known to function in a regulatory cascade with neurogenin and also to positively regulate itself in mammalian neuron specification. We seek to verify this relationship in C. elegans in order to better understand the regulatory context of our novel cnd-1 target genes.

600B A worm specific CRISPR guide RNA selection tool. S. Filbotte1, V. Au1, I. Chaudhry1, M.L. Edgley1, H. Hutter2, E. Li-Leger1, D.G. Moerman1 1) Zoology, University of British Columbia, Vancouver, British Columbia, CA; 2) Department of Biological Sciences, Simon Fraser University, Burnaby, BC, CA.

CRISPR/Cas9 is the current technology of choice for genome editing. This is due to its versatility, as it is an RNA guided system where a 20-base crRNA and a tracrRNA (together called a guide RNA - sgRNA) direct a Cas9 nuclease to the target sequence providing high specificity with minimal secondary site effects. At the target site the endonuclease makes a double-stand cut of the DNA, which can then be resolved through Non-Homologous End Joining (NHEJ) or Homology Dependent Repair (HDR). CRISPR-Cas9 technology was first adapted for C. elegans in 2013 and since then the community has produced increasingly more sophisticated methods to mutate, delete and tag genes.

Several web tools exist to help select guide RNA but at the Vancouver gene knockout facility we found that none of those tools were specific enough for our CRISPR applications. We therefore designed our own C. elegans specific guide RNA selection tool. Since using this tool improved our success rate we decided to make it available to the community at large. The guides have been pre-calculated and assessed for the whole C. elegans genome using the best practices as could be found in the literature. We intend to keep the web site up-to-date as those best practices evolve over time and as we get access to more data regarding guide efficiency and off target effects in the worm. We urge users to inform us of their experience using these guides to help us improve the efficacy of guides designed using the site.

The relevant web application can be accessed at http://genome.sfu.ca/crispr with a user interface similar to that of the web site for the Million Mutation Project.


WormBase is an international consortium responsible for the ongoing maintenance and enrichment of the genome and genes belonging to the free living nematode C. elegans and other closely related nematodes or a select few seen as having evolutionary, medical or agricultural significance. This involves gathering, incorporating and curating a large collection of scientific data types from a variety of sources.

C. elegans was the first multicellular organism to be fully sequenced and was published in 1998 with both a coding and non-coding set of genes. WormBase has continued to improve the genome and gene set correcting errors that are highlighted through publications, 3rd party data submissions and the biggest challenge, new technologies. This poster aims to highlight challenges and opportunities for discovery in this area of curation. Sequence based data types and technologies are used to confirm or allow curators to improve the quality of gene structures and their resulting products to give as complete a representation of the gene as possible. This is particularly important as WormBase is responsible for submitting
the C. elegans reference gene set to the public archives every 2 months where it is used in external resources such as UniProt and the RefSeq canonical sequence databases.

602A Efficient identification of candidate gene mutations by mapping-by-sequencing in the non-model nematode Oscheius tipulae.

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Oscheius tipulae is a common free-living nematode in the same clade as the parasitic taxa Heterorhabditis and strongylids, and closer to the model species Caenorhabditis elegans than the outgroup Pristionchus pacificus. This hermaphroditic species is thus informative for comparative genetics, developmental and evolutionary studies. However, the genetic toolbox for non-model organisms such as O. tipulae is still underdeveloped.

In model species with fully assembled and annotated genomes, mapping-by-sequencing has become a standard method to map and identify phenotype-causing mutations. Candidate variants are pinpointed using a cross to a divergent mapping strain and sequencing of a pool of mutant segregants (e.g. ref. 1). Chemical mutagenesis (EMS) performed about 15-20 years ago by Marie-Anne Félix's lab on the O. tipulae strain CEW1 generated several vulval development and other morphological mutants, including dumby, roller and uncoordinated phenotypes (ref 2-6). Using the mapping-by-sequencing approach, a draft genome sequence for O. tipulae CEW1 and crosses with the divergent wild isolate JU170, we have identified for the first time relevant candidate genes for these phenotypes in O. tipulae. Our success suggests that a draft assembly with multiple scaffolds per chromosome is sufficient to perform mapping-by-sequencing. Other recent genetics tools such as CRISPR/Cas9 system have been developed and widely used on Caenorhabditis species to perform targeted mutagenesis, and we propose to confirm candidate genes by developing CRISPR/Cas9 system on O. tipulae.

We argue here that the mapping-by-sequencing approach and genomic analysis tools can be easily used in non-model organisms. This brings non-model species firmly into genomics-enabled science, and provides tools to investigate the biology of non-model species and improve our understanding of evolution and developmental mechanisms.


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To advance our understanding of development and physiology, it is important to gain detailed insight into the spatial and temporal expression patterns of all genes. Such a comprehensive overview of gene expression patterns will identify cell type and organ specific genes. It will also provide a framework for studying how gene expression patterns change upon perturbation.

RNA sequencing (RNA-seq) is ideally suited for genome-wide analysis of gene expression. However, RNA-seq by itself does not provide information on where genes are expressed in the organism. A solution to this problem is to include spatial information by sectioning the organism along one of the body axes and to perform RNA-seq on each of the separate sections.

We have developed a serial-section RNA-seq (serial-seq) method for C. elegans. In brief, we cryo-section a young adult animal from the head to the tail into 20 micrometer thick slices, resulting in a total of 40 to 50 slices. Each slice is then sequenced using the sensitive CEL-seq method and the sequencing data is aligned to create an anteroposterior gene expression map. Using this approach, an average of 8794 genes (43% of coding genes) (n=8) can be detected along the length of the animal. These include genes that are expressed in major tissues such as the germline, but also genes that are known to be expressed in only single neuron pairs in the head, demonstrating the high sensitivity of our approach. Furthermore, we found that these expression patterns are highly reproducible among independently sectioned animals.

What makes serial-seq particularly powerful in C. elegans is its relatively simple and invariant anatomy. Because each slice contains only a limited number of cells, we can predict which cells are in the different slices and use this information to identify cell type specific genes. Furthermore, we have developed an algorithm that enables us to search for genes with specific expression patterns. Using known marker genes as a starting point, we have identified genes that are specifically expressed in the germline and sperm, in organ structures such as the pharynx, the intestine, the vulva, the spermatheca and the male reproductive tract, and in neurons of the nerve ring and tail ganglia. The ability to identify cell type specific genes is an important advantage over RNA-seq of isolated cells, which does not distinguish between specific and more generally expressed genes.

In summary, we have generated high-resolution anteroposterior gene expression maps of C. elegans, including maps of hermaphrodites, males and germline deficient mutants. In addition to providing an expression pattern resource, serial-seq is a powerful method for studying gene expression pattern changes at the whole-genome level.

604C High throughput chemical genomics in C. elegans to screen for novel anthelmintics and their targets. H. Fahs1, F. Refai1, R. White1, P. Cipriani1, G. Butterfoss1, R. Kalloush1, M. Kallassy2, F. Piano1, K. Gunsalus1 1) New York University Abu Dhabi; 2) Saint-Joseph University - Lebanon.

Biologically active chemicals are the basis of most known therapeutics and are powerful tools to study cell biological
processes. However the discovery of new bioactive compounds and characterization of their molecular targets remain a challenge. We are using small molecule and natural products to identify novel compounds that affect animal development and study their modes of action. We established a high-throughput automated platform for chemical and functional genomic screening that accommodates both cell-based and whole-organism assays. We are focusing on broad-spectrum anthelmintics using the free-living nematode models *C. elegans* and the distantly related *P. pacificus*. Given the short life cycle of the worm, our platform enables one person to screen 20,000 chemicals per week and perform one genome-wide RNAi screen every three weeks. We validated our approach in a pilot screen of an FDA-approved drug library, which confirmed the effects of known anthelmintics on *C. elegans* and/or *P. pacificus*. We screened a library of ~32,000 small molecules, selected using a computational approach to predict bioavailability in nematodes and identified numerous candidate molecules that will be assayed for toxicity in mammalian cells. To uncover molecular targets of bioactives and mechanisms of resistance, we will use forward and reverse genetic screens to identify suppressors (or enhancers) of chemical sensitivity. We have also screened a *Bacillus thuringiensis* (Bt) library of 300 uncharacterized strains isolated in Lebanon and the UAE. Bt is a spore-forming bacterium that synthesizes crystal inclusions, certain of which show species-specific toxicity against insects, nematodes (i.e. Cry5B), and cancer cells. Bt crystal toxins therefore constitute a promising alternative to chemical anthelmintics. We found 95 strains that hinder the development of worms, and among them 50 strains that act through a Cry5-independent mechanism. Virulence factors will be characterized by DNA sequencing combined with functional genomic assays to elucidate their mechanisms of action.

605A Unc-68 functions in chemosensory nerve cells. C. Ferreira1, M-A Shaw2, I Hope1  1) School of Biology, Faculty of Biological Sciences, University of Leeds, LS2 9JT, UK; 2) Leeds Institute of Biomedical and Clinical Sciences, St James’s University Hospital, Leeds, LS9 7TF, UK.

Previously, we introduced single amino acid changes equivalent to those found in human RyR1, causing myopathic conditions, into the *C. elegans* version of the ryanodine receptor, UNC-68. The locomotory response of these *C. elegans* strains to increasing concentrations of caffeine was assessed. Caffeine is used, along with halothane, in malignant hyperthermia diagnostics. Locomotion in young wild type adults is stimulated by low concentrations and inhibited by high concentrations of caffeine. While all the model strains exhibited increased sensitivity to halothane, only certain model strains demonstrated the caffeine inhibition of locomotion. As individuals age, the locomotion in most strains is attenuated and the caffeine inhibition accentuated. Notably, for late onset axial myopathy (LOAM) model strains, caffeine began to stimulate locomotion in aging adults, and the degree of stimulation increased progressively with age. *C. elegans* caffeine-resistant strains had been found to have mutations in *osm-3* and *che-3*, required specifically for chemosensory nerve cell function. The role of these genes in the differential response to caffeine attributed to the *unc-68* variants was tested by RNAi knockdown. Both the stimulation and inhibition of locomotion by caffeine was either lost or reduced for all model strains and wild type controls. Furthermore, the progressive stimulation of locomotion in response to increasing concentrations of caffeine in old adults of the LOAM models was also eliminated with knockdown of these genes. Given the generally-accepted refractility of nerve cells to RNAi knockdown, we have now assayed *osm-3* and *che-3* null *C. elegans* mutants, in the same assay. Locomotion of young and old adults of *osm-3* and *che-3* mutants show neither the stimulation nor inhibition by caffeine. This raises the possibility that the influence of the UNC-68 amino acid changes on the caffeine response could actually be within the chemosensory nerve cells, rather than in muscle cells as anticipated.

Seeking to distinguish if the sensitivity to the UNC-68 amino acid changes lay in the sensory cells or in the longer-term, downstream response, we used behavioural assays to interrogate the immediate response to caffeine and other noxious chemicals. All model strains retained the ability to back away from noxious chemicals suggesting the chemosensory cells are not broadly compromised. Individuals with the LOAM-associated *UNC-68* variant specifically, however, seemed unable to sense 40mM caffeine. Furthermore, while young adults of the LOAM model were able to sense 20mM caffeine, this ability was lost in older adults. *osm-3* and *che-3* mutants failed to react to either 20mM or 40mM caffeine.

606B Muscle-specific transcriptome rearrangements during the progression of duchenne muscular dystrophy in *C. elegans*. H.C. Geissel1,2, C. Nguyen3, S. Blazie1,2, K. Kotagama1,2, M. Mangone2  1) Molecular and Cellular Biology Graduate Program, School of Life Sciences 427 East Tyler Mall Tempe, AZ 85287 4501; 2) Virginia G. Piper Center for Personalized Diagnostics, The Biodesign Institute at Arizona State University, 1001 S McAllister Ave, Tempe, AZ, USA; 3) Barrett Honors College, Arizona State University, 751 E Lemon Mall, Tempe, AZ 85281.

Duchenne muscular dystrophy (DMD) is a lethal, X-linked disease characterized by progressive muscle degeneration. The condition is known to be driven by mutations in the dystrophin gene that result in production of a truncated, non-functional dystrophin protein. However, the resulting changes in muscle-specific genetic networks that take place in the absence of dystrophin remain unknown, as they are potentially obscured by the chronic inflammatory response elicited by muscle damage associated with the disease.

*C. elegans* have a mild inflammatory response that allows for the characterization of the transcriptome rearrangements affecting disease progression independently of inflammation. The mutant *C. elegans* strain dys-1(eg33) contains a nonsense mutation in the dystrophin gene leading to the production of a truncated DYS-1, which mimics some aspects of human DMD.

In effort to better understand the dynamics of transcriptome rearrangements during DMD progression, our lab has optimized an approach (PAT-seq) to isolate and sequence high-quality body muscle-specific transcriptomes from dys-1(eg33) worms at different stages of the disease.
We have crossed dys-1(eg33) with worms expressing pab-1 fused to 3xFLAG under the control of the myo-3 promoter, and characterized the extent to which this chimeric strain mimics the human version of DMD. To validate this strain, we performed survival curves, genomic DNA sequencing, and the muscle specific RNA silencing of several worm orthologs of the human dystrophin complex, including dystrophin (dys-1), dystrobrevin (dyb-1), and dystroglycan (dgn-1). Our results will allow for the comprehensive identification of transcriptome rearrangements that could ultimately serve to identify new therapeutic targets for the treatment of DMD.

607C Sibling Subtraction Method: A novel approach to identify causal mutations by WGS.  B. Joseph1, N. Blouin2, D. Fay1 1) Department of Molecular Biology, University of Wyoming, Laramie, WY; 2) Wyoming INBRE Bioinformatics core, Department of Molecular Biology, University of Wyoming, Laramie, WY.

Sequencing technologies have made it possible to carry out whole genome sequencing (WGS) to identify the genetic variants present in mutant organisms. In addition, methods have been developed that simultaneously facilitate mapping of the causal mutation. Currently, two principal strategies for “mapping by sequencing” are used by C elegans researchers. In one approach, mutants are crossed to the polymorphic HA strain, which provides extensive SNP mapping information and localization of the causal mutation. This approach, however, is not highly practical for complex genetic backgrounds, such as the case with genetic suppressors, and the numerous polymorphisms present in the HA strain can alter phenotypes in ways that are unpredictable. A second method, EMS density mapping, exploits of the higher concentration of signature-EMS alterations in regions surrounding causal mutations following multiple backcrosses. The resolution of EMS density mapping, however, can be quite low and this method is not applicable for alleles that arise spontaneously or through other methods. We recently obtained ~30 strong suppressors nekl-2; nekl-3 double-mutant larval lethality. Our screens included a novel strategy using counter-selection that was enabled by the peel-1 toxin. Notably, neither the nekl-2 or nekl-3 single mutants used for this screen display phenotypes on their own, nor did the majority of obtained suppressors. In order to identify the causal mutations that suppress nekl-2; nekl-3 lethality, we designed and implemented a novel refinement strategy we call the Sibling Subtraction Method (SSM).

For our approach, suppressed strains were backcrossed several times to ensure Mendelian inheritance and to partially remove background mutations. While performing the final backcross, we isolated strains that were homozygous for the suppressor mutation as well as strains that were non-suppressed (i.e, homozygous wild type at the suppressor loci). These isolates were combined to create a Suppressed DNA pool and a Non-suppressed Comparitor DNA pool, respectively. Following WGS, alignment, and filtering steps, common variants present in both the Suppressed and Comparitor pools were subtracted. This resulted in a very small number of candidate variants (~5) that caused non-synonymous coding changes in each of the suppressor strains. Notably, we were able to subtract more than 95% of background variants using this method. The bioinformatics analysis was performed using Cloudmap workflows in the Galaxy web platform, which are freely available and widely used by the C elegans field. Our strategy was validated on five suppressor strains and, most importantly, is generally applicable for all varieties of mutations and genetic backgrounds. As such, SSM largely obviates the need to use the HA strain and provides better candidate refinement than EMS density mapping methods.


Parkinson’s Disease (PD) is the most common age-associated movement disorder worldwide, and its prevalence is increasing in our aging population. While several well-studied genes have been associated with PD, 70-95% of PD cases have unknown origins, reflecting the need for innovative approaches to identify causal PD genes. Existing unbiased approaches like GWAS, although powerful, are hampered by statistical limitations. Furthermore, rapid candidate gene testing in humans is unrealistic, mammalian model systems are not amenable to high-throughput testing, and invertebrate models have not been utilized for unbiased screening of PD-like phenotypes. To solve these problems, we developed a novel approach that efficiently extracts cell lineage-specific signals from the compendium of C. elegans expression data and generates network representations of tissue- and cell-type-specific functional similarity, then combined that information with genome-wide disease studies to identify candidate PD genes, followed by high-throughput candidate testing of age-dependent motility defects in C. elegans. We discovered that reduction of many of our top-priority candidate Parkinson’s disease genes in C. elegans caused age-dependent thrashing phenotypes that are not typical of aging worms. Furthermore, reduction of the branched chain amino acid (BCAA) transferase bcat-1 caused severe age-dependent spasm-like “curling” through its induction of neurodegeneration in cholinergic neurons, despite the fact that bcat-1 reduction in non-neuronal tissues extends lifespan. Our integrative cross-species and unbiased functional analyses revealed possible roles for cholinergic signaling and BCAA metabolism in PD, and illustrate the importance of evaluating quality-of-life metrics in addition to longevity.

609B Genetic screens for identification of the telomerase RNA component in C. elegans.  E. Kim, J. Lee Department of Biological Sciences, Institute of Molecular Biology and Genetics, Seoul National University, Seoul, Seoul, KR.

In the linear chromosome replication, DNA polymerase cannot complete the replication of chromosome ends, thus cells are faced with ‘end replication problem’. Shortened chromosome ends can be considered as DNA damage, and chromosome fusion may occur through DNA repair pathways. Therefore, eukaryotic cells, which have linear chromosomes, have specific repetitive sequences at the end of chromosomes to protect them: ‘telomeres’. Telomeres can be maintained by telomerase, a reverse transcriptase conserved in different organisms, from ciliates to human. The telomerase holoenzyme is composed of telomerase catalytic subunit, RNA component that acts as a template for lengthening of 3’ single-stranded telomeric DNA, and several
accessory proteins. *Caenorhabditis elegans* also has telomerase for telomere maintenance, but only the gene encoding telomerase catalytic subunit, *trt-1*, has been identified. As the telomerase RNA component of *C. elegans* has not been identified, we are performing experiments to identify it with two approaches: by candidate approach based on telomere template sequences using informatics, and by the forward genetic screen used in identification of telomerase catalytic subunit, *trt-1* (1). We found several mutant lines that showed weak telomere signals in telomere dot blot. We will confirm telomere defects of these lines and identify corresponding genes.


610C **Serial-section RNA sequencing: genome-wide gene expression pattern analysis in C. elegans males and hermaphrodites.** A.L.P. Ebbing, Á. Vértesy, M.C. Betist, A. van Oudenaarden, H.C. Korswagen Hubrecht Institute, Utrecht, NL.

RNA sequencing (RNA-seq) is ideally suited for genome-wide analysis of gene expression. However, when a whole organism is used for RNA isolation and sequencing, the data will show the overall level of gene expression, but not where such genes are expressed in the organism. A solution to this problem is to provide spatial information by sectioning the organism along one (or more) of the body axes and to perform RNA-seq on each of the separate sections.

We have developed a serial-section RNA-seq (serial-seq) method for *C. elegans* (see also the other abstract of Ebbing, Korswagen and co-workers). In brief, we cryo-section a young adult animal from the head to the tail into 20 micrometer thick slices, resulting in a total of 40 to 50 slices. Each slice is then sequenced using the sensitive CEL-Seq method and the sequencing data is aligned to create a high-resolution anteroposterior gene expression map.

We have generated expression maps for 4 young adult hermaphrodites, 4 young adult males and 2 germline deficient *glp-1* mutants. In these datasets, an average of 8794 genes (43% of coding genes) can be detected along the length of the animal. These include genes that are expressed in major tissues such as the germline, but also genes that are known to be expressed in only single neuron pairs in the head, demonstrating the high sensitivity of our approach. Furthermore, we found that these expression patterns are highly reproducible among independently sectioned animals.

To explore these gene expression maps, we have developed an algorithm that enables us to search for genes with specific expression patterns. Using the expression patterns of known marker genes as a starting point, we have identified genes that are specifically expressed in the germline and sperm, in organ structures such as the pharynx, the intestine, the vulva, the spermatheca and the male reproductive tract, and in neurons of the nerve ring and tail ganglia. Furthermore, we have found extensive sex-specific differences in gene expression in the germline and sperm, in the male reproductive tract and in the male specific CEM neurons. We will present an overview of the method and discuss our findings on cell, organ and sex-specific gene expression patterns.

611A **Dynamic changes of gene-body H3K4me3 with age in C. elegans somatic cells.** Mintie Pu1, Minghui Wang2, Siu Sylvia Lee3  1) Molecular Biology & Genetics, Cornell University, Ithaca, NY; 2) Computational Biology Service Unit, Cornell University, Ithaca, NY.

Recent studies demonstrate that altered histone modifications can profoundly impact longevity. However how histone modification changes modulate aging and how aging alters histone modifications are still largely unclear. To gain insights into the histone modification pattern during aging, we monitored the genomic locations and abundance of several histone modifications involved in gene transcriptional regulation in the somatic cells of *C. elegans* at different ages. Previously we found that the genome-wide pattern of H3K36me3 is largely stably maintained during aging and that H3K36me3 marking plays an important role in maintaining gene expression stability and lifespan. We have now surveyed the genome-wide pattern of H3K4me3 through similar aging time points. We identified specific regions of H3K4me3 marking that show dynamic change with age and that these H3K4me3 changes are highly correlated with gene expression alterations. Interestingly, the H3K4me3 regions that change with age and are accompanied by RNA expression alterations largely mark gene-bodies, contrary to the canonical marking of H3K4me3 around transcriptional start sites (TSS). Comparison with modENCODE data suggest that the gene-body marking of H3K4me3 that tend to change with age are not marked by H3K4me3 during early development but become apparent during late larval and/or adult stages, whereas the H3K4me3 marking around TSS are clearly established during early development and remain largely stable with age. Our study suggests an intriguing possibility of adult-specific H3K4me3 marking that are more susceptible to age-dependent change, which may have important implications for age-dependent RNA expression dynamics.

612B **Hybrid assembly of the genome of the entomopathogenic nematode *Steinernema carpocapsae.** Marissa Macchietto1, Lorrainey Serra2, Bryan Rodriguez2, Ali Mortazavi2,3  1) Institute of Health Informatics, University of Minnesota, Minneapolis, MN, USA; 2) Department of Developmental and Cell Biology, University of California, Irvine, CA 92697, USA; 3) Center for Complex Biological Systems, University of California, Irvine, CA 92697, USA.

Entomopathogenic nematodes from the genus *Steinernema* are lethal insect parasites that quickly kill their insect hosts with the help of their symbiotic bacteria. *Steinernema carpocapsae* is one of the most well-studied entomopathogens, due to its broad lethality to diverse insect species, and to its effective commercial use as a biological control agent for insect pests. For this reason, it has become an important genetic model for studying parasitism, pathogenesis, and symbiosis. We used a newly
published hybrid assembly pipeline to assemble the best genome of *S. carpopcapae* to date, comprising 86,259,276 bp in 86 scaffolds, with an N50 of 4.03 Mb, from a combination of 75X coverage Pacbio and 130X coverage Illumina reads. We found that 90% of the genome is represented by the 22 largest scaffolds. RNA-seq data from 17 developmental stages spanning the embryo to adult stages were used to help predict 22,295 gene models, a major reduction in the number of genes from the previously published assembly by Dillman et al. 2015, which has 28,313 genes, and an increase in the number of genes relative to a high contiguity *S. carpopcapae* Breton strain assembly (N50=1.24), which has 16,333 genes. Using this new genome, we infer the potential chromosomal origins of our scaffolds by comparing them to *C. elegans* using shared one-to-one orthologs and find that many of the largest scaffolds correspond primarily to single chromosomes in *C. elegans*. We also investigate a potential large 1.2 Mb duplication in the genome, and delve into gene expression differences between male and female stage nematodes. This new genome and more accurate set of annotations will provide a good foundation for new comparative genomic and gene expression studies.

### 613C Repeat region inversion probes: a high-throughput estimation of rDNA locus copy number in Caenorhabditis elegans

C.A. Mok, E.A Morton, C. Queitsch, R.H. Waterston  Genome Sciences, University of Washington, Seattle, WA, USA.

In *C. elegans* the major rDNA locus lies on the right end of LGI. A 7.2 kb repeat contains the rrm-1(18s rRNA), rrm-2(5.8s rRNA), and rrm-3(26s rRNA) gene. The N2 laboratory strain has been estimated to contain between 80-100 copies (Sulston and Brenner 1974; *C. elegans* sequencing consortium 1998). However, the copy number was found to vary between 55-130 copies of this region across 2007 mutagenized strains using whole genome sequencing (WGS) (Thompson et al. 2013). In contrast, wild isolates have estimated copy numbers of the LGI locus ranging as low as 68 and as high as 418 (Thompson et al. 2013). Although the standard laboratory strain N2 is usually considered to be isogenic, we wondered if recombination errors in meiosis might produce variable copy numbers of this repeat that could be associated with phenotypic differences observed in areas such as lifespan, stress response, and mutation penetration. Two methods for estimating rDNA copy number are contour-clamped homogeneous electric field (CHEF) gels and WGS. CHEF gels, the gold standard in rDNA copy number estimation, require large numbers of worms to generate a sample. WGS, however, requires far less sample input but is costly with large sample numbers. Since examining the effect of rDNA copy number on phenotype variation requires working with small populations and multiple biological replicates, we devised a targeted sequencing approach that operates well with low sample input and is cost-efficient. Briefly, the method uses molecular inversion probes (MIPs, Hiatt et al. 2013) to sample regions of the rDNA locus as well as single-copy sites of the *C. elegans* genome. These probes are used in a Repeat Region Inversion Probe (RRIP) capture reaction combining genomic DNA samples with a special normalization plasmid containing single-copy versions of the rDNA locus and the other RRIP genomic targets. The plasmid versions, however, contain single nucleotide variants to distinguish these from the endogenous loci. After sequencing, reads from both plasmid and genomic DNA are analysed to generate an rDNA copy number estimate. We investigated the accuracy of the RRIP approach by testing wild isolate genomic samples that were previously sequenced by WGS in the Million Mutation Project. Our RRIP approach provides similar estimates of rDNA copy numbers with only a fraction of the reads required for WGS. Thus, RRIP is able to estimate rDNA copy number from small amounts of genomic DNA, for low sequencing cost, in a short period of time (3-day turnaround). With RRIP we can study small populations, potentially at a timescale of a single generation to investigate the influence of rDNA copy number variation in lifespan and overall health.

### 614A Improving helminth genome curation in WormBase

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*Brugia malayi* is a clade III parasitic nematode and one of the causal agents of filariasis. Its whole lifecycle can be maintained in a laboratory and cultured strains are available through a strain repository. It is often used as model organism for parasitic filarial nematodes and host/parasite and host/endosymbiont analysis. In addition, it is the oldest helminth species in WormBase and often used as model to trial improvements of curation processes.

As part of the WormBase core genome curation and through collaborations with the *B. malayi* community, the assembly was greatly improved over the previous hybrid short read and Sanger sequencing WGS approach. The inclusion of optical mapping, as well as long-range PacBio data, allowed the scaffolding of the previous fragmented assembly into whole chromosomes, close gaps, as well as reduce heterozygosity and resolve repetitive regions. This finally allows the analysis of whole genome rearrangements and chromosomal synteny maps.

In addition curation from the previous assembly was preserved and updated to accommodate new data and WormBase paper mining extended to cover parasitic worms. This work allows WormBase to better cover the needs of the helminth community and can also used to improve other core species curation efforts.

### 615B Functions of regulatory elements during C. elegans development

J. Serizay, W. Q. Seow, N. Huang, M. Chesney, J. Ahringer  Gurdon Institute, Cambridge, GB.

Transcription is controlled by different types of accessible regulatory elements: typically, transcription initiates at a promoter located upstream of the transcribed sequence and its activity can be regulated by one or several enhancers, usually located in cis but potentially far from the transcribed region. These regulatory elements are characterized by specific marks such as histone modifications and are often bound by transcription factors. Such regulatory elements control the correct pattern of gene expression, which could be ubiquitous or show spatial and/or temporal regulation. Enhancers control the activity of promoters
through poorly understood mechanisms. It has been shown that enhancers often make physical contact with promoters, supporting models where promoter activity is controlled by enhancer-promoter interactions. Such interactions can span kilobases and up to a megabase, bringing in close spatial proximity two linearly distant regulatory elements. Notably, alterations in the chromatin interaction landscapes have also been found in cancer cells. Thus, defining the contacts made between enhancers and promoters is important for understanding gene regulation. We aim to bring insights into how regulatory elements function and the nature and roles of their interactions during C. elegans development.

To study the control of gene expression in development, we have developed a FACS based method to purify nuclei from specific cells and tissues. We made a GFP reporter anchored to the outer nuclear membrane using a fragment from UNC-83. Nuclei are isolated from strains expressing this transgene under the control of tissue-specific promoters (muscle, epidermis, intestine, neuronal and germ line specific promoters), then GFP+ nuclei are sorted by FACS, enriching for populations with a purity up to 93%. To characterize the tissue specific control of gene expression, we are currently identifying which regulatory elements are used in different tissues by mapping chromatin accessibility (using ATAC-seq) and by performing RNA-sequencing from a range of tissue-specific sorted nuclei.

We have also designed a novel method, called ARC-C, to identify genome-wide interactions between regulatory elements at a resolution of 500 bp. Using this technique, we have identified >30,000 interactions between C. elegans regulatory elements. We are now applying this method to tissue specific samples to study the developmental control and functions of promoter-enhancer connections.


Metazoan Transcriptional Regulatory Factors (TFs) bind to genomic response elements, controlling gene expression precisely to establish and maintain tissue-specific cellular identity. Importantly, TFs also display remarkable plasticity, governing distinct gene networks in different tissues and developmental stages. For example, the conserved C. elegans TF, NHR-25, regulates two different developmental programs, vulva patterning and molting, in larvae. To characterize NHR-25 activity, we used CRISPR methods to introduce GFP, 3xFLAG and auxin inducible tag (AID) into the endogenous NHR-25 gene. The multiple tags simultaneously enabled live imaging of NHR-25 in L3 larvae, ensuring NHR-25 expression in both the developing vulva and seam cells, and ChIP-seq. We found that 75% of our NHR-25 enrichment sites overlapped with those in the modENCODE ChIP-seq NHR-25 dataset at a similar developmental time. However, we found an order of magnitude fewer enrichment peaks for NHR-25, suggesting that even modest overexpression of TFs results in substantial promiscuous binding. To identify the primary gene targets of NHR-25, we transiently depleted NHR-25 for four hours through addition of auxin, resulting in degradation of NHR-25 through the AID tag, and carried out RNA-seq. Genes that changed in expression in response to this short depletion of NHR-25 were identified by mapping to NHR-25 functional promoter network elements responsible for regulating specific target genes in different tissues.


Studies in the nematode Caenorhabditis elegans have shown that exposure to as-synthesized and transformed (sulfidized) silver nanoparticles (sAg-NPs) can cause various toxic effects, but genomic effects that can be induced by prolonged exposure over multiple generations have not been investigated. A previous multigenerational study of continuous exposure of C. elegans to AgNO3, Ag-NPs and sAg-NPs showed that increased sensitivity, in terms of reproductive toxicity from Ag, occurred as early as the second generation for AgNO3 and Ag-NPs, but not sAg-NPs. This suggested that Ag-NPs may cause mutations or epimutations. In this study, we used wild type N2 C. elegans as a model organism to determine if mutations and/or epimutations contribute to the observed multigenerational effects.

Exposure was carried out using sub-lethal concentrations (EC30 for reproduction) of AgNO3, Ag-NPs and sAg-NPs in simulated soil pore water for 10 generations with the parent generation (F0) unexposed for all groups. Four replicates were used per treatment, from which DNA was extracted from an entire brood of a single worm for whole genome DNA sequencing by NextSeq. Analysis of sequencing data revealed no significant differences in the total number of mutations (SNPs, deletions or insertions) from the controls. This suggests that induced germine mutations may not be responsible for the increased sensitivity observed in multigenerational exposures. We hypothesized that epigenetic mechanisms are involved.

Increased DNA methylation (6-methyladenine), an epigenetic marker, which was recently discovered in C. elegans, has been shown to negatively impact reproduction over multiple generations. We are currently investigating DNA methylation as a potential mechanism by which reproductive toxicity can be passed on to subsequent generations after exposure to Ag-NPs. Preliminary experiments using slot blots show that there is a potential increase in DNA methylation after 2 generations of exposure to pristine Ag-NPs. Interestingly, there seems to be a decrease in DNA methylation for AgNO3 exposed worms. In addition to confirming these results and testing sAg-NPs, we will also examine if the higher levels of DNA methylation persist in subsequent generations after cessation of exposure.

618B The genetic basis of difference in 3D genomic architecture between C. nigoni and C. briggsae. X. Wei1,3,4, R. Ma1,2, Y. Zha1,2, S. Chen1, M. Zhang1,5,6, J. Zeng5, X. Liu1 1) MOE Key Laboratory of Bioinformatics, Center for Synthetic and Systems Biology, School of Life Sciences, Tsinghua University, Beijing, China; 2) Institute for Interdisciplinary Information
massive reannotation of the wormbase C. briggsae gene set.

About a third of the existing gene structures were already correct, a third have been changed and a third are either expressed at too low a level to determine the correct structure, or are damaged by poor quality assembly in that region, or might be pseudogenes, or otherwise have proved intractable.

About 200 C. elegans genes had their structures corrected based on homology evidence from the new C. briggsae structures.

Several interesting genes with unusual conserved structures have been discovered during the process of manual assessment of the gene set. Among these are two genes using a putative non-canonical initiation codon (CBG08614, F26D11.1 and CBG20564, C37C3.8). There are about 40 genes where the locus produces two non-overlapping isoforms but they do not alternate of TAD boundaries. As a result, we only have observed 8 TAD boundaries specific for C. nigi and 6 for C. briggsae. Interspecial crossing of C. nigi and C. briggsae gives rise to fertile hybrids, providing a chance to examine the evolution of 3D genome structure and its genetic basis. We did HiC analysis of their F1 hybrids. Our preliminary data have revealed 10 TADs in their F1 hybrids. All of them are conserved between C. nigi and C. briggsae. We are generating more HiC data of F1 hybrids to reveal how the region of the parental species-specific boundaries change in F1 hybrid.

Stage-specific gene expression in the larval stages of Caenorhabditis angaria for comparative transcriptomic analysis of Caenorhabditis development.

Caenorhabditis angaria (AKA Caenorhabditis sp. 3 PS1010) is a gonochoristic species of entomopathogenic nematodes whose genome has been sequenced. Larval development of C. angaria is conserved across many diverse species of nematodes and involves four distinct stages. We hope to characterize gene expression and miRNA differences important to nematode development by sequencing the transcriptome of individual nematodes from each larval stage using the SmartSeq2 protocol. Sequencing individual nematodes will allow us to analyze the heterogeneity of expression at each stage in development, an impossible task when sequencing multiple nematodes in one sample. The gene expression and miRNA profiles for the larval stages in C. angaria when compared to those in C. elegans could reveal conserved genes and pathways necessary for larval development. Progression through the larval stages includes an increase in size and organ development. By tracking expression profiles across the life of the worm, we can distinguish genes regulating the development of different organs. Male and female expression differences can be characterized and used to establish factors for sex determination and development in nematodes. The timing for the lifecycle of C. angaria, measured using identifiable larval stage markers, such as vulval development, and size, reveals a longer life cycle than C. elegans. Sequencing the transcriptome of individual C. angaria can lead to insights into evolution, development, and sex determination in nematodes.

Gene Regulation and Genomics - Novel Genetic Technologies
Epistasis analysis using RNA-seq. D. Angeles-Albores\textsuperscript{1,2}, C. Puckett Robinson\textsuperscript{1,2,3}, B.J. Williams\textsuperscript{1}, B.A. Wold\textsuperscript{1}, P.W. Sternberg\textsuperscript{1,2} 1) Division of Biology and Biological Engineering, Caltech, Pasadena, CA; 2) Howard Hughes Medical Institute; 3) Department of Neurology, Keck School of Medicine, University of Southern California.

RNA-seq analysis of transcriptomes is commonly used to identify genetic modules that respond to perturbations. In single cells, transcriptomes have been used as phenotypes, but this concept has not been applied to whole-organism RNA-seq. Linear models can quantify expression effects of individual mutations and identify epistatic effects in double mutants in individual genes, resulting in thousands of epistasis measurements. Interpreting these high-dimensional measurements is not intuitive. We thus developed a single coefficient to quantify epistasis from vectorial phenotypes that accurately reflects the underlying genetic interactions. To demonstrate the power of our approach, we sequenced four single and two double mutants of \textit{C. elegans}. From these mutants, we successfully reconstructed the known hypoxia pathway that was derived from classic double mutant analyses in which EGL-9 and VHL-1 inhibit the activity of HIF-1. In addition, we uncovered a class of 31 genes that have opposing changes in expression in \textit{egl-9(lf)} and \textit{vhl-1(lf)} but for which the \textit{egl-9(lf);vhl-1(lf)} mutant has the same expression phenotype as does \textit{egl-9(lf)}. These changes violate the classical model of HIF-1 regulation, but can be explained by postulating a role of hydroxylated HIF-1 in transcriptional control, opposing the function of non-hydroxylated HIF-1 at selected targets. As a second example, we used transcriptional profiling to explore the role of sperm in regulation of \textit{C. elegans} life cycle, and identified a substrate-dependent pathway linking \textit{fog-2} and aging through sperm depletion. Our ongoing work demonstrates the usefulness of transcriptomic phenotypes for interpreting epistatic interactions and resolving molecular pathways in detail. We believe our method holds promise for quantitative assays that involve multiple molecular species resulting in measurements of complex phenotypes.

Making tetraploid stable strains to investigate the causes and consequences of whole genome polyploidization. E. Clarke, Malikha Khidoyatova, Anne Jose, M. Schvarzstein Biology, City University of New York, Brooklyn College, Brooklyn, NY.

Whole genome polyploidization has been implicated as a key step in development of cancer and drug resistance. In addition, polyploidization is important in nature for adaptation, speciation, organogenesis, wound healing, and biological scaling. Our understanding of the roles and consequences of polyploidization in multicellular organisms has been limited by the fact that whole genome polyploidy could not be easily induced in laboratory model systems. Nigon was the first to generate \textit{Caenorhabditis elegans} tetraploid strains, but this method is strenuous and inefficient, yielding only a handful of tetraploid strains in the last 30 years.

One way in which tetraploidy arises in nature is by the generation of diploid gametes, which upon fertilization form a tetraploid organism. We have developed an efficient scheme based on the finding that the meliosis-specific cohesin component \textit{rec-8} mutant produces diploid gametes. In \textit{rec-8} mutants asymmetric segregation of chromatids in the second spermatocyte division yields both anucleate and diploid sperm. \textit{rec-8} mutant oocytes fail to extrude the second polar body, giving rise to diploid female gametes. Our method utilizes transient RNAi for \textit{rec-8} to generate tetraploids from any diploid strain, possibly by generating diploid gametes that may give rise to a tetraploid stable strain. \textit{rec-8} RNAi treatment of diploid hermaphrodites of any genotype for two generations occasionally yields Lon animals, which are characteristic of polyploid \textit{C. elegans}. These Lon animals can give rise to stable tetraploid strains. Using this methodology several complex strains have been generated. These include strains containing balancers, fusion chromosomes, chromosomal inversions and fluorescent markers have been generated. Manipulation of ploidy within a single species will enable us to inquire the role of genome size on development, intracellular and cellular scaling, animal size, cell division, and gene dose. Analysis of some of the tetraploid strains will be presented.

Applying CRISPR-Cas9 Technology and Whole Genome/Amplicon Sequencing to the \textit{C. elegans} Knockout Project. Vinci Au\textsuperscript{1,2}, Iasha Chaudhry\textsuperscript{1}, Mark L. Edgley\textsuperscript{1,2}, Lisa Fernando\textsuperscript{1}, Stephane Flibotte\textsuperscript{1}, Erica Li-Leger\textsuperscript{1,2}, Su Wang\textsuperscript{1}, Donald G. Moerman\textsuperscript{1,2} 1) Department of Zoology, University of British Columbia, Vancouver, BC, CA; 2) Michael Smith Laboratories, University of British Columbia, Vancouver, BC, CA.

The \textit{C. elegans} Gene Knockout Facility generates null mutations in \textit{C. elegans} for distribution worldwide, with the goal of obtaining knockouts for every Open Reading Frame (ORF) in the genome. To date, 67% of the ORFs in the genome (13,559 ORFs) are represented by null mutations. Our immediate priority is to complete knockouts for all members of important gene families such as transcription factors, kinases, phosphatases, and GTPases. We are also attempting to generate deletions of homologous gene clusters. These tandem gene knockouts may provide insight into the function of a particular group of related genes when a single gene knockout has no detectable phenotype.

We are currently using two different methods to achieve our goals: CRISPR-Cas9 genome editing, and whole genome (WGS) and amplicon sequencing of EMS/ENU-mutagenized worm populations. The CRISPR-Cas9 method allows targeting of specific genes, while WGS/amplicon sequencing, albeit less specific, is quicker and more cost effective. The CRISPR-Cas9 system allows us to simultaneously make targeted deletions and insert selectable markers to track mutations. This method is applicable both for single gene knockouts and for tandem gene knockouts. The amplicon sequencing approach uses populations of mutagenized worms that are harvested at the F3 generation. We do WGS on half of each population to identify SNVs of interest. The other half of the population is single and used for isolation of the mutation, using PCR amplicons that are barcoded, pooled, and sequenced on an illumina sequencer. This identifies single sub-populations that are homozygous or heterozygous for the SNV of interest. We have generated 308 mutagenized F3 isolates, and have performed WGS on 73 of these populations. Mutations in 451 genes with no previously known null alleles have been identified in this way. To date, we have successfully isolated new knockouts in 19 genes using the amplicon sequencing method, and 42 genes using the CRISPR-Cas9 approach. Finished knockouts are sent to the Caenorhabditis Genetics Center at the University of Minnesota, from which strains are distributed to labs around the world.

CRISPR/Cas9 genome engineering strategies allow the directed modification of the *C. elegans* genome to introduce point mutations, generate knock-out mutants and insert coding sequences for epitope or fluorescent tags. Three practical aspects however complicate such experiments. First, the efficiency and specificity of single-guide RNAs (sgRNA) cannot be reliably predicted. Second, the detection of animals carrying genome edits can be challenging in the absence of clearly visible or selectable phenotypes. Third, the sgRNA target site must be inactivated after editing to avoid further double-strand break events. We describe here a strategy that addresses these complications by transplanting the protospacer of a highly efficient sgRNA into a gene of interest to render it amenable to genome engineering. This sgRNA targeting the *dpy-10* gene generates genome edits at comparatively high frequency. We demonstrate that the transplanted protospacer is cleaved at the same time as the *dpy-10* gene. Our strategy generates scarless genome edits because it no longer requires the introduction of mutations in endogenous sgRNA target sites. Modified progeny can be easily identified in the F1 generation, which drastically reduces the number of animals to be tested by PCR or phenotypic analysis. Using this strategy, we reliably generated precise deletion mutants, transcriptional reporters, and translational fusions with epitope tags and fluorescent reporter genes. In particular, we report here the first use of the new red fluorescent protein mScarlet in a multicellular organism. *wrmScarlet*, a *C. elegans*-optimized version, dramatically surpassed TagRFP-T by showing an 8-fold increase in fluorescence in a direct comparison.

625C Strategies for improving the efficiency of precise genome editing in multiple nematode species using CRISPR/Cas9. B. Farboud1, A. Severson2, B. Meyer1 1) HHMI and Dept. of MCB, UC Berkeley, Berkeley, CA; 2) Dept. of Biological, Geological and Environmental Sciences, Cleveland St. University, Cleveland, OH.

The ease of programming Cas9 to bind and cleave DNA sequences with few constraints has led its widespread use in *C. elegans* research; however, many limitations for Cas9 genome editing remain. We have improved the efficiency of Cas9 mutagenesis by addressing difficulties in five areas: 1) inserting DNA sequences greater than 3kb, 2) incorporating precise mutations at a long distance from the DSB, and 3) inserting mutations near the DSB. Additionally, 4) we developed a method to increase the efficiency of Cas9 mutagenesis, and 5) improved the rate of mutant recovery in multiple nematode species.

1) Large insertions (1.5 to 8kb) can be introduced in the genome by making two Cas-mediated DSBs and providing a repair template spanning the two DSBs. Insertions have been made with high efficiency (35%) without using selectable markers.

2) To target changes far from a DSB, two Cas9-mediated DSBs straddling the site of mutagenesis and a repair template spanning the two DSBs promote efficient replacement of intervening sequences. The distance between a DSB and the first mutant bases can be > 1 kb.

3) We found that the position of a desired change relative to the location of the PAM affects the rate of incorporation. If the desired change is 5' of the PAM, a single-strand DNA (ssDNA) repair template, that corresponds to the protospacer strand will yield high efficiency incorporation. If the desired change is 3' of the PAM, a ssDNA repair template should correspond to the spacer strand to incorporate a change. Use of a ssDNA repair template that corresponds to the PAM strand drastically reduces the frequency of changes 3' of the PAM.

4) The rate of Cas9-mediated mutagenesis is enhanced when a *C. elegans* hermaphrodite is mated prior to microinjection of Cas9, because Cas9 more efficiently targets the paternal chromosome. Efficiency of both imprecise and precise repair increases, and the rate of homozygous mutations is enhanced nearly 10 fold.

5) To facilitate recovery of Cas9 generated mutants in multiple nematode species, two highly conserved genes were developed as co-CRISPR/co-conversion markers. Mutations in *ben-1* confer resistance to benomyl and hence mobility when grown on benomyl, making *ben-1* a good co-conversion marker. Conversion of *zen-4(cle10ts)* to *zen-4(+)* creates viable embryos and hence can be used as a stringent selection marker.

626A EMS vs ENU: Does mutagen choice matter? E. Hulber1,2, E. Jorgensen1,2 1) Department of Biology, University of Utah, Salt Lake City, UT; 2) Howard Hughes Medical Institute.

In 1974 when Sydney Brenner performed the first mutagenic screen in *C. elegans*, he advocated for the use of the mutagen EMS (ethyl methanesulfonate). Other model systems, however, more typically use ENU (N-ethyl-N-nitrosourea) to induce mutation. By whole-genome sequencing of mutagenized F1 individuals, we demonstrate that EMS and ENU have dramatically different mutagenic profiles. While EMS is heavily biased toward G>A transitions, ENU has a broader distribution. These differing mutagenic profiles manifest themselves as dramatically different profiles of codon changes and amino acid changes. EMS introduces slightly more nonsense mutations, likely causing null alleles. On the other hand, only a limited set of rather conservative amino acid substitutions are introduced by EMS, limiting its usefulness. ENU has a much broader distribution of amino acid substitutions, which can aid in discovering unexpected details of protein functions. Our analysis also demonstrates how the non-random structure of the codon table serves as a barrier to dramatic amino acid changes, a possible influence in the evolution of the universal codon table.

627B Genome editing using Cas9 ribonucleoprotein complexes and linear repair templates. A. Paix, A. Folkmann, G. Seydoux Molecular Biology and Genetics, HHMI, Johns Hopkins University School of Medicine, Baltimore, MD.

The ability to introduce targeted edits in the genome of model organisms is revolutionizing the field of genetics. State-of-the-art methods for precision genome editing use RNA-guided endonucleases to create double-strand breaks (DSBs) and DNA templates containing the edits to repair the DSBs.

Following this strategy, we have developed a protocol to create precise edits in the *C. elegans* genome. The protocol takes
advantage of two innovations to improve editing efficiency: direct injection of CRISPR-Cas9 ribonucleoprotein complexes and use of linear DNAs with short homology arms as repair templates. The protocol requires no cloning or selection, and can be used to generate base and gene-size edits in just 4 days. Point mutations, insertions, deletions and gene replacements can all be created using the same experimental pipeline.

In this workshop, we will present our method and the rules governing successful genome editing in C. elegans

628C Use of 3D printing and magnets to create a novel cost-effective fluorescence dissecting stereomicroscope system. Andrew Papp, John Biondo Titech Research, Los Angeles, CA.

Various fluorophores are used to label molecules of interest to determine the presence, location, and timing of gene expression, and gene-gene interactions, as well as neuronal activity. This labeling can be accomplished through antibody binding, the creation of transgenic organisms with fluorescent fusion proteins, reporter genes, or ion-responsive fluorescent proteins.

Fluorescence from these labels can be conveniently observed in live specimens through the use of a fluorescence dissecting stereomicroscope. Such microscopes typically use expensive, cumbersome, epifluorescence illumination systems powered by short-lived mercury arc lamps and requiring filter cubes with expensive dichroic mirrors.

We previously demonstrated that it is possible to get adequate illumination and detection using LED light sources and oblique illumination (Papp and Biondo, 2015). Several engineering challenges to making a practical version of such a system have been overcome through the use of multifunctional 3D-printed parts to hold filters and aim light beams. Users may need to switch back and forth between observation of different fluorophores quickly and many thousands of times. Strong small magnets were used to create wear-free detents to precisely position the optics and also to allow tool-free installation and changing of modules for the detection of different fluorophores.


629A An improved INTACT protocol for C. elegans cell-type-specific genomic profiling reveals novel insights into neuronal development. D.P. Rahe1, H. Sun1, E. Leyva-Diaz1, O. Hobert1,2 1) Biological Sciences, Columbia University, New York, NY; 2) Howard Hughes Medical Institute.

Cell-type-specific profiling allows for the detailed analysis of a desired tissue, without the background of the rest of the organism. In the worm, this has long been hampered by the lack of robust and specific tools. The INTACT technique uses a genetically encoded nuclear membrane tag to allow the immunoprecipitation (IP) of entire nuclei. Through changes from the published method to the lysis, nuclear purification, and IP steps of the protocol, we were able to increase the purity and specificity of the technique. We will describe our efforts to demonstrate the potential of this technique, using INTACT to perform RNA-seq on individual ASER or ASEL neurons. By performing INTACT at different stages we can gain insight into the changes in these sensory neurons during development, and demonstrate that this method may be used for profiling in the most stringent conditions (one single, small cell in the entire organism). We next chose to answer another longstanding question of our lab: what is the gene expression profile of a neuron lacking its terminal fate-specifying transcription factor (TF)? We will describe efforts to profile ASE, AIY, AWA, and ADL neurons lacking these key TFs to uncover interesting patterns of terminal selector function. We will compare the use of this method with another existing method for cell-type-specific profiling, namely FACS of fluorescent-labeled dissociated cells. By comparing our pan-neuronal RNaseq profile to those previously published using the FACS technique, we will be able to demonstrate benefits and drawbacks of each method. Lastly, we discuss the ability to use this technique in conjunction with other genomic profiling methods, such as ATAC-seq and ChIP-seq.


C. elegans has many tools for genome modification. However, the precise insertion of larger DNA constructs is still relatively laborious and difficult. In hopes of making large integrations at predetermined genomic sites easier, we have adapted the fC31 integrase system for use in microfluidics. By comparing our pan-neuronal RNAseq profile to those previously published method to the lysis, nuclear purification, and IP steps of the protocol, we were able to increase the purity and specificity of the technique. We will describe our efforts to demonstrate the potential of this technique, using INTACT to perform RNA-seq on individual ASER or ASEL neurons. By performing INTACT at different stages we can gain insight into the changes in these sensory neurons during development, and demonstrate that this method may be used for profiling in the most stringent conditions (one single, small cell in the entire organism). We next chose to answer another longstanding question of our lab: what is the gene expression profile of a neuron lacking its terminal fate-specifying transcription factor (TF)? We will describe efforts to profile ASE, AIY, AWA, and ADL neurons lacking these key TFs to uncover interesting patterns of terminal selector function. We will compare the use of this method with another existing method for cell-type-specific profiling, namely FACS of fluorescent-labeled dissociated cells. By comparing our pan-neuronal RNaseq profile to those previously published using the FACS technique, we will be able to demonstrate benefits and drawbacks of each method. Lastly, we discuss the ability to use this technique in conjunction with other genomic profiling methods, such as ATAC-seq and ChIP-seq.

631C Multigene analysis of aging markers in Caenorhabditis elegans using directed evolution and high-throughput microfluidics. W. Zhuo1, Y Zhao2, H. Lu1,3, P. McGrath2,3 1) Chemical and Biomolecular engineering, Georgia Institute of Technology, Atlanta, GA; 2) School of Biology, Georgia Institute of Technology, Atlanta, GA; 3) Bioengineering, Georgia Institute of Technology, Atlanta, GA.

Most complex diseases and traits have a strong heritable component and are usually controlled by a complicated network of genes with various effect sizes. Classical forward genetic methods only identify single-large effect genes and are insufficient in explaining the intricate mechanisms underlying multigenic traits such as aging. To further understand the genetic architecture and mechanisms of aging, we are developing a new approach to generate, isolate and accumulate small effect genetic changes that influence aging in the model organism Caenorhabditis elegans via the use of automated, microfluidic technologies. Prior
research has found the expression level of microRNA mir-71 to be predictive of animal’s lifespan. We have optimized an automatic microfluidic sorter to accurately screen and isolate mutagenized animals with a subtle increase in Pmir-71::GFP expression. Mutant animals with small effect genetic changes are difficult to identify due to an overlap in their phenotypic distribution with the biological noise of wildtype animals. To overcome this challenge, we iteratively applied selective pressure for increased Pmir-71::GFP expression using our sorter over several generations to enrich the percentage of desired mutant animals. We plan to perform multiple mutagenesis-sorting experiments to accumulate small effect genetic mutations that will help us understand the aging genetic network. Once proven, this approach can be generalized to investigate other multigenic traits in model organisms.

**Gene Regulation and Genomics - RNA Interference and noncoding RNAs**

632A  A novel small RNA labeling approach uncovers the tissue- and cell-specific microRNome of C. elegans.  C. Alberti1, S. Ameres2, L. Cochella1  1) Research Institute of Molecular Pathology (IMP), Vienna, Austria; 2) Institute of Molecular Biotechnology (IMBA), Vienna, Austria.

The implementation of distinct gene expression profiles is essential for accurate animal development. While post-transcriptional gene regulation by miRNAs plays an essential role in this process, uncovering the biological function of most of them remains challenging. This is, at least partly, due to the highly specific expression patterns of many miRNAs, which suggest that many of those may have functions in specialized cellular contexts. Obtaining miRNA expression profiles with high – ideally single– cell resolution thus becomes crucial.

We report a novel approach to obtain tissue-specific and even single-cell miRNomes in C. elegans, without the need for previously sorting out the cells of interest. The strategy is based on cell-specific 3′ terminal methylation of miRNAs, achieved by selective expression of the small RNA duplex-specific methyltransferase HEN1. Because methylated miRNAs are resistant to chemical ribose oxidation, tissue-specific miRNAs can be recovered by established methylation-specific cloning protocols, followed by high-throughput sequencing. Here, we show that this strategy displays high specificity and sensitivity. Using this method, we have obtained the miRNomes of larger tissues of the worm but also of individual neuronal pairs. These data already provide an insightful view into the miRNA requirements of different cell types.

Chemical small RNA-tagging overcomes the current challenges in tissue- and cell-type-specific miRNA profiling and provides novel entry points to understanding the regulation of gene expression in the course of animal development.

633B  The conserved kinase SGK-1 affects small regulatory RNA function in C. elegans.  Amelia F Alessi1, Himani Galagali1, Sungki Hong1, Ken Inokido1, James J Moresco3, John R Yates III3, Kevin J Ranke3, Xantha Karp1, Alexander Soukas5, John K Kim1  1) Department of Biology, Johns Hopkins University, Baltimore, MD; 2) Life Sciences Institute, University of Michigan, Ann Arbor, MI; 3) Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA; 4) Department of Biology, Central Michigan University, Mount Pleasant, MI; 5) Center for Genomic Medicine, Endocrine Division and Diabetes Unit, Massachusetts General Hospital, Boston, MA.

Small regulatory RNA pathways play essential, conserved roles in diverse biological processes, including in development, cellular differentiation, germline integrity, and viral defense. While fundamental insights into the mechanisms of small RNA biogenesis and gene silencing have been made, the interplay between functionally distinct small RNA pathways, some of which share common factors, remains incompletely understood. We have discovered that the conserved serine/threonine kinase SGK-1 genetically interacts with both the microRNA (miRNA) and small interfering RNA (siRNA) pathways in an opposing fashion. Loss of sgk-1 suppresses let-7 miRNA mutant defects and attenuates the potency of feeding RNAi, which silences targets via siRNAs. Loss of sgk-1 also promotes the accumulation of mature let-7 and silencing of the let-7 target, lin-41 mRNA, in a let-7 hypomorphic mutant. Likewise, sgk-1 gain-of-function mutants enhance miRNA mutant defects and enhance the efficacy of feeding RNAi. We hypothesize that SGK-1 kinase activity acts to “balance” miRNA and siRNA functions. SGK-1 is orthologous to the mammalian Serum and Glucocorticoid-inducible Kinases (SGKs) and has conserved roles in mediating metabolism, growth, and cellular stress responses downstream of TOR and insulin signaling pathways. Interestingly, we observe that loss of rict-1, which encodes the TORC2 component Rictor, also suppresses a let-7 miRNA mutant defect, suggesting that SGK-1 function in small RNA pathways may be mediated through TORC2. Our data suggest that SGK-1 may directly interact with components of the miRNA-Induced Silencing Complex (miRISC). In silico analysis also revealed SGK-1 phosphorylation motifs in factors of both pathways. Our current research is focused on (A) defining the mechanism by which sgk-1 differentially affects the miRNA and siRNA pathways, including exploring if protein(s) in these pathways are direct SGK-1 substrates, and (B) investigating if SGK-1 impact on small RNA pathways is regulated by conserved energy and stress sensing pathways.

634C  The Role of Small RNA Pathway Machinery in Mitosis and Cell Cycle.  A. Asundi1, J. Rohrberg1, A. Suzuki2, A. Goga1, N. L'Etoile1  1) Cell and Tissue Biology, UCSF, San Francisco, CA; 2) Department of Oncology, Astellas Pharma Inc. Tokyo 103-8411, Japan.

Small RNAs have recently emerged as interesting potential candidates for regulating various cell processes from differentiation to non-autonomous cell communication. We hypothesize that small RNAs may also have a role in regulating mitosis and the cell cycle, particularly in rapidly dividing cell populations. We have approached this question using both an in vivo model (C. elegans reproductive tract) and an in vitro model (oncogenic mammalian epithelial breast cells) to examine cell proliferation in the absence of two conserved proteins in the small RNA pathway: the double stranded RNA import channel, SIDT-1/SID-1 and the nuclear RNAi protein NRDE-2.
The adult *C. elegans* germ line contains the only population of mitotically dividing stem cells in the organism and is a tractable system by which to study the influence of small RNA machinery on proliferating germ cell (PGC) population and reproduction. Various canonical cell signaling pathways including, TGF-b (Dalfo et al. 2012), IGF-1/insulin (Michaelson et al. 2010) and GLP-1/Notch (Kimble et al. 1997) are known to regulate the number of PGCs. To test whether small RNA signals may also play a role in maintaining PGC number, our lab examined PGC number and brood size in *sid-1* and *nrde-2* null mutants. We found that PGC number is significantly reduced in *nrde-2* null mutants as compared to wild type worms. In addition, the brood size of these worms is significantly decreased as compared to wild type. We also find that although *sid-1* null mutants have a significantly reduced number of PGCs, the number of *sid-1* mutant progeny is comparable to that of wild type.

We hypothesized that since *sid-1* and *nrde-2* are conserved between *C. elegans* and mammals, these proteins might also function in regulating mitosis in mammalian cells. We used siRNA to knockdown NRDE-2 or SITD-1 in MCF10A breast epithelial cells that have been transformed to overexpress the oncogene Aurora B Kinase (AUBK). We have shown that in NRDE-2 siRNA treated AUBK overexpressing MCF10A cells, the number of metabolically active cells significantly decreases as compared to control siRNA treated cells. Similarly, treating AUBK overexpressing cells with SITD-1 siRNA significantly decreases metabolic cell activity. However, untransformed MCF10A cells treated with NRDE-2 siRNA or SITD-1 do not show a significant decrease in viable cell number when compared to control siRNA treated untransformed MCF10A cells.

Taken together, these data suggest that the small RNA pathway may be required maintain mitotic integrity, particularly in rapidly dividing cell populations, such as the *C. elegans* stem cell niche and cancerous cell populations.

635A  The TRIM-NHL protein NHL-2 is a Novel Co-Factor of the CSR-1 and WAGO 22G-RNA PathwaysThe TRIM-NHL protein NHL-2 is a Novel Co-Factor of the CSR-1 and WAGO 22G-RNA Pathways. Gregory Davis1, Shikui Tu2, Rhys Colson1, Menachem Gunzburg3, Michelle Francisco3, Debashish Ray4, Joshua Anderson1, Monica Wu5, Quaad Morris3, Timothy Hughes3, Jacqueline Wilce1, Julie Claycomb1, Zhiping Weng1, Peter Boag1 1) Development and Stem Cells Program, Monash Biomedicine Discovery Institute and Dept. of Biochemistry and Molecular Biology, Monash University, Melbourne, VIC, Australia; 2) Program in Bioinformatics and Integrative Biology, University of Massachusetts Medical School, Worcester, MA, USA; 3) Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada.

Proper regulation of germline gene expression is essential for fertility and maintaining gonadal integrity. In the *C. elegans* germline, a diverse repertoire of regulatory pathways promote the expression of endogenous germline genes and limit the expression of deleterious transcripts to maintain genome homeostasis. TRIM-NHL proteins have emerged as conserved hubs of gene regulation across tissues and species, interfacing with multiple facets of RNA and protein regulatory pathways to ensure proper gene expression. Initially studied for its role in modulating miRISC activity via *let-7* and *lsy-6* in the soma, here we show that the conserved TRIM-NHL protein, NHL-2, plays an essential role in the *C. elegans* germline, modulating germline chromatin and meiotic chromosome organization. We show that NHL-2 localizes to germ granules, and uncover another role for NHL-2 in small RNA biology: as a co-factor in the biogenesis of both positively and negatively acting germline small RNA pathways (22G-RNAs). We show that NHL-2 physically and genetically interacts with the positively acting CSR-1 22G-RNA pathway, and our data support a model whereby NHL-2 is required for the activity of the RNA-dependent RNA Polymerase complex, which synthesizes 22G-RNAs. Furthermore, we demonstrate that NHL-2 is a bona fide RNA binding protein and, along with RNA-seq data from *nhl-2(ok818)* mutants, these results point to a small RNA-independent role for NHL-2 in regulating transcripts at the level of RNA stability. Collectively, our data implicate NHL-2 as an essential hub of gene regulatory activity in both the *C. elegans* germline and soma.


*Caenorhabditis elegans* contains twenty-five Argonautes, of which, only ALG-1 and ALG-2 are known to interact with miRNAs. ALG-5 belongs to the AGO subfamily of Argonautes that includes ALG-1 and ALG-2, but its role in small RNA pathways is unknown. We analyzed by high-throughput sequencing the small RNAs associated with ALG-5, ALG-1, and ALG-2, as well as changes in mRNA expression in *alg-5*, *alg-1*, and *alg-2* mutants. In contrast to ALG-1 and ALG-2, which associate with the majority of miRNAs and have general roles throughout development, ALG-5 interacts with only a small subset of miRNAs and is specifically expressed in the germline. Our results provide a near-comprehensive analysis of miRNA-Argonaute interactions in *C. elegans* germ line and reveal distinct and overlapping roles for ALG-5, ALG-1, and ALG-2.

637C Divergent Roles of the miRNA Argonaute Proteins in *C. elegans* Aging. L.B. Chipman, I.A. Nicastro, A.P. Aalto, J.P. Broughton, A.E. Pasquinelli  Division of Biological Sciences, University of California San Diego, La Jolla, CA.

Although highly related proteins often perform redundant functions, there are rare cases of homologous proteins taking on opposing roles in certain contexts. We discovered one such example where the activities of Argonaute-like-gene 1 (*alg-1*) and *alg-2* diverge in adult *C. elegans*. These Argonaute (AGO) proteins are specific to the miRNA pathway and seem to perform overlapping and complementary roles in regulating gene expression during embryogenesis and larval development. Surprisingly, we found that loss of *alg-1* leads to a shorter lifespan and loss of *alg-2* results in an extended lifespan. Gene expression analyses revealed that distinct sets of genes are mis-regulated in each of the AGO mutant backgrounds. Consistent with the longevity phenotypes of *alg-1* and *alg-2* mutant animals, many of the differentially expressed genes are regulated by the insulin/IGF-1 signaling (IIS) pathway. Furthermore, genetic experiments demonstrate that the long lifespan of animals deficient in insulin receptor activity (*daf-2* mutants) is partially dependent on *alg-1*, while the extended lifespan of *alg-2* mutants requires the FOXO DAF-16 transcription factor. These findings prompt the question of how two proteins that are over 80% identical in amino acid sequence and exhibit similar expression patterns and functions during development take on opposing roles in
adulthood. To address this problem, we have used CRISPR to fuse fluorescent tags to the endogenous \textit{alg-1} and \textit{alg-2} genes, which will enable detailed analyses of the expression and activity of these AGOs in aging animals. These strains will allow us to test the hypothesis that in adults ALG-1 and ALG-2 bind distinct miRNAs and targets, which contributes to their opposing longevity roles. To understand the molecular basis for the different activities of these two AGOs, regulatory and coding sequences will be swapped between \textit{alg-1} and \textit{alg-2} to identify the elements responsible for their divergent roles in adult animals. Overall, I aim to elucidate how two miRNA AGOs promote opposite longevity fates in \textit{C. elegans}.

\textbf{638A Investigating the role of MUT-2 in transgenerational gene silencing triggered by neuronal double-stranded RNAs.} Y.S. Choi\textsuperscript{1,2}, Lanele Edwards\textsuperscript{1,2}, Aubrey DiBello\textsuperscript{1,3}, Antony Jose\textsuperscript{1}, Students of FIRE-TBI 1) U. of Maryland College Park, College Park, MD; 2) NIH, Bethesda, MD; 3) Tufts School of Medicine, Boston, MA.

Studies in \textit{C. elegans} have shown that dsRNA expressed in neurons can transmit a signal to the germline of an animal to cause silencing of a germline gene in that animal and in its descendants. The molecular details of how mobile RNA is processed, exported, and transmitted from neurons to the germline are poorly understood. Tissue-specific rescue experiments using repetitive transgenes suggest that MUT-2, a putative RNA nucleotidyltransferase, is sufficient in the sending cells for gene silencing by mobile dsRNA. One hypothesis suggested by this finding is that forms of RNA that are modified by MUT-2 move between cells. Alternatively, use of repetitive transgenes could have led to misexpression in receiving cells and MUT-2 might function only in receiving cells. Distinguishing between these possibilities requires the development of better reagents and methods for the analysis of MUT-2 and small RNAs. In the absence of MUT-2, transposons are activated, leading to progressive mutagenesis in the background, which could complicate interpretations. Indeed, when we generated new deletions of \textit{mut-2} using CRISPR-based genome editing and analyzed three independently propagated \textit{mut-2(-)} deletion lines, we did not detect the previously reported reduced brood size. To examine possible changes in small RNAs that result from loss of \textit{mut-2}, we have developed a sensitive northern blotting approach that can detect RNAs as short as 14 nt and thus effectively complement RNA-seq (1).

Finally, we are defining the neurons that can export dsRNA to elicit the most effective and enduring transgenerational gene silencing through a large-scale effort called the Transgenerational Brain Initiative using cohorts of \textasciitilde35 undergraduates each year. This effort will create and characterize a collection of strains expressing dsRNA, reporter genes, and MUT-2 from single copy transgenes in different subsets of neurons. Collectively, with these tools, we will establish where MUT-2 acts to initiate transgenerational gene silencing triggered by neuronal dsRNA.


\textbf{639B The germline DEAD-box protein, GLH-1, interacts with PRG-1 and a JNK-related kinase, KGB-1, to promote epigenetic gene silencing, and to preserve an RNA interference response that is robust to low temperatures.} Siyuan Dai\textsuperscript{1,2}, En-zhi Shen\textsuperscript{1,3}, Craig Mello\textsuperscript{2,3} 1) Interdisciplinary Graduate Program, University of Massachusetts Medical School, Worcester, MA; 2) RNA Therapeutic Institute, University of Massachusetts Medical School, Worcester, MA; 3) Howard Hughes Medical Institute.

\textit{C. elegans} is exquisitely sensitive to dsRNA-induced gene silencing. During the RNAi response the Argonaute RDE-1 is loaded with an initial small-RNA (sRNA) silencing signal (produced by dicing of a dsRNA trigger). This initial sRNA signal is then amplified upon target recognition by cellular RNA-dependent RNA polymerase factors. Interestingly, in the germline, the dsRNA response competes with priRNA/PRG-1-initiated silencing pathways for the same downstream amplification and silencing machinery. This means that wild-type germlines must balance competing signals from distinct Argonaute pathways (RDE-1 and PRG-1) in order to maintain robust capacity to respond dsRNA and priRNA silencing triggers. In an effort to search for PRG-1 binding factors we identified GLH-1 and GLH-4, two homologous DEAD-box proteins localized in \textit{C. elegans} P granules. Genetic tests utilizing a priRNA-sensitive gene silencing assay revealed that \textit{glh-1} and \textit{glh-4} are both required to maintain PRG-1-dependent transgene silencing. Surprisingly, we also found that GLH-1 mutants are deficient in their RNAi response when exposed to dsRNA at low temperature (15°C). Similarly, we found that a JNK-related kinase, KGB-1, previously reported as a regulator of GLH-1 (Development 2007, 134: 3383-3392) is also defective in low-temperature RNAi. The cold-sensitive defects of \textit{glh-1} and \textit{kgb-1} single mutants were suppressed in double mutants with \textit{prg-1}. These findings imply that the priRNA pathway may become hyper-activated at low temperatures in the absence of the GLH and KGB factors thus sequestering shared downstream machinery away from the dsRNA response. The GLH proteins and KGB-1 have both been shown to be required for proper P-granule assembly and localization. Thus the defects observed in Argonaute pathways could reflect indirect affects of P-granule disruption in these mutants. In an effort to address this question we used CRISPR to introduce point mutations predicted to disrupt GLH ATPase activity. As hoped these mutations preserve P-granule integrity, but nevertheless cause strong cold-sensitive RNAi defects. Thus GLH-1 activity appears to be required for both PRG-1-dependent silencing and to prevent aberrant PRG-1 activity that interferes with the RDE-1/dsRNA response. Further work is required to understand how GLH-1 and KGB-1 collaborate to regulate small-RNA silencing pathways in the germline and how they promote robustness to temperature.

\textbf{640C 3' supplemental base pairing can determine functional specificity of \textit{let-7} family microRNAs in \textit{C. elegans.}} Y. Duan\textsuperscript{1,2}, I. Veksler-Lubinsky\textsuperscript{1}, V. Ambros\textsuperscript{1} 1) Program in Molecular Medicine, UMass Medical School, Worcester, MA; 2) Interdisciplinary Graduate Program, UMass Medical School, Worcester, MA.

MicroRNA (miRNA) are endogenous non-coding RNA molecules that exist in all multicellular eukaryotes. MiRNAs are grouped into families based on the identity of nucleotides 2-8 from the 5' end, which are also referred to as the miRNA seed. The seed region is essential for miRNA targeting, while the non-seed region, or 3' supplemental, can also contribute to target specificity.
Let-7 family miRNAs have been shown to exist in almost all bilateral animals, and are often present in multiple copies in the genome. Strikingly, almost all species that have let-7 family miRNAs contain at least one copy of a particular let-7 isoform (referred to as let-7a) that shares almost 100% identical sequence across bilaterians. Since the let-7 family microRNAs have the same seed region sequences, the deep conservation of the non-seed region of let-7a suggests that let-7a may have unique target sites and/or other characteristics not shared by other let-7 family miRNAs. We investigated the specificity of in vivo functions of the let-7a non-seed sequences by swapping the let-7a non-seed region at the C. elegans let-7 locus with the non-seed region of the C. elegans let-7 family paralog, mir-84. The let-7(mir-84 swap) mutant expresses mir-84 with a developmental profile, and to levels, similar to wild type let-7. However, let-7(mir-84 swap) animals exhibit retarded heterochronic phenotypes and vulva defects characteristic of let-7(f). This result suggests the existence let-7a-specific target sites or other interactions that are determined by the 3' supplemental of let-7a, and which cannot be effectively engaged by mir-84. We postulated that lin-41 could be one let-7a target that contributes to the phenotypes above, since the let-7 sites in the lin-41 3' UTR exhibit extensive non-seed complementarity specific to let-7. Accordingly, we mutated the let-7 complementatory sites (LCSs) in the endogenous lin-41 3' UTR to match the 3' region of mir-84. These lin-41 LCS mutations should restore full 3' supplemental pairing of mir-84 to the lin-41 3' UTR sites, but nevertheless they only partially rescued the mir-84 swap heterochronal phenotypes. This result suggests that other targets, besides lin-41, interact with let-7 by 3' supplemental base pairing, and contribute to the control of developmental timing in C. elegans. Our future direction will be to focus on identifying other let-7a-specific targets that are regulated by 3' supplemental base pairing to let-7a, and to characterize the base-pairing patterns and/or other features associated with these interactions.

641A Investigating the biosynthesis and functions of newly identify isiRNA in C. elegans. Trang Duong Cell & Tissue Biology, UCSF, San Francisco, CA.

Small non-coding RNAs of 20-30 nucleotides can target both chromatin and transcripts in a sequence-specific manner to regulate gene expression. The first small regulatory RNA was discovered in C. elegans in 1993 (Lee, Feinbaum, & Ambros, 1993). Recent progress in high-throughput sequencing has uncovered an astounding landscape of small RNAs. Various small RNAs have been found and can be categorized into three main classes according to their mechanism of biogenesis and the libraries. We used fastX-clipper, fastX-trimmer, then align to the WS253 reference genome using bowtie 2 program, and lastly a custom python script to keep only anti-sense reads. Our analysis across hundreds of libraries from dozen of different laboratories revealed that there is another class of siRNA that mapped to intronic regions of the gene (isiRNA). Though abundant, the function and biogenesis of these isiRNAs have yet to be described in C. elegans. We investigated the characteristics of introns that have isiRNA mapping to them, and we found that the median of these introns is 10-fold longer compared to the median of genomic introns. Through characterizing genes that have isiRNA mapping to them, we discovered that genes with alternative splicing have higher probability of having isiRNA mapping compared to genes without alternative splicing. By analyzing high-throughput sequences of co-immunoprecipitation libraries we found that this isiRNA bound specifically to some endo-siRNA Argonautes but not to miRNA Argonautes. My current study is focusing on understanding the biogenesis splicing. By analyzing high-throughput sequences of co-immunoprecipitation libraries we found that this isiRNA bound specifically to some endo-siRNA Argonautes but not to miRNA Argonautes. My current study is focusing on understanding the biogenesis splicing. By analyzing high-throughput sequences of co-immunoprecipitation libraries we found that this isiRNA bound specifically to some endo-siRNA Argonautes but not to miRNA Argonautes. My current study is focusing on understanding the biogenesis splicing.

642B Characterization of a new Argonaute interactor involved in the miRNA silencing pathway. Pierre-Marc Frédéric1,2, Guillaume Jannot1,2, Laure Villoing1,2, Isabelle Banville1,2, Martin J. Simard1,2 1) St-Patrick Research Group in Basic Oncology, CHU de Québec-Université Laval Research Centre (L’Hôtel-Dieu de Québec); 2) Laval University Cancer Research Center, Québec, Canada.

MicroRNAs are small non-coding RNA that regulates the expression of several genes. The miRNA pathway effector complex, the miRISC (miRNA induced silencing complex), is formed by the association of an argonaute protein with a miRNA. The subsequent binding of the miRISC to its mRNA target can either repress translation or degrade the mRNA. To further understand the miRNA gene regulation mechanisms, it is necessary to characterize new important molecular partners involved in the miRNA silencing.

Using different genetic and molecular screens, we identified new miRISC interactors. Among them, we found a DnaJ chaperone protein as an interactor of a C. elegans miRNA-specific Argonaute ALG-1 in the yeast two-hybrid system. Different genetic assays indicate that the DnaJ chaperone is important for the microRNA pathway in animals. Interestingly, observations made with Drosophila cells extract suggested that Heat Shock Proteins or HSPs are required for small RNA loading onto Argonaute proteins (Iwasaki and al, Molecular Cell, 2010). It is also known that DnaJ chaperone proteins can provide specificity to HSPs in order to guide them to specific proteins. We therefore hypothesize that the DnaJ chaperone interacting with ALG-1 recruits HSPs proteins in order to permit loading of miRNAs on this Argonaute. To test this model, we will first demonstrate that DnaJ protein interacts with ALG-1 associated to the loading complex in vivo with the newly generated animals expressing endogenously HA-tagged DnaJ gene. We will next test whether DnaJ is required for: 1) the interaction of HSPs with ALG-1 and; 2) the loading of miRNA onto ALG-1 using both RNAi and KO animals. Taken together, this study will provide mechanistic insights on how miRNA (and potentially others small RNAs in worms) are loaded specifically onto the proper Argonaute proteins.
**643C**  *mir-243, mir-51 y mir-52 are required for diapause formation in C. elegans as a transgenerational defense mechanism against bacterial pathogens.*  
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¹) Center for Genomics and Bioinformatics, Universidad Mayor, Chile; ²) Fundación Ciencia y Vida, Chile; ³) Universidad de Talca, Chile.

*C. elegans* feeds on a variety of bacteria, both pathogenic and non-pathogenic, and under starvation, high population density, and other stresses enters diapause by forming the dauer larvae. Our previous work showed that *C. elegans*, also forms dauers as a mechanism of transgenerational defense against the pathogens *P. aeruginosa* PAO1 and *S. Typhimurium* MST1. We proposed that in the bacteria-worm communication, bacterial dsRNAs trigger an RNAi-dependent process, generating endo-siRNA accumulation in the germ line subsequently transported to the progeny to promote diapause formation in *C. elegans*. To understand which genes (small RNAs and mRNA) accumulate in response to pathogens, we performed a comparative differential expression analysis of the worm transcriptome under pathogenesis and on non-pathogenic bacteria. We found that *mir-243, mir-51* and *mir-52* are overexpressed by 6.46, 2.27 and 2.26 fold respectively, in response to *P. aeruginosa* PAO1. In vivo gfp expression confirms the overexpression of the three miRNAs in worms fed with pathogens compared to non-pathogenic bacteria. Importantly, animals with mutations in *mir-243, mir-51* and *mir-52* do not form dauers in response to *P. aeruginosa* PAO1, strongly suggesting a role for these small RNAs in the behavioral response to pathogenesis. To construct the gene networks underlying dauer formation under pathogenesis, we used the miRNA-mRNA interactions predicted by five softwares simultaneously (INTA, RNA duplex, RNAplex, RNAup and PI Ta), and overlapped them with an additional layer formed by the transcription factors, known to regulate the miRNAs that appeared differentially expressed in our experiments.

**Reference:**  
1. Ni JZ, Chen E, Gu SG. BMC Genomics. 2014. PMID: 25534009  

**644A**  *The triggering mechanism of C. elegans germine nuclear RNAi at the native targets.*  
J. Ni, N. Kalinava, S. Gu  
Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ.

We wish to understand the molecular mechanisms that distinguish “self” and “non-self” DNA in the germline genome of *C. elegans*. Nuclear RNAi refers to a set of small RNA-guided chromatin-based gene-silencing pathways (e.g., heterochromatin formation and transcriptional silencing), and plays an essential role in genome surveillance in yeast, plants, and animals. We previously identified a large set of genomic loci that are targeted by germine nuclear RNAi in *C. elegans* [1,2]. The triggering mechanisms at these native targets appear to be highly complex and are poorly understood. We are using two different approaches to resolve this gap. (1) We are identifying aberrant features associated with germine nuclear RNAi-targeted transcripts. To this end, we are using an unbiased RNA-seq approach to characterize both polyA and non-polyA transcripts in both wild type and mutant animals. In addition, subcellular localization of the native target transcripts are being examined using single-molecule FISH and RNA-seq combined with biochemical fractionation. (2) We are using CRISPR-mediated genome editing to test whether the silencing responses at native targets are indeed triggered by the aberrant features that are identified in (1). To date, we have found that “self” and “non-self” transcripts differ in RNA-processing, as well as subcellular localization. By using CRISPR, we have identified cis-regulatory elements that are required for the silencing response.

**Reference:**  
1. Ni JZ, Chen E, Gu SG. BMC Genomics. 2014. PMID: 25534009  

**645B**  *Identification of a Novel Endogenous Small RNA Pathway Specifically Targeting the 3′ UTRs of mRNAs.*  
J.W.J. Randolph, L. Li, W. Gu  
Cell Biology and Neuroscience, University of California Riverside, Riverside, CA.

We are reporting a novel WAGO small RNA pathway which specifically targets the 3′ UTR of hundreds of functionally important genes. In *C. elegans*, endogenous small RNAs, 22G-RNAs, bind Argonautes to regulate almost all germline genes. There are two major 22G-RNA-mediated pathways in *C. elegans* germline cells: One is mediated by Argonaute CSR-1 and plays important roles in chromosome segregation and embryonic development; the other is mediated by multiple Argonautes, WAGOs, and plays critical roles in silencing transposons, pseudogenes, viruses, and some functional genes. In all these small RNA pathways, 22G-RNAs are generated by RNA-dependent RNA polymerases (RdRPs) using mRNAs and other RNAs as templates. Usually these 22G-RNAs are generated from both coding regions and UTRs of RNAs. Here we are reporting a novel small RNA pathway which specifically targets the 3′ UTRs of hundreds of genes, many of which have been well studied and play important roles in germline and embryonic development. Our preliminary results indicated that these genes are targeted both by CSR-1 and WAGO Argonautes. However, CSR-1 majorly targets the 5′ UTR and coding regions, while the WAGO Argonautes only target the 3′ UTRs. Interestingly, the WAGO-22Gs are not dependent on rde-3, which is usually required for generating 22Gs in other WAGO-dependent pathways including exogenous RNAi pathways. Our RNA-seq results suggest that these 22G-RNAs may be involved in silencing the target RNAs. We are currently using genetics, high-throughput sequencing and ribosome profiling to investigate if these small RNAs are only generated from the 3′ UTR regions and if these small RNAs are involved in translation regulation. We are also analyzing if these 22G-RNAs affect miRNA-mediated gene regulation at the 3′UTR of RNAs. In all, we are reporting a novel WAGO-mediated 22G pathway which specifically targeting the 3′UTR of hundreds of functional genes and this pathway is different from the canonical WAGO pathway since RDE-3 is not required for the 22G biogenesis.

**Reference:**  
[1,2]. The triggering mechanism of...
transcriptional and post-transcriptional regulatory mechanisms have been described in a tissue and cell-specific manner, it still remains unknown how such a level of complexity can be achieved. In terms of post-transcriptional regulation, microRNAs have been proposed as key elements in the last years due to their essential role in animal survival and embryonic development. However, unlike transcription factors, whether these molecules are involved in the proper development and/or function of organs remains unclear.

The pharynx of C. elegans is to date the only organ in which the lineage of every one of its cells is known. This rhythmically pumping organ, proposed as functional homolog of the heart, has been a powerful system to study organogenesis. Although, some genetic pathways that control the morphogenesis or cell fate specification in this organ have already been elucidated, there are still many open questions.

One of the shared genetic networks between the pharynx and the vertebrate heart includes the miRNA mir-1. We have identified a defective pumping behavior in mir-1 mutant animals. The animals display higher pump and inter-peak interval (IPI) duration and consequently, a lower pumping frequency, when compared to wild type worms. We therefore performed a search against different mir-1 targets, and identified 5 members of the vha family as potential regulators of pharyngeal muscle cells development or function. The relationship between this vacuolar ATPase family and the fusogen protein EFF-1 suggest that regulation of the vha genes may impact the proper fusion and morphogenesis of muscle cells in the pharynx. Thus, our results suggest a role for mir-1 in pharynx development that, at the same time, is essential for the proper function of this organ. By analyzing the role of conserved microRNAs in the pharynx we expect to find conserved and essential pathways in heart development.

647 A  Forward genetic screen to identify regulators of microRNA strand choice.  D. Haskell, L. Li, A. Zinovyeva  Division of Biology, Kansas State University, Manhattan, KS.

Precisely controlled gene regulation is essential for proper organism development and homeostasis. A species of small non-coding RNAs called microRNAs (miRNAs) play a critical role in post-transcriptional regulation of gene expression. miRNAs regulate gene expression by binding to partially complementary sites in mRNA targets, repressing their translation, or targeting the mRNA for degradation through recruitment of miRNA induced silencing complex (miRISC) components. One of the key steps in miRNA biogenesis is Dicer-mediated processing of the hairpin precursor miRNA to generate a double stranded miR:miR* duplex. As the miRNA duplex loads into Argonaute, the orientation of the duplex loading dictates which miRNA strand will become the more abundant, mature miRNA. Most of the time, this process of mRNA strand selection is highly asymmetric, with one miRNA strand dominantly selected. It is known that 5’ nucleotide (nt) identity and thermodynamic stabilities of the miR:miR* duplex are important determinants of small RNA strand selection in vitro. Interestingly, evidence recently emerged that miRNA strand choice may be regulated in vivo, with miR and miR* levels changing in response to physiological or environmental cues, suggesting the miRNA duplex extrinsic factors may play a role in miRNA strand selection. We have generated a sensor that is designed to differentially express fluorescent proteins based on the relative abundance of miR and miR* strands of a given miRNA. We will use this sensor to conduct a forward genetic screen to identify factors that may be important for miRNA strand selection in vivo.


648 B  Functional screen to identify modulators of miRNA activity.  Shilpa Hebbar, Anna Zinovyeva  Division of Biology, Kansas State University, Manhattan, KS.

Regulation of gene expression is essential for normal physiology and development. One of the ways cells regulate gene expression is through a class or non-coding RNAs called microRNAs (miRNAs). miRNAs associate with Argonaute proteins to form miRNA Induced Silencing Complexes (miRISCs) to post transcriptionally repress gene expression by targeting mRNA 3’UTRs through partial sequence complementarity. Proteins associated with C. elegans miRISCs were identified using Argonaute ALG-1 immunoprecipitation and 2’-O-methyl oligonucleotide-mediated miRNA-specific pulldown experiments followed by MUDPIT proteomics. We hypothesized that factors that co-precipitate with ALG-1 and/or miRNA-specific miRISCs play a role in regulating miRNA biogenesis or function. To identify which miRISC interactors are important for miRNA activity and/or processing, we are carrying out functional assays in several genetically sensitized backgrounds. Specifically, we are utilizing RNAi to knock down the putative miRISC interactors in animals with compromised miRNA activity (such as Isy-6(ot150), mir-48 mir-241 (ndf51), and mir-35-41 (ndf50) mutants). We hypothesize that enhancement or suppression of the phenotypes associated with reduction of miRNA function in these mutants will indicate potential role(s) of the candidate genes in miRNA regulation. We will report on our progress in identifying which physical interactors of ALG-1/miRISCs are important for modulating miRNA activity.

649 C  Identification of a new modulator of the microRNA pathway.  Lucile Fressigné1,2, Gabriel Bossé1,2, François Houle1,2, Martin J. Simard1,2  1) St-Patrick Research Group in Basic Oncology, CHU de Québec - Université Laval Research Centre (L'Hôtel-Dieu de Québec); 2) Laval University Cancer Research Center, Québec, Canada.

Small non-coding RNAs have emerged as major players in gene regulation. Among them, microRNAs (miRNAs) are
endogenous 21-22 nucleotides-long non-coding RNAs which are partially complementary to target messenger RNAs (mRNAs). miRNA associates with proteins of the Argonaute family (ALG-1 and ALG-2) to form the core of the microRNA induced silencing complex (miRISC). Upon association between miRISC and the miRNA target, this complex represses mRNA translation or recruits other factors to trigger its degradation.

While several genetic and molecular approaches have uncovered players of the microRNA pathway implicated in its biogenesis and function, it is still yet unknown how this pathway is modulated. In the recent years, we have contributed to shed light on this aspect by discovering that DCS-1/XRN complex controls the level of miRNA in animals (Bossé et al. Mol Cell, 2013 and Meziane et al. Scientific Report, 2015). In association to this complex, we identified a phosphatase called PPM-2. Our genetic data support that the loss of ppm-2 phenocopies the alteration of the microRNA pathway in C. elegans. Interestingly, ppm-2 genetically interacts with argonaute genes and their protein levels are affected in absence of ppm-2. Our data currently suggest that PPM-2 controls the fate of Argonaute (such as recycling or degradation) and thus contributes to the modulation of the miRNA pathway.

650A A miRNA role in neuronal calcium dynamics.  K. Kagias1, A. Norris2, V. Leon3, J. Calarco3, Y. Zhang3 1) Department of Organismic and Evolutionary Biology, Center for Brain Science, Harvard University, Cambridge, USA; 2) Department of Cell and Systems Biology, University of Toronto, Toronto, CANADA.

Micro RNAs (miRNAs) are small RNA molecules that regulate gene expression post-transcriptionally. A number of miRNAs encoded in the human genome are implicated in the pathology of different psychiatric disorders. The pleiotropic effect on the neuronal tissues reported for some of these mammalian miRNAs likely reflects their essential functions in fundamental neuronal processes, such as synaptic transmission and plasticity. Here, we investigate the C. elegans homologues of these conserved miRNAs, namely the mir-250 (the homologue of the human mir-128), the mir-45 and the mir-61 (the homologues of the human mir-134), and the mir-270 and the mir-269 (the homologues of the human mir-31), exhibit restricted neuronal expression overlapping in the RIA interneuron. Previous work show that RIA regulates a number of complex behaviors, including learning, and that RIA axons generate dynamic calcium activities underlying specific behavioral outputs. Preliminary analysis of a deletion mutant that lacks the clustered miRNAs mir-61 and mir-250 and a deletion mutant that lacks mir-269 reveals the critical role of these miRNAs in regulating the calcium dynamics of RIA. Furthermore, mir-61/250 and mir-269 seem to also needed for the aversive learning, for which RIA plays an essential regulatory role. Whole transcriptome analysis of isolated RIA neurons uncovers potential mechanisms underlying the functions of these miRNAs in regulating RIA calcium dynamics by identifying a mitochondrial uniporter encoding gene, as well as its regulatory units, as the downstream targets of the miRNAs. We are currently characterizing this calcium-regulating pathway in RIA. Our work will provide insights into the functions of these phylogenetically conserved miRNAs in basic neuronal functions.

650B H3K9me3 plays a complex role in RNAi-mediated transcriptional silencing and transgenerational epigenetic inheritance in C. elegans.  N. Kalinava, J. Ni, E. Chen, K. Peterman, S. Gu  Molecular Biology & Biochemistry, Rutgers University, Piscataway, NJ.

In many organisms, nuclear siRNA can guide transcriptional silencing and/or heterochromatin formation at the homologous genomic regions. Such heterochromatin response can be maintained over several generations in C. elegans. However, the function of the heterochromatin response remains elusive.

We use biochemical, genetic and whole-genome approaches to investigate the requirements of H3K9me3 heterochromatin for initiation and maintenance of transcriptional silencing in germline nuclear RNAi pathway in C. elegans.

First we identified three histone methyltransferase (HMT) – MET-2, SET-25 and SET-32 that, in combination, are required for the full H3K9me3 response in the germline nuclear RNAi pathway. Our results showed that H3K9me3 is not required for the heritable RNAi. In addition, complete depletion of H3K9me3 in met-2 set-25;set-32 mutants does not result with transcriptional de-silencing of nuclear RNAi targets. Therefore, the maintenance of transcriptional silencing by nuclear RNAi pathway can occur in H3K9me3-independent manner [1]. We recently developed a CRISPR-based system to examine the establishment of transcriptional silencing by nuclear RNAi at the native targets. This work revealed additional complexity for the silencing mechanism at the native targets in that H3K9me3 and RNAi play distinct roles at the establishment and maintenance stages of transgenerational silencing. We will report these novel findings at the meeting.

Reference:

652C A robust double RNAi pipeline for high-throughput screening based on bacterial conjugation.  M. Kazmierczak, B. Tursun  Berlin Institute for Medical Systems Biology (BIMSB), MDC, Berlin, DE.

The knock down of genes by RNAi has been fundamental to identify inhibitors of induced cell transdifferentiation in C. elegans (Tursun et al., 2011). Bacteria strains expressing dsRNA targeting specific genes can be fed to the worm allowing straightforward whole-genome RNAi screens of the 20,000 genes in the C. elegans genome. However, most biological processes are regulated by more than one gene raising the need for simultaneous knock down of two or more genes. Two approaches are currently available for double RNAi knockdown, two bacteria strains expressing specific dsRNA can be mixed and grown together in one well or alternatively, a new bacterial clone can be generated carrying a plasmid on which two RNAi targets of interest are 'stitched' together. We found in our lab that the results of double RNAi by mixing bacteria are highly variable. In contrast, the second approach of using stitched RNAi clones yields a high reproducibility of results, but it is for obvious reasons not suitable for a whole-genome approach since it would require generating 20,000 new plasmids containing both targets on the same construct.
Such an approach becomes even more impracticable if different combinations of simultaneous knock downs should be carried out.

We have developed a protocol using bacterial conjugation mediated by the ‘Fertility Factor’ (F) Episome which we generated by recombineering in order to combine two different RNAi plasmids in a single bacterium. The objective was to be able to transfer a single RNAi plasmid to a large number of bacteria carrying different RNAi clones in one step in a high-throughput manner for large scale ‘double’ or even ‘triple’ RNAi screens. For proof-of-concept we tested a previously described synthetic lethality upon double RNAi against *rpn-10* and *rpn-12* by feeding L4 hermaphrodites with a mixture of the respective bacteria (Takahashi et al., 2002). We combined both RNAi plasmids for *rpn-10* and *rpn-12* in a single bacterium by conjugation and we were able to recapitulate the previously reported results. Moreover, in our analysis the synthetic lethality was more robust when generated using conjugated double RNAi bacteria as compared to mixed bacteria. Currently, we are using the conjugation-mediated double RNAi approach for a large-scale screen in order to identify factors safeguarding cellular identities.

Double RNAi by conjugation allows testing for additive effects of knocking down two genes simultaneously and thus further increases the versatility of RNAi screens in the context of different biological processes.


653A The microRNA mir-243 regulates innate immunity in *C. elegans*.  Abdul Hakkim Rahamatullah, Martin Newman, Sakhimala Jagadeesan, Rhonda Feinbaum, Raja Mohammed Khader, Gary Ruvkun, Frederick Ausubel  Harvard Medical School/ Massachusetts General Hospital, Boston, MA.

MicroRNAs (miRNAs) are small non-coding RNA molecules involved in RNA silencing and post-transcriptional regulation of gene expression and are predominantly found in plants and animals. The role of miRNAs in regulating infection and immunity is poorly understood. We identified 8 miRNAs that are differentially expressed upon infection with *Pseudomonas aeruginosa* PA14 compared to worms that are fed with the normal food *E. coli* OP50. Among the 8 different miRNAs, mir-243 was significantly upregulated upon PA14 infection. To further understand the role of mir-243 in immune regulation, we constructed a mir-243 null mutant using CRISPR and found that the mir-243 null mutant was immunocompromised. The argonaute Rde-1 interacts with mir-243 and we found that both mir-243 and rde-1 are essential for pathogen resistance. Y47H10A.5 is a small protein with unknown function and is one of the known targets for mir-243. Silencing Y47H10A.5 using RNAi in mir-243 null rescues the immune compromised phenotype suggesting that the elevated levels of Y47H10A.5 could be the trigger for the death of the worm. Taken together it is plausible that mir-243 might act as an “Immune-Break” to regulate innate immunity.

654B Investigating the tissue specific roles of the Argonaute family proteins ALG-1 and ALG-2 in *C. elegans*.  K. Kotagama1,2, S.M. Blazie1,2, H.C. Geissel1,2, M. Mangone1,2,3  1) Molecular and Cellular Biology Graduate Program, School of Life Sciences 427 East Tyler Mall Tempe, AZ 85287 4501; 2) The Biodiscovery Institute at Arizona State University 1001 S McAllister Ave, Tempe, AZ 85287-5601; 3) Virginia G. Piper Center for Personalized Diagnostics, The Biodiscovery Institute at Arizona State University, 1001 S McAllister Ave, Tempe, AZ, USA.

The Argonaute family proteins ALG-1 and ALG-2 execute the miRNA pathway in *C. elegans* by targeting the 3'untranslated regions (3'UTRs) of mature miRNAs to induce translational repression. The loss of both proteins leads to embryonic lethality during morphogenesis, with clear defects in muscle and epidermal tissue formation, suggesting an essential role for ALG-1 and ALG-2 in tissue development.

We recently showed that alternative polyadenylation (APA), a mechanism by which the same gene is expressed with different 3'UTR isoforms, is pervasive at the tissue specific level in *C. elegans*. Genes can escape regulation by miRNAs through APA events, suggesting that the interface between the miRNA pathway and APA has biological relevance at the tissue specific level. These interactions remain poorly characterized.

While the role of ALG-1 in the miRNA pathway at the organismal level has been thoroughly explored, the contributions made by ALG-2 are still unclear. Its activity could explain the robustness of miRNA pathway in development, and allow us to better understand the relationship between these closely related proteins. In addition, the tissue specific localization and expression dynamics of ALG-1, ALG-2 and their associated miRNAs are still not fully understood.

To deconvolve the tissue specific miRNAome, and the contribution of ALG-1 and ALG-2 during development, we established a novel approach to isolate and sequence high-quality tissue specific miRNAs and their target transcripts. We prepared transgenic strains expressing tissue specific GFP tagged ALG-1 or ALG-2 in three of the largest somatic tissues in worm: the pharynx (*myo-2*), body wall muscle (*myo-3*) and intestine (*ges-1*). Our approach produces an interface between the tissue specific APoAome and miRNAome during development, and allows us to better understand the roles of miRNAs in producing and maintaining tissue identity.

655C New triggers, old targets: Searching for bacterial small RNAs that elicit an interkingdom RNAi-based behavioral response.  Marcela Legüe1, Carlos Caris1, Alberto Martin2, Lidia Verdugo1, Andrea Calixto1  1) Centro de Genómica y Bioinformática. Universidad Mayor, Santiago, CL; 2) Fundacion Ciencia y Vida. Computational Biology Laboratory, Santiago,
The host-microbe interaction depends on molecular patterns and damage signals that reciprocally modulate bacterial colonization and the host immune response, triggering changes in gene expression in both players. Bacterial small RNAs (sRNAs) are emerging actors of this inter-domain crossstalk, but many questions about the mechanisms by which they affect host transcriptomic profiles remain unanswered. Our group observed that C. elegans enters diapause, after two generations of feeding on P. aeruginosa PAO1 and S. Typhimurium MST1, as an RNAI-dependent defensive mechanism against infection. We hypothesize that bacterial sRNAs trigger this behavior by targeting host RNAs. Our aim is to identify which sRNAs from P. aeruginosa PAO1 sensed by C. elegans, are able to trigger an RNAI mediated transcriptomic change through targeting host RNAs. We compared the transcriptomic profiles of naive bacteria (never exposed to worms) with those residing in C. elegans intestine for one, and two generations, using simultaneous worm-bacteria RNAseq. Sequencing enriched for small RNAs was done using Illumina Trueq, yielding an average of 2.5 M reads from naive bacteria and 1.2 M reads from intestinal bacteria (bacteria-worm samples) trimmed and qualified filtered (Q = 30; length = 17). After mapping (Bowtie, Bowtie2, Segemehl/Lack, Star) reads from bacteria-worm samples were separated in silico (Picard tools), counted, and annotated using NRDR database and SRF prediction (Structure RNA Finder). Differentially expressed genes between naive and experienced bacteria using Deseq2 identified 23 small RNAs from P. aeruginosa PAO1 overexpressed in contact with host (15 in F1, 19 in F2), far less than the 88 sRNAs from E. coli OP50 (61 in F1, 63 in F2). Targets were predicted by IntaRNA, RNA duplex, RNAplex, RNAup and PITA softwares, with 12956 RNA-RNA interactions consistently predicted by all five. Pre-miRNAs constituted the most frequent target class in the host (1214). Prediction of structure and target; analyzes of functional RNA motifs, sites and their homology (RegRNA 2.0), will clarify functional roles and specificity of sRNAs expressed in pathogens.

In summary, there is a set of sRNAs uniquely overexpressed in pathogens, candidates of triggering a behavior response in host. We propose that there is a bidirectional dynamic interaction between bacteria and C. elegans that involves the activation of signaling pathways in the host, in which the RNAi machinery may play a central role. This work is the base for future validation studies.

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**656A Untangling the Role of H3K9 Methylation in Transgenerational Small RNA Inheritance.** Itamar Lev, Uri Seroussi, Hilla Gingold, Roberta Bril, Sarit Anava, Oded Rechavi. Department of Neurobiology, Wise Faculty of Life Sciences and Sagol School, Tel Aviv University, Tel Aviv 6997801, Israel.

In recent years transgenerational inheritance of acquired traits has been described in various species. Both small interfering RNAs (siRNAs) and chromatin modifications have been implicated in the maintenance of such heritable responses. In plants and yeast, a self-reinforcing feedforward loop was described, where nuclear siRNAs direct histone methylation at pericentromeric regions and in return, the siRNA machinery is recruited to pericentromeric methylated histones to synthesize additional siRNAs. In Caenorhabditis elegans, double-stranded RNA-induced RNA interference (RNAi) can produce long-term heritable silencing responses that involve the production of "secondary" siRNAs and histone methylation of the targeted locus. Recently, we showed that MET-2, a histone-H3 Lysine-9 mono/di-methyltransferase, suppresses transgenerational RNAi inheritance indirectly by affecting small RNA biogenesis. The current work discusses how chromatin modifications affect small RNA biogenesis and the different requirements for heritable silencing of transgenes and endogenous genes.

**657B A conserved RNA binding protein modulates microRNA activity during C. elegans development.** L. Li, Y. Wang, M. Kranick, A. Zinov'yeva. Division of Biology, Kansas State University, Manhattan, KS.

MicroRNAs are small non-coding RNAs that post-transcriptionally regulate gene expression. MicroRNA activity is essential for animal development as alterations in microRNA expression or function have been associated with many developmental pathologies and diseases, making microRNAs of great interest as diagnostic and therapeutic tools. The 21–23 mature single-strand microRNA brings the microRNA Induced Silencing Complex (miRISC) to target mRNAs via imperfect complementary base-pairing, resulting in mRNA silencing through translational repression and/or mRNA degradation. However, little is known about how microRNA function itself is regulated by specific developmental and physiological signals. To address how cellular factors affect microRNA-mediated gene repression, we used proteomics approaches and identified a conserved RNA binding protein, HRPK-1, as a novel microRNA interacting factor. Reduction or loss of hrpk-1 function results in a series of developmental phenotypes, including defects in fertility, embryonic development, cell fate, and organ morphogenesis: all phenotypes reminiscent of defects seen in specific microRNA mutants. Knockdown of hrpk-1 by RNAi enhances embryonic lethality of mir-35-41 mutants, cell fate defects of a reduction-of-function allele of lsb-6, and heterochronic phenotypes associated with mir-48 mir-241 mutants, suggesting that HRPK-1 may function as a positive regulator of microRNA activity. To further characterize hrpk-1, we used CRISPR/Cas9 genome editing to generate additional hrkp-1 alleles and to tag hrpk-1 with GFP at its endogenous locus. Interestingly, hrkp-1 mutants show no reduction in mature microRNA production, suggesting that HRPK-1 function may not be required for microRNA biogenesis. microRNA-specific 2′-O-methyl-oligonucleotide pulldown experiments suggest that HRPK-1 co-precipitates with some but not all miRNAs. In addition, absence of HRPK-1 does not negatively affect mature miRISC formation. We will present our current model for HRPK-1 role in microRNA-mediated gene regulation and our progress towards testing that model.

**658C The Roles of RNA Polyphosphatase PIR-1 in RNA interference.** Lichao Li, James Randolph. Cell Biology and Neuroscience, University of California, Riverside, Riverside, CA.

RNA interference (RNAi) is a highly conserved process in eukaryotes that is responsible for transposon silencing, gene regulation and antiviral response. During RNAi process, double-stranded RNAs (dsRNAs) are cleaved by Dicer into 20-26 nucleotide long primary short interfering RNAs (siRNAs). Then these primary siRNAs guide specific RNA binding proteins,
Argonautes, to target mRNAs and recruit RNA-dependent RNA polymerases (RdRPs) to produce secondary siRNAs (22G-RNAs), which directly silences target mRNAs. Previous Dicer Immunoprecipitation discovered an RNA polyphosphatase PIR-1 interacting with Dicer, who may participate in RNAi but the mechanism is unrevealed. The project is to investigate the functions and mechanism of PIR-1 in RNAi pathways.

First, we elucidated that the in vitro activity of recombinant C. elegans PIR-1 purified using bacteria is to remove β and γ phosphates from the 5′ end of triphosphorylated RNA molecule. And Electrophoretic Mobility Shift Assay (EMSA) shows that catalytically dead PIR-1(C150S) has no RNA polyphosphatase activities but binds the substrate tightly. Second, we investigate the role of PIR-1 in antiviral RNAi. Orsay virus propagation enrichment in infected pir-1 null mutant suggests that PIR1 is significant for antiviral RNAi response to Orsay virus. dsRNA extraction and strand-specific qPCR results show that viral dsRNAs are depleted in pir-1 null mutant but not in other RNAi deficient mutants such as rde-1, rde-3 or dcr-1, which indicated that PIR-1 is involved in dsRNAs processing. We are constructing the pir-1; dcr-1 double mutant to understand the specific roles of PIR-1 in dsRNAs stability and cleavage. Since catalytically dead PIR-1 still binds substrate RNAs, we are preparing FLAG-tagged catalytically dead PIR-1 using CRISPR/Cas9 to identify the in vivo substrate of PIR-1.

Third, our preliminary study revealed that PIR-1 is required for synthesis of endogenous siRNAs (26G-RNAs) which targets thousands of genes in germline cells and early embryos. We are using genetics and high-throughput sequencing to dissect the role of PIR-1 in these RNAi-mediated pathways.

659A  DsRNA activity in Caenorhabditis elegans is independent of sid-1, sid-2, and endocytosis when delivered using dsRNA-chitosan polyplex nanoparticles.  S.S. Lichtenberg, O.V. Tsyusko, J.M. Urnire  Plant and Soil Science, University of Kentucky, Lexington, KY.

RNAi-based products are rapidly coming to maturity as the latest generation of pesticides. Unfortunately, naked dsRNA suffers from rapid environmental degradation and poor uptake in many target species. Complexation of dsRNA with a nanoparticle carrier is one means of overcoming some of these limitations. Polyplex nanoparticles, composed of a cationic polymer and dsRNA, have shown considerable potential as dsRNA delivery vehicles. In this work, we demonstrate that chitosan-dsRNA polyplexes are approximately ten times as potent as bare dsRNA at silencing a gfp transgene in Caenorhabditis elegans. Furthermore, we show that dsRNA-chitosan polyplex activity is independent of pathways for environmental uptake of naked dsRNA (the sid-1 and sid-2 pathway). Pharmacological inhibition of clathrin-dependent and caveolin-dependent endocytosis also fails to inhibit activity of dsRNA-chitosan polyplexes, but diminishes the activity of naked dsRNA. These data suggest that the uptake of dsRNA-chitosan polyplexes proceeds via an endocytosis-independent mechanism. Such a route could allow dsRNA to bypass endosomes, thereby avoiding endosomal degradation of dsRNA. This may partly explain the enhanced potency of dsRNA-chitosan polyplexes relative to naked dsRNA.

660B  The miR-44 family of microRNAs is necessary for normal fertility in C. elegans.  Katherine Maniates, Benjamin Olson, Allison Abbott  Department of Biological Science, Marquette University, Milwaukee, WI.

While microRNAs regulate diverse developmental and physiological processes in C. elegans, specific functions for most individual miRNAs have not yet been identified. One challenge to understanding the specific functions is that many individual microRNAs mutants do not display readily observable defects. We have identified the miR-44 family of microRNAs which have many penetrant observable defects in loss of function mutants. Some of the observed phenotypes of the mir-44 family mutants include reduced brood size, high levels of unfertilized oocytes, and fertilization defects, likely due to defects in sperm function. The miR-44 family comprises four miRNAs: mir-44, mir-45, mir-61, and mir-247 that share a common seed sequence and thus are predicted to regulate shared target mRNAs. Interestingly, miR-44 and miR-45 share an identical mature sequence and are located only ~9kb apart on chromosome II precluding the generation of double mutants. Using CRISPR/Cas9, we generated a mir-44/-mir-45 double mutant that we are currently analyzing. Our preliminary data indicates that mir-44/-mir-45 mutants have significantly decreased brood size and increased levels of unfertilized oocytes. Since unfertilized oocytes can be due to defects in either the sperm or oocytes, to test if mir-44/45 function in spermatogenesis, we crossed mir-44/-mir-45; him-8 males with unc-17(e245) hermaphrodites and saw that they generated lower levels of cross progeny. This is consistent with defects in spermatogenesis or sperm function. The mir-44/-mir-45 mutant is currently being analyzed to identify specific processes during spermatogenesis that require the miR-44 family. The goal is to then identify pathways and targets that are regulated by the miR-44 family in the process of spermatogenesis.

661C  The early embryonic mir-35 family of microRNAs regulates sex determination and viability through overlapping target genes.  K. McJunkin  National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD.

Gene expression during early embryogenesis is largely controlled by post-transcriptional mechanisms. However, the roles that microRNAs play in controlling biological processes at this stage of development are poorly understood. We have probed the function of the mir-35 family of microRNAs, which is essential for embryonic development. We show that the mir-35 family regulates the sex determination pathway at multiple levels through its target genes encoding suppressor-26 (sup-26) and NHL (NCL-1, HT2A, and LIN-41 repeat) domain-containing-2 (nhl-2), two RNA-binding proteins. Although sex determination is established zygotically after the establishment of dosage compensation, the effect of the mir-35 family on this pathway is partially maternal. This maternal effect on an inherently zygotic process suggests that the mir-35 family may ensure a window of naivete in early development, preventing premature sex-specific gene expression. In this way, the mir-35 family plays a role in developmental timing, analogous to other essential microRNAs families expressed during larval development.
In addition to the role this network plays in controlling sex determination, the repression of nhl-2 by mir-35 family members appears to be essential for development. Genome editing events affecting the single mir-35 family seed match in the nhl-2 3'UTR are negatively selected. This is an exciting and rare example of a single microRNA binding site that is essential for development. Here we present our recent progress in elucidating the nature of the deleterious effects of nhl-2 derepression, as well as our efforts to discover other targets whose repression by mir-35 family members is essential.


An organism’s survival is rooted in its ability to sense and respond to stimuli. C. elegans is able to detect and interpret a variety of environmental cues, and is further able to integrate and disseminate this information to somatic and germline cells. Much of this communication is accomplished through canonical neuronal signaling. However, previous work in the field suggests this is not the only method. Mobile RNA signals, such as exogenous RNA interference (RNAi) and endogenous small interfering RNA (siRNA) are generated using template mRNAs and non-coding RNAs by a complex RNA interference (RNAi) machinery. In mammals, biochemical and crystal structure studies show that LIN-28 achieves this negative regulation by binding to the let-7/let-7* stem loop of either primary-let-7 or pre-let-7. Interestingly, the post-transcriptional repression that LIN-28 imposes upon let-7 biogenesis is conserved in C. elegans, but the stem-loop sequence is not. Recent studies have demonstrated that in C. elegans LIN-28 binds to the primary-let-7 transcript downstream of the let-7/let-7* stem loop to repress let-7 biogenesis. In addition to LIN-28 repression, other mechanisms of post-transcriptional regulation of let-7 biogenesis have also been described. First, let-7 complimentary sequences in its primary transcript have been described to promote its own biogenesis. Second, trans-splicing of pri-let-7 has been shown to remodel its secondary structure to facilitate processing. This work aims to further characterize how these various modes of post-transcriptional control of let-7 biogenesis are integrated to achieve robustly regulated expression of let-7 in the context of the heterochronic pathway.

662B  Post-transcriptional regulation of the microRNA let-7 in C. elegans.  Charles Nelson, Victor Ambros  Program in Molecular Medicine, UMass Medical School, Worcester, MA.

let-7 is a microRNA whose sequence, function, and regulation are well conserved throughout the animal kingdom. LIN-28 is an RNA binding protein that negatively regulates let-7 biogenesis. In mammals, biochemical and crystal structure studies show that LIN-28 achieves this negative regulation by binding to the let-7/let-7* stem loop of either primary-let-7 or pre-let-7. Interestingly, the post-transcriptional repression that LIN-28 imposes upon let-7 biogenesis is conserved in C. elegans, but the stem-loop sequence is not. Recent studies have demonstrated that in C. elegans LIN-28 binds to the primary-let-7 transcript downstream of the let-7/let-7* stem loop to repress let-7 biogenesis. In addition to LIN-28 repression, other mechanisms of post-transcriptional regulation of let-7 biogenesis have also been described. First, let-7 complimentary sequences in its primary transcript have been described to promote its own biogenesis. Second, trans-splicing of pri-let-7 has been shown to remodel its secondary structure to facilitate processing. This work aims to further characterize how these various modes of post-transcriptional control of let-7 biogenesis are integrated to achieve robustly regulated expression of let-7 in the context of the heterochronic pathway.

664C  Dissecting the Role of DRH-3 in RNA Interference in C. Elegans.  An-Phong Nguyen, Yesica Mercado Ayon, Lichao Li  Cell Biology and Neuroscience, UCR, Riverside, CA.

RNA interference (RNAi) plays crucial roles in regulating many important biological processes using short interfering RNAs (siRNAs). In C. elegans, siRNAs, 22G-RNAs, are generated using template mRNAs and non-coding RNAs by a complex containing RNA-dependent RNA Polymerase (RdRP) and Dicer Related Helicase 3 (DRH-3). Our previous observations indicated that DRH-3 is essential to the biogenesis of siRNAs mapped to the 5’ UTRs and the protein coding regions but not to the very 3’ end of 3’ UTRs. Therefore, in the drh-3 mutants, 22Gs are still being made at the 3’ UTRs of RNAs.

We speculate that siRNA generation starts from the 3’ UTRs of target RNAs using a machinery containing DRH-3 and RdRPs, and DRH-3 is required for the translocation of this machinery towards the 5’ end of target RNAs to make more siRNAs. We propose two models to explain the function of DRH-3. In model 1, DRH-3 unwinds the siRNAs generated by RdRP from template RNAs, and facilitates the loading of siRNAs into Argonautes including WAGO-1 and/or WAGO-9. Without DRH-3, siRNAs are only generated at the 3’ UTRs but cannot be unwound and loaded into Argonautes. As a result, RdRP cannot move along template RNAs and the cloned 22Gs are localized to the 3’ UTR of RNAs. In model 2, DRH-3 is required to unwind mRNA secondary structure ahead of RdRPs to allow the translocation of RdRPs. Without DRH-3, the siRNAs are still only generated in 3’ UTRs but can be loaded into Argonautes.

To distinguish these two models, we generated two drh-3 mutants with a transgene that contains either a FLAG-tagged WAGO-1 or FLAG-tagged WAGO-9, both of which binds DRH-3 dependent siRNAs in wild type cells. Using immunoprecipitation followed by high-throughput sequencing, we aim to detect whether the 3’UTR-siRNAs in the drh-3 mutants bind these two Argonautes, as predicted by model 1. We have generated these strains, performed the immunoprecipitation, and created the high-throughput sequencing library. We are performing high-throughput sequencing and bioinformatics analysis.

665A  Regulation of Argonaute Protein Localization.  D.A. Nguyen, C.M. Phillips  Department of Biological Sciences, University of Southern California, Los Angeles, CA.

Small RNAs exist in great abundance to regulate many cellular processes, such as gene expression, chromosome
MicroRNAs (miRNAs) are small (~22 nucleotide long) non-coding RNAs which regulate gene expression in a wide variety of eukaryotes and carry out functions that range from control of development to the regulation of adult homeostasis. Recent research has uncovered multiple miRNAs with potential roles in human diseases such as cancer, diabetes and neurodegenerative diseases. Parkinson’s Disease is an aging-associated neurodegenerative disease with no known cure and is characterized by the loss of dopaminergic (DA) neurons during aging with associated pathologies such as resting tremors and bradykinesia. A lack of early diagnosis of Parkinson’s Disease (PD) is a current biomedical challenge of high relevance. The average age for the diagnosis of PD is 62 and only about 2% of diagnoses are made before the age of 40. The discovery that a-synuclein proteotoxicity is a cause of PD has generated targeted deletions of these sequences to test whether they are required for sub-cellular localization. Also, post-translational modifications of Argonaute proteins (ex. methylation) on these repetitive stretches could also play a role in regulating Argonaute protein localization. We are examining this possibility by creating point mutations in the potential modified residues. These experiments will provide insight into how these critical regulators of gene expression are positioned correctly within the cell.

666B  Requirement of small RNAs for transgenerational inheritance of the acquired stress resistance in C. elegans.  E. Okabe, S. Kishimoto, M. Uno, E. Nishida  Graduate School of Biostudies, Kyoto University, Kyoto, Japan.

As organisms are constantly exposed to many environmental stresses, dealing with stresses is an intrinsic protective mechanism to adapt oneself to the environment. Through exposure to mild stresses, animals increase stress resistance and viability, and thereby live longer. We have recently shown that the acquired stress resistance in the parental generation can be transmitted to the subsequent generations in C. elegans. However, the molecular mechanisms underlying the transgenerational inheritance of the acquired stress resistance remain largely unknown. In this study, we show that small RNAs play an important role in the transgenerational inheritance of the acquired stress resistance. Worms exposed to hyperosmosis during larval stages increase the oxidative stress resistance, and the increased stress resistance in the parental generation can be transmitted to the offspring. Our results show that a loss-of-function mutation in hrde-1, the gene encoding a major Argonaute protein that promotes multigenerational RNAi inheritance in the germline, has no effect on the increased stress resistance of the parental generation, but results in the suppression of the increase in stress resistance of the F1 descendants. Loss-of-function mutations in nrde-1 and sid-1, the genes encoding proteins that comprise the RNAi silencing machinery, also result in the suppression of the increase in stress resistance of the offspring. Furthermore, the increased stress resistance of both the parental generation and the offspring is suppressed in sid-1, rsd-2 and rsd-6 mutants, which are defective in dsRNA uptake. These results suggest that small RNAs play roles in acquiring the increase in stress resistance and transmitting the increased stress resistance to the offspring.

667C  Function of MicroRNAs in a C. elegans Model of Parkinson’s Disease.  D. Piscitelli, A. de Lancastre  Quinnipiac University, Hamden, CT.

MicroRNAs (miRNAs) are small (~22 nucleotide long) non-coding RNAs which regulate gene expression in a wide variety of eukaryotes and carry out functions that range from control of development to the regulation of adult homeostasis. Recent research has uncovered multiple miRNAs with potential roles in human diseases such as cancer, diabetes and neurodegenerative diseases. Parkinson’s Disease is an aging-associated neurodegenerative disease with no known cure and is characterized by the loss of dopaminergic (DA) neurons during aging with associated pathologies such as resting tremors and bradykinesia. A lack of early diagnosis of Parkinson’s Disease (PD) is a current biomedical challenge of high relevance. The average age for the diagnosis of PD is 62 and only about 2% of diagnoses are made before the age of 40. The discovery that a-synuclein proteotoxicity is a cause of PD has generated targeted deletions of these sequences to test whether they are required for sub-cellular localization. Also, post-translational modifications of Argonaute proteins (ex. methylation) on these repetitive stretches could also play a role in regulating Argonaute protein localization. We are examining this possibility by creating point mutations in the potential modified residues. These experiments will provide insight into how these critical regulators of gene expression are positioned correctly within the cell.
Gene regulation via non-cell autonomous acting small RNAs in C. elegans.  
Rachel Posner1, Ekaterina Star1, Sarit Anava1, Eran Azmon1, Shahar Bracha1, Hila Gingold1, Oliver Hobert2, Oded Rechavi1 1) Department of Neurobiology, Wise Faculty of Life Sciences & Sagol School for Neuroscience, Tel Aviv University, Tel Aviv, Israel; 2) Columbia University Medical Center, Department of Biochemistry and Molecular Biophysics, Howard Hughes Medical Institute, New York.

Exposure of C. elegans to artificial dsRNA can trigger exogenous small RNA-mediated silencing that transmits from somatic cells to the germline, and persists for multiple generations. However, it is unknown whether endogenous types of small RNAs (miRNAs, piRNAs and endo-siRNAs) produce systematic responses and what type of effect they may have on gene regulation. Our recent results suggest that endogenous siRNAs act in a non-cell autonomous manner, orchestrating gene silencing between tissues. We will present a dissection of the major components of the RNAi pathway required for non-cell autonomous gene regulation by endo-siRNAs and provide evidence possibly linking the regulation of physiological phenotypes to small RNAs mobilizing between tissues.

The double-stranded RNA binding protein RDE-4 can act cell autonomously during feeding RNAi in C. elegans.  
P. Raman1, S. Zaghab1,2, E. Traver1,3, A.M. Jose1 1) Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD-20742; 2) University of Maryland School of Medicine, Baltimore, MD 21201; 3) Hofstra Northwell School of Medicine, Hofstra University, Hempstead, NY 11549.

Long double-stranded RNA (dsRNA) can silence genes of matching sequence upon ingestion in many invertebrates and is therefore being developed as a pestcide. Such feeding RNA interference (RNAi) is best understood in the worm C. elegans, where movement of short dsRNAs between cells has been inferred using repetitive transgenes designed to express the long dsRNA-binding protein RDE-4 under a muscle-specific promoter. Here we show that expression of repetitive transgenes can inhibit feeding RNAi and that using repetitive transgenes to express rde-4(+) in intestine, hypodermis, or neurons can enable silencing in rde-4(-) tissues. However, silencing was also observed in animals with dsRNA import restricted to one tissue and processing restricted to another tissue, suggesting misexpression from repetitive transgenes. Furthermore, in animals with single-copy rescue of RDE-4, feeding RNAi was restricted to the tissue with RDE-4 expression. Thus, it is possible that movement of short dsRNAs between cells requires features of expression from repetitive transgenes such as high levels of expression or production of novel protein isoforms. Alternatively, consistent with the requirement for long dsRNA to trigger RNAi in insects, these results suggest that the entry of long dsRNA could be a necessary first step for feeding RNAi in animal cells.

SGK-1 and the regulation of microRNA activity after dauer.  
K. J. Ranke1, L. T. Savage1, A. F. Alessi2, J. K. Kim2, X. Karp1 1) Biology, Central Michigan University, Mt. Pleasant, MI; 2) Biology, Johns Hopkins University, Baltimore, MD.

The ability of a multicellular organism to maintain adult stem cells is crucial to survival in an erratic environment. These stem cells can divide to produce cells with different fates, but many stem cells predominately exist in a state of quiescence or cell cycle arrest. We are interested in understanding how stem cells maintain their multipotency during quiescence. C. elegans is a convenient model to address this question as it offers multipotent stem cell-like lineages, such as hypodermal seam cells. Furthermore, C. elegans demonstrates prolonged quiescence in an alternate developmental stage known as dauer that can be used as a model for stem cell quiescence. In inhosipitable conditions, L2 staged larvae molt into the stress-resistant and quiescent dauer larva stage. If conditions improve, dauer larvae advance to L3 and resume development normally. A network of heterochronic genes encoding transcription factors and microRNAs tightly regulates seam cell fate at each stage of development. Notably, this regulation differs between larvae that develop through dauer and those that develop continuously. Specifically, the phenotypes of many heterochronic mutants, including those with compromised microRNA activity, develop normally after dauer. A key aspect of the post-dauer suppression of these mutants is that microRNA activity is enhanced after dauer by unknown mechanisms. To characterize factors that affect the post-dauer microRNA pathway, we compare their effect on microRNA mutant phenotypes during continuous and post-dauer development. The serine/threonine kinase SGK-1 is a novel regulator of microRNA activity during continuous development. We are investigating the role of SGK-1 in modulating post-dauer microRNA activity using hypomorphic alleles of the let-7 microRNA. At the non-permissive temperature, let-7(n2853) mutants display phenotypes such as bursting and failure to adopt adult seam cell fate upon reaching reproductive maturity. These phenotypes occur whether worms have developed through dauer or continuously. Mutations that impact microRNA activity will suppress or enhance the let-7 phenotypes. We are using both loss and gain-of-function sgk-1 alleles in combination with let-7 alleles to determine the role for sgk-1 in affecting microRNA activity after dauer, using bursting and seam-cell fusion as a read-out of microRNA activity. Thus far, our data indicate that sgk-1 is a negative regulator of microRNA activity during both continuous and post-dauer development. Therefore, sgk-1 can impact microRNA activity after dauer.

Differential requirements for an RNA-dependent RNA polymerase during RNA interference within C. elegans somatic cells.  
S Ravikumar, S Devanapally, AM Jose  Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD- 20742, USA.

Double-stranded RNA (dsRNA) from diverse sources can trigger RNA interference in C. elegans. These include dsRNA transcribed within bacteria that worms ingest, in vitro transcribed dsRNA introduced into worms by ingestion or injection, and in vivo transcribed dsRNA made in the same cell as the target gene to be silenced or in a distant cell that exports dsRNA to the cell with the target gene. It is generally assumed that all these forms of dsRNA delivery engage the same mechanism to cause gene silencing. Intriguingly, we found that the need for an RNA-dependent RNA polymerase (RdRP) for silencing the same target gene can differ based on the mode of delivery of dsRNA. While ingested dsRNA expressed in bacteria and intracellularly
transcribed dsRNA both required the RdRP RRF-1 to silence a multi-copy transgene expressing GFP in intestinal cells, gfp-dsRNA from neurons did not require RRF-1 for silencing the same target or a single-copy transgene expressing GFP in all cells. However, not all neuronally expressed dsRNA caused RRF-1-independent silencing in a distant tissue. Silencing of the endogenous muscle gene unc-22 by unc-22-dsRNA from neurons was entirely RRF-1 dependent. These differential requirements could reflect differences in amounts of dsRNA being delivered, molecular features of dsRNA being delivered, molecular features of the target gene, or tissue-specific variation in silencing mechanisms. To explicitly test each possibility, we will generate strains with different dsRNA sources and target gene contexts using CRISPR-based genome editing. For example, we have created a chimeric target composed of both unc-22 and gfp to test if having both targets as a single transcript eliminates the differences in RRF-1 requirement for silencing by unc-22-dsRNA or gfp-dsRNA from neurons. Results of these experiments and subsequent analyses will be presented at the meeting.

672B Dissection of the molecular and sub-cellular surroundings of WAGO-3 in the germline of Caenorhabditis elegans. J. Schreier1, S. Dietz2, S. Hellmann1, F. Butter2, R. F. Ketting1 1) Biology of non-coding RNA, Institute of Molecular Biology, Mainz, Germany; 2) Quantitative proteomics, Institute of Molecular Biology, Mainz, Germany.

Argonaute proteins represent the central component of every RNA-interference (RNAi)-related pathway. The genome of C. elegans encodes in total 27 Argonaute proteins. Interestingly, almost half of them belong to the worm-specific Argonaute (WAGO) sub-clade, which is highly diversified and unique to nematodes. Upon association with their small RNA cofactors, called 22G RNAs, WAGO proteins both localize to the nucleus or cytoplasm and function in multiple RNAi-related pathways. However, little is known about their individual molecular functions, target transcripts, protein interactions, tissue specificities or subcellular localizations.

Using the CRISPR/Cas9-mediated genome editing system, we edited the endogenous wago-3 locus by inserting an N-terminal gfp::3xflag tag. Fluorescence microscopy shows that WAGO-3 is specifically and globally expressed in the germline and localizes to perinuclear granules. Notably, the spacial distribution of WAGO-3 within the germline strongly depends on mutator genes like mut-7 and mut-16. Experiments addressing these aspects of WAGO-3 cell-biology in more detail will be presented.

In order to characterize the direct molecular surroundings of WAGO-3 in more detail, we performed immunoprecipitation experiments coupled to label-free quantitative mass spectrometry in wild-type and selected Mutator mutant backgrounds. From these experiments a set of novel proteins has been identified that may regulate or drive the activity of WAGO-3. These results will serve as basis for further analyses and enable the comparison of WAGO-3 with other endogenously edited WAGO proteins to further elucidate specificities and functional redundancies among the WAGO sub-clade.

673C Global Changes in Non-Coding RNA Expression during Heat Stress. W.P. Schreiner, J.S. Chen, K Wong, A.P. Aalto, D Pagliuso, A.E. Pasquinelli Division of Biological Sciences, University of California San Diego, La Jolla, CA, 92161.

Outside of the laboratory in the natural world, organisms are subject to various environmental insults that, if not properly addressed, can have lethal consequences. Organisms employ a carefully orchestrated and conserved Heat Shock Response (HSR) in order to cope with the potentially lethal effects of increased temperature. While the transcriptional response to HS is well-established, post-transcriptional control mechanisms involved in the HSR are yet to be fully investigated. We are particularly interested in the role of non-coding RNAs (ncRNAs) in modulating gene expression in response to heat stress. This focus stems from our discovery of nearly one hundred novel long ncRNAs (lncRNAs) induced by heat shock in C. elegans. Additionally, we have found several miRNAs to be differentially regulated by heat stress. While changes in miRNA expression and a functional role for some of these miRNAs in mitigating the HSR have been previously published, their targets are yet to be established. We aim to determine how HS induces the expression of long and short ncRNAs and how they might contribute to the HSR.

Preliminary evidence shows that Heat Shock Factor 1 (HSF-1) is required for the expression of some novel lncRNAs, indicating that this transcription factor directs the production of ncRNAs as well as protein-coding mRNAs. Additionally, we find that the levels of some IncRNAs continue to be elevated long after the heat shock episode, raising the possibility that these RNAs provide resistance to future exposures to heat stress. Overall, these studies have revealed that heat shock causes extensive remodeling of the ncRNA landscape, providing new candidates for regulating the HSR.

674A Towards establishing when double-stranded RNA can move between cells in C. elegans. Nathan Shugarts, Winnie Chan, Antony Jose Cell Biology and Molecular Genetics, University of Maryland, College Park, MD.

Extracellular RNAs are found in the circulation of plants and animals. While RNAs that move between cells can regulate physiology and development in plants, identities and roles of such RNAs in animals are unknown. Evidence for the movement of RNA between cells has been obtained in C. elegans through transgenic expression of double-stranded RNAs (dsRNAs) within tissues. Specifically, when a transgene is used to express dsRNA in one tissue, silencing of a matching gene can be detected in other tissues. This silencing requires the dsRNA-selective importer SID-1, suggesting movement of dsRNA between cells. However, multiple models can be envisioned to explain the observed silencing. For example, dsRNA within a cell could be packaged during early embryogenesis into intracellular vesicles that are then inherited through cell divisions, such that dsRNA can later enter into the cytosol and cause silencing. In this model, dsRNA is produced in one cell and causes silencing in another without becoming extracellular. Alternatively, an embryonic cell could deliver dsRNA into an adjacent embryonic cell. In this model, dsRNA enters the extracellular space before the formation of the pseudocoelem. Finally, cells in mature tissues could release dsRNA into circulation and distant tissues could then import dsRNA. In this model, dsRNA transits through the pseudocoelem. Because each model requires a different developmental setting, determining when the SID-1-dependent
movement of dsRNA is supported in \textit{C. elegans} could constrain possibilities.
To restrict production, export, and import of dsRNA to specific periods during development, we are implementing optogenetic methods that can enable precise control of these processes. To control export, we will damage neurons expressing dsRNA at different time points during development using the photosensitizer protein miniSOG. Expressing miniSOG in neurons and exposing the worm to blue light will generate reactive oxygen species, causing neuronal damage. Evaluating silencing after the resultant damage will indicate when in development neurons producing dsRNA must be functional to observe gene silencing in a non-neuronal tissue. Preliminary data suggest that the extent of silencing differs depending on the targeted gene and the developmental time point when worms are exposed to light. Finally, to control production of dsRNA and its import, we will use a light-activated gene expression system to express dsRNA in neurons and the dsRNA-selective importer SID-1 in non-neuronal tissues of \textit{sid-1(-)} worms, respectively. Progress towards implementing these optogenetic systems will be presented at the meeting.

\textbf{675B} \textbf{A probable link between primary small RNA methylation and secondary siRNA amplification.} J. Sverdson\textsuperscript{1}, R. Tucci\textsuperscript{2}, B. Montgomery\textsuperscript{2}, K. Brown\textsuperscript{1}, T. Montgomery\textsuperscript{1,2} \textsuperscript{1) Cell and Molecular Biology, Colorado State University, Fort Collins, CO; 2) Department of Biology, Colorado State University, Fort Collins, CO.}

In \textit{C. elegans}, primary small RNAs initiate both endogenous and exogenous RNAi by directing the association of target mRNAs with a secondary small interfering RNA (siRNA) amplification complex. The siRNA amplification complex contains an RNA-dependent RNA polymerase as well as several MUT/RDE proteins with unknown function. There are two classes of primary small RNAs, priRNAs and ERGO-1 class 26G-RNAs, that trigger mRNA entry into the MUT/RDE-dependent siRNA amplification pathway. Both priRNAs and ERGO-1 class 26G-RNAs are methylated at their 3' ends by the 2'-O methyltransferase henn-1, which is thought to be required for protection from exonuclease-mediated degradation. However, other small RNAs, including miRNAs, CSR-1 class 22G-RNAs, and ALG-3/4 class 22G-RNAs are not methylated. It is possible that primary small RNA methylation is in some way linked to secondary siRNA amplification. If so, primary small RNAs produced through the exogenous RNAi pathway, which are biochemically distinct from endogenous small RNAs, should also be methylated. To test this, we sequenced small RNAs from animals undergoing RNAi using an approach that allows for enrichment of methylated small RNAs. Indeed, primary, but not secondary, exogenous siRNAs are also methylated. Exogenous primary siRNAs bind to the Argonaute RDE-1. RDE-1 also binds a subset of endogenous small RNAs, most notably miRNAs. Although miRNAs are not typically methylated, our results indicate that those that associate with RDE-1 are methylated. We are currently exploring the mechanistic link between primary small RNA methylation and secondary siRNA amplification and the role of Argonautes in dictating which small RNAs are methylated.

\textbf{676C} \textbf{Intranuclear targets of Argonaute proteins controlling homologous pairing of meiotic chromosomes.} H. Tabara\textsuperscript{1,2}, S. Mitani\textsuperscript{1}, Y. Kohara\textsuperscript{2}, K. Nagata\textsuperscript{1} \textsuperscript{1) Univ. of Tsukuba, Fac. of Med., Japan; 2) National Institute of Genetics; 3) Tokyo Women's Med. Univ.}

Homologous pairing of chromosomes is a key event in meiosis. It provides a basis for crossover recombination, as well as for the spatial and temporal arrangement to ensure exact separation of the homologous chromosomes at the end of meiosis I. In \textit{C. elegans}, homologous pairing of chromosomes starts from pairing center (PC) sequences, and subsequently the pairing of non-PC regions is established by the synaptonemal complex (SC). However, it is not well understood how homologous sequences of non-PC regions are recognized during the SC formation. The shape of SC is layer-axes, in which two lateral elements sandwich a central region. The lateral elements are thought to contain a meiosis-specific cohesin complex consisting of HIM-1, COH-3/4 and several other components.

We previously analyzed the double-mutant of \textit{csr-1} and its paralog, which correspond to Argonaute proteins with Slicer activity to cleave target RNAs. In the pachytene nuclei of the Argonaute double-mutant, a non-homologous abnormal formation of SC was observed. The similar abnormality was observed also in the \textit{ego-1} mutant, which corresponds to an RdRP synthesizing some 22G-RNAs. In the pachytene region of wild-type germ cells, our immuno-staining detected CSR-1 and EGO-1 not only in the cytoplasm but also in the nuclei. CSR-1 is considered to function for silencing of target mRNAs in the cytoplasm, but we think that the intranuclear role of CSR-1 is not clear yet.

To obtain insight into the role of CSR-1 in the accurate SC formation, we analyzed and compared the chromosomal distributions of CSR-1, HIM-1 and COH-3/4 by ChiP-seq experiments. The ChiP signals of CSR-1 and cohesin components were rich in repeat sequences and detected with a similar distribution on chromosomes. Among the repeat sequences, the most typical targets of CSR-1, HIM-1 and COH-3/4 were \textit{CeRep55} repeats. \textit{CeRep55} is a class of minisatellite sequences, which form clusters present on all chromosomes.

We found that \textit{CeRep55} clusters express long non-coding (Inc) RNAs as well as 22G-RNAs interacting with CSR-1 and its paralog. Our \textit{in situ} hybridization experiments detected the \textit{CeRep55} IncRNAs on chromosomes. The signals of \textit{CeRep55} IncRNAs anchoring to COH-3/4 regions were observed often as dumbbell-like shapes, each of which could connect homologous chromosomes. Interestingly, the number of \textit{CeRep55} IncRNA signal regions with dumbbell-like shapes in the Slicer-type Argonaute double-mutant was decreased to less than half of those in the wild type. The Slicer-type Argonaute proteins may be involved in homolog recognition during the SC formation, by targeting repeat-derived IncRNAs.

\textbf{677A} \textbf{MUT-16 promotes formation of a small RNA amplification complex through its C-terminal intrinsically disordered region.} D.C. Anderson, C.J. Uebel, C.M. Phillips \textsuperscript{Department of Biological Sciences, University of Southern California, Los Angeles, CA.}

*Small RNAs* are a diverse set of regulatory RNAs, which are bound by members of the Argonaute protein family. They
regulate gene expression in a wide range of organisms and the fundamental mechanisms governing RNA silencing are conserved across most eukaryotes. Small RNAs regulate fully or partially complementary mRNAs at the level of transcription, translation, and RNA stability. By regulating both endogenous and foreign RNAs, small RNAs maintain proper gene expression, silence deleterious RNA products, and play critical roles in development, chromosome segregation, fertility, and viral defense. We previously characterized the MUT-16 protein, which is essential for RNA silencing. MUT-16 nucleates Mutator foci, a perinuclear RNA silencing compartment found adjacent to P granules in germ cells. MUT-16 recruits numerous other proteins required for RNAi and small RNA biogenesis to Mutator foci, yet no proteins have been shown to be required for MUT-16 localization. The relationship between P granules and Mutator foci is still under investigation, but their close juxtaposition suggests a role for RNA and protein sorting between compartments during the multi-step process of mRNA recognition and RNA silencing.

To elucidate the role of MUT-16 in assembly of the Mutator foci, we have used CRISPR to generate targeted mut-16 deletions. Each in-frame deletion removes between 61 and 114 amino acids (6-11% of the protein). All deletion strains have been assayed for RNAi competence in both germline and somatic tissues. While the majority of the protein is essential for RNAi in germline cells, we were surprised to discover that only a few key regions of the protein are required for RNAi in somatic tissues. These differences may be a result of distinct molecular requirements for RNAi in the germline compared to the soma (for example, MUT-14 is required for germline RNAi but not somatic RNAi) or in the nature of the Mutator protein complexes themselves in each tissue. We had previously observed that while MUT-16 forms foci in germ cells, no foci can be observed in somatic cells despite a requirement for MUT-16 in these tissues.

Furthermore, while some of the deletions abolish recruitment and localization of other known small RNA factors, most do not affect MUT-16 localization and Mutator foci formation. In contrast, we have identified three contiguous deletions (260 total amino acids) of MUT-16, which are essential for in vivo Mutator foci formation. This region corresponds to a portion of the intrinsically disordered region found at the C-terminus of MUT-16. These results indicate a role for the intrinsically disordered region of MUT-16 in the formation of these essential RNA silencing foci.


Circular RNAs (circRNAs) have recently been identified as a natural occurring family of wide-spread and diverse endogenous non-coding RNAs (reviewed in Cortés-López and Miura, Yale J. Biol, 2016). They are remarkably stable RNA molecules mostly generated by backsplicing events from known protein-coding genes, and are abundantly expressed in many animals, from C. elegans (Ivanov et al, Cell Rep, 2015; Memczak et al, Nature 2013), to humans. Recent reports suggest functional roles for circRNAs, such as regulating transcription, binding proteins, and as microRNA sponges. CircRNAs accumulate during aging on a genome-wide level in Drosophila and mice (Westholm et al, Cell Rep, 2014; Gruner H. et al, Sci Rep, 2016), but their potential role and function in the aging process is not known. We profiled for the first time genome-wide circRNA changes during aging in C. elegans. Using total RNA-seq of whole C. elegans from L4-staged larvae, Day-1, Day-7 and Day-10 adults, we identified 1166 circRNAs including 575 novel annotations. Individual circRNAs were validated by Northern blot, qRT-PCR, and Sanger sequencing. We identified a massive and progressive accumulation of circRNAs during aging, which is independent of linear RNA changes from shared host genes. More specifically, 290 circRNAs were significantly upregulated in Day-10 compared to Day-1 adults, whereas only 6 were downregulated. This includes a >20-fold enrichment in a circRNA derived from the gene for the CREB homolog chr-1, as verified by qRT-PCR. Other genes in longevity-associated pathways produce age-accumulated circRNAs, including daf-16 and daf-2. Unlike in other species, the age-accumulation trend appears not to be confined to neurally expressed genes. Given the progressive accumulation of circRNAs and their resistance to decay, we propose that stability in post-mitotic tissues is the main driver of the age-upregulation trend. We are currently examining whether circRNA accumulation patterns change in mutants with altered lifespan phenotypes. Taken together, our characterization of age-accumulated circRNAs lays the foundation for future investigations into the functions of circRNAs and how they might play a role in the aging process.

Neurobiology - Behavior

679C The deterministic dynamics of random search in C. elegans. Tosif Ahamed, Greg Stephens, Ichiro Maruyama OIST Graduate University, Onna-son, Okinawa, JP.

Foraging C. elegans actively modulate their search strategy to effectively sample their environment. These decisions are based on statistical distributions of food patches in the environment, sensory cues and their recent experience. Examples of C. elegans search behaviors include area restricted search, salt chemotaxis and roaming-dwelling. Studies based on centroid measures have suggested a random search framework to explain these behaviors. Within this framework, worms sample an environment by executing straight runs punctuated randomly by sharp turns made by reversals, omega turns or a combination of both. When the frequency of sharp turns is high, worms perform a diffusive search, densely sampling a small area. On the other hand when the frequency of sharp turns is small, they perform a ballistic search, going straight for a long period of time. In this study we take another look at random search in C. elegans by analyzing the continuous dynamics in the low dimensional space of C. elegans postures given by the eigenworm representation. To our surprise, we find that the randomness in random search is largely a result of deterministic chaos instead of sensory or motor noise. Thus, the chaotic nature of the dynamics implies that the behavior of the worms is not random, but predictable for a finite period of time. This prediction window is governed by a quantity called the Maximal Lyapunov Exponent (MLE), which is positive for chaotic systems. Highly chaotic systems have a small prediction window due to a large MLE, while, weakly chaotic systems have a big prediction window resulting from a small
MLE. We find that for worms engaged in an area restricted search, the MLE gradually reduces with time as the worms transition from a diffusive search strategy to a ballistic search strategy. Moreover, the reversal frequency of these worms is positively correlated with the MLE, consistent with the observation that worms get more predictable as they reduce their reversal frequency. Furthermore, the variation in MLE enough to describe most of the random search behavior, suggesting the intriguing possibility that only one degree of freedom underlies control of C. elegans foraging behavior. Finally, we use this theoretical framework to probe the biology of random search by studying the roles of monoamines dopamine and serotonin in foraging. In summary, we extend past theoretical work on C.elegans random search by including detailed postural dynamics and find that deterministic chaotic dynamics underlie the apparent randomness of C.elegans search strategies. Our analysis also suggests that the MLE of the dynamics is potentially the only parameter that the worm controls to modulate its search strategy and decide how predictable or unpredictable it wants to be.

680A Effects of engineered nanomaterials on the behavior and embryonic development of the nematode, Caenorhabditis elegans. Melissa Antonio, J. Constante, A. Hartoonian, S. Hibbard Biological Sciences, California Baptist University, Riverside, CA.

Since C. elegans are soil-dwelling organisms in the environment we are interested in probing the question of how the exposure of specific engineered nanomaterials (ENMs), namely molybdenum disulfide and graphene oxide, may pose a threat to living organisms. Although the use of ENMs is increasing, concern of adverse effects on soil communities is also rising. Not only are ENMs toxic to various organisms in soil, “but can bioaccumulate, trophically transfer and even biomagnify in some systems” (1). Molybdenum, for example, is a metal that is continuously used for plant growth and an essential component for nitrogen fixation and nitrate reduction (2). In addition, graphene, a carbon nanomaterial, has been widely used in various ways, such as pollution prevention and remediation. However, as the production and application amount is increasing, it is also released into the environment resulting in potential risks of ecological environment and human health (3). Of course, the use of an ENM such as molybdenum and graphene may provide various benefits, but little is known on the potentially negative effects they may have on the environment. Therefore, it is crucial that the behavior of ENMs in soils, and ultimately in organisms that thrive in the soil such as C. elegans, is well studied and understood to prevent negative impacts to the environment. The data we have collected thus far consists of preliminary findings testing the affects of heavy metals, such as CdCl2, CuSO4, and ZnSO4, on the mobility/behavior of toxicity mutants and wild type N2 strains. We will soon proceed with exposing both strains to the ENMs mentioned above and observe potential affects on behavior and embryonic development.


Changing behavior at the right time is crucial for animals to survive in constantly fluctuating environments. In particular, the latency of behavior changes is critical; animals fail to discriminate a long-lasting change from a transient noise if the latency is too short, while they fail to feed and avoid dangers if it is too long. However, molecular and neural mechanisms underlying the regulation of the latency of behavior changes mostly remain elusive. In thermotaxis behavior of C. elegans, animals migrate toward the past cultivation temperature associated with food when put on a thermal gradient without food. A few hours after cultivation temperature is shifted, animals change their preferred temperature to a new cultivation temperature. Here we show that a mutation of slo-2 gene that encodes a BK-type Ca2+-dependent K+ channel, which was confirmed to be a gain-of-function (gf) mutation by electrophysiological analyses, decelerates the change in preferred temperature in thermotaxis behavior. We further found that the behavior change of the animals double mutant for slo-2 and slo-1 that encodes another BK-type K+ channel was faster, suggesting that SLO-1 and SLO-2 act redundantly to decelerate behavior change. While gf mutations of human slo-2 homolog cause epilepsies, we found that the expression of C. elegans slo-2 with analogous mutations decelerates the change of thermotaxis more than that of wild type slo-2, implicating similar molecular mechanisms underlying human epilepsies and C. elegans thermotaxis. A major thermosensory neuron AFD increases intracellular Ca2+ concentration in response to a temperature increase near the past cultivation temperature. While the onset temperature of AFD response also changed after cultivation temperature was shifted, the slo-2(gf) mutation decelerated the change in AFD responses. Furthermore, a mutation in cng-3 gene that encodes a cyclic nucleotide-gated cation channel suppressed the decelerated phenotypes of both thermotactic behavior and AFD responses caused by the slo-2(gf) mutation. We also showed by co-immunoprecipitation experiments that SLO-2 and CNG-3 physically interact, suggesting that Ca2+ influx through CNG-3 channel might locally activate SLO-2, which leads to decelerate updating of thermal memory stored in AFD thermosensory neurons and thereby the timing of behavior changes. Our study would serve as a starting point toward comprehensive understanding of neural bases of behavior changes.

682C Analyses of the regulation of forgetting by the food signals in the olfactory learning of C. elegans. M. Arai, A. Inoue, T. Ishihara Department of Biology, Faculty of Science, Kyushu University, Fukuoka, JP.
Animals can retain various kinds of information as memories to respond more effectively to environments. In addition, the memories should be forgotten depending on the environments, because some information is valuable only in particular conditions. Therefore, the memory retention should be adequately regulated by environmental conditions, although the regulatory mechanisms of forgetting remain unclear.

*C. elegans* shows adaptation to diacetyl, which is one of the odorants sensed by AWA sensory neurons. We demonstrated that the TIR-1/JNK-1 pathway in AWC sensory neurons accelerated forgetting of the adaptation to diacetyl. In wild-type animals, the adaptation is sustained for less than 4 hours on food, whereas, in *tir-1* loss-of-function mutant, the adaptation persisted more than a day in the same condition. However, when, after conditioned with diacetyl, animals were incubated without food, both wild-type and *tir-1* (II) animals were recovered from the adaptation within 4 hours. Furthermore, *ceh-36* mutant, which does not have functional AWC neurons, showed the prolonged retention of the adaptation regardless of with or without food. Therefore, we postulated that food signals, which are inhibited by TIR-1/JNK-1 pathway in AWC, inhibit forgetting. To elucidate the mechanisms for the regulation of the forgetting by the food signals, we carried out suppressor screening from *tir-1*.

We checked about 10,900 haploid genomes to search for the mutants showing the normal forgetting even when animals were cultivated on food after adaptation although *tir-1* (II). We found that mutations in *dgk-1*, which encodes a diacylglycerol kinase-1 involved in acetylcholine release by phosphorylation of diacylglycerol (DAG), suppress the phenotype of *tir-1*. Next, we examined whether the known DAG pathway is involved in the forgetting of diacetyl adaptation using the double mutants with *tir-1*. The behavioral assays after recovery showed that mutations in *dgk-3*, *goa-1* (*Goa*), and *egl-30* (gft) (*Gqa*) suppressed the *tir-1* phenotype similarly to *dgk-1*. These results suggest that the food signal regulates the forgetting of olfactory adaptation through the DAG pathways.

**683A  Behaviour in an eggshell.**  *Evan Ardiel*, Hari Shroff  NIBIB, NIH, BETHESDA, MD.

With its small and well-documented connectome, *C. elegans* has emerged as a powerful system for the modeling of complex behaviours from sensory input to motor output. Adult locomotion has been the focus of much research, but the emergence of coordinated behaviour during embryogenesis remains almost completely unexplored. Spontaneous muscle contractions begin about 2 h after the transition from proliferation to organogenesis (~8 h post-fertilization), before the appearance of neuromuscular junctions. Over the next 6-7 h, the embryo’s length approximately doubles and myogenic contractions give way to more coordinated behaviour mediated by the nervous system. The embryo’s small size, entangled posture, and rapid movements have traditionally complicated the collection and interpretation of behavioural data during this developmental period. To describe postural changes over the final hours of embryogenesis, we used light–sheet fluorescence microscopy for fast and gentle 3D imaging of a strain expressing a fluorescent marker in the seam cells. We were able to capture embryonic volumes at 3 Hz for more than 5 h without delaying hatch time. We tracked the labelled cells over the 50000+ volumes and used the lateral positions of the seam cells to construct a simple, dynamic model of the embryo in the eggshell. We observed distinct behavioural states and trajectories as the embryo developed. Detailed description of movement during the three-fold stage promises to yield insights into motor circuit assembly.

**684B  Investigation of the behavioral and cellular basis for magnetotaxis in *C. elegans*.**  Chance Bainbridge\(^1\), Jocelyn McDonald\(^1\), Zachary Benefield\(^1\), Samantha Padia\(^1\), Lucas Barickman\(^1\), Janelle Jackson\(^1\), Ken Nguyen\(^2\), David Hall\(^2\), Andrés Vidal-Gadea\(^1\)  1) School of Biological Sciences, Illinois State University, Normal, IL; 2) Albert Einstein College of Medicine, New York, NY.

The magnetic field of the Earth provides directional information to organisms across many taxa (from bacteria to mammals). However, the cellular and molecular mechanisms necessary for magnetic field detection in animals remain poorly understood, in part, because of the genetic, behavioral, and neurological intractability of the species studied. We previously showed that *C. elegans* readily orients to magnetic fields of earth-strength through a pair of identified sensory neurons (AFDs). This opens the door for the use of this behaviorally and genetically tractable species in the study of magnetotransduction. Much remains to be understood regarding how *C. elegans* transduces magnetic information, how this information is encoded, and how it ultimately affects the animal’s behavior. Our lab studies the behavioral, cellular, and molecular basis of magnetotransduction in *C. elegans*. By recording animals orienting to magnetic stimuli, we determined that *C. elegans* orients to magnetic fields by modulating the frequency of deep bends during locomotion. We found that the integrity and number of AFD sensory villi are critical for magnetic orientation in *C. elegans*. Magnetotransduction and magnetic orientation in *C. elegans* function light-independently. We used streptavidin coated magnetic beads and atomic force microscopy to track the potential presence of endogenous magnetic particles in *C. elegans*. The behavioral, cellular, and genetic tractability of *C. elegans* offer a unique valuable opportunity to elucidate the mechanism through which animals harness the magnetic field of the earth to enhance their survival.

**685C  PI3K/Akt signaling influences neuronal function in *C. elegans*.**  L. Barkema, M. Williams, S.J.B. Fretham  Dept of Biology, Luther College, Decorah, IA.

Highly active cells such as neurons must effectively mitigate the cost of high energy demands. One way cells accomplish this is via the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway which integrates external and internal cues such as growth factors, nutrients, energy levels, and oxidative stress to regulate growth, plasticity, and stress responses. Disrupted PI3K/Akt signaling has been identified in several neurological conditions although its mechanistic role is not clear. In order to better understand how PI3K/Akt signaling contributes to neuronal function and disease this study assessed neuronal function in wild type (WT) and mutant *Caenorhabditis elegans* (elegans) cultured on standard NGM agar or NGM agar supplemented with iron (ferric ammonium citrate). Iron is an essential co-factor for many metabolic enzymes but accumulates in the nervous system in several neurodegenerative conditions leading to oxidative stress. Consistent with previous studies, in standard culture
conditions PI3K/Akt mutants demonstrated an inverse relationship between pathway activity and lifespan, as well as altered mRNA expression of DAF-16 target genes aqp-2 and sod-3 measured using quantitative PCR. Excess iron reduced lifespan in WT animals and in daf-16 and daf-18 (PTEN) mutants with increased upstream PI3K/Akt activity and reduced FOXO activity but not in age-1 (PI3K) mutants with decreased PI3K/Akt signaling. Furthermore in standard conditions there was an inverse relationship between PI3K/Akt activity and dopamine-dependent basal slowing behavior. Exposure to excess iron resulted in further impairment of dopamine-dependent behavior in daf-16 mutants. These observations suggest optimal dopaminergic function depends on PI3K/Akt signaling integrity and that PI3K/Akt integrity may be disrupted by iron dyshomeostasis.


Glutamate is a critical neurotransmitter involved in cognition, memory, and learning. Although much research has been conducted to examine glutamatergic signaling pathways, the functional role of glutamate transporters (GLTs) in behavior, learning, and memory remains largely unexplored in C. elegans. We tested wildtype C. elegans and C. elegans with GLT deletions on a battery of behavioral tests including nose-poke, smell on a stick, and chemotaxis in response to attractants and repellants to examine how different transporters affect basic behaviors. Thus far, we have examined C. elegans with deletions of GLT-3 located in canal cells, GLT-1 located in muscle cells, GLT-4 located on presynaptic neurons, and GLT-5 located within the pharyngeal region. We then conducted non-associative and associative learning paradigms. Compared to wildtypes, GLT-3; GLT-1 knockouts were deficient in all basic behavioral tasks besides chemotaxis in response to a chemorepellent, GLT-4 knockouts were deficient in all basic behavioral tasks besides response to the smell of an aversive chemical, and GLT-5 knockouts were deficient in response to the smell of an aversive chemical and chemotaxis in response to a chemorepellant, however they exhibited a hyperactive chemotactic response to a chemorepellent. Mutants with GLT-3; GLT-1 or GLT-5 deletions displayed normal habituation, a type of non-associative learning, despite deficient basic behavioral task performance, however mutants with GLT-4 glutamate transporter deletions were unable to show habituation due to an abnormal initial response. All mutants displayed associative learning, however GLT-4 mutants had less of an extreme response to the aversive chemical prior to conditioning and expressed less association to that same chemical in the post-learning period. We next examined exposure to the addictive substances nicotine and ethanol as a type of memory. We evaluated the ability for different mutant strains to express drug-seeking behavior and initial preference for these drugs. We then tested whether the mutants would associate the drugs with an aversive chemical as we demonstrated in wild types. Our data suggest that basic behaviors may rely on different glutamate transporters than learning and memory do. These differences may be attributable to differences in transporter localization.

687B  A mutant GPCR receptor npr-14 alters locomotion, egg laying and quiescence in C. elegans.  William Bendena, Foroozan Torki, Lian Zara, Ian Chin-Sang  Dept Biology, Queen’s Univ, Kingston, ON, CA.

Sleep is an evolutionary conserved behavior which in most species is essential for survival. However, the mechanisms involved in the genetic regulation of sleep remain poorly understood. C. elegans exhibit a number of sleep-like behaviors: a) lethargus which is a developmental period between molts, b) satiety which occurs after feeding and c) after long durations of thirsting in liquid media. We have identified a C. elegans G-protein coupled receptor, NPR-14, that is involved in the above behaviors by functioning through cGMP protein kinase (EGL-4) - dependent and independent pathways. The NPR-14 GPCR works together with other signaling pathways that have been implicated in sleep regulation including Notch, GABA, and insulin. An npr-14 null mutation results in anachronistic quiescence especially in the presence of food. The mutants also displays egg-laying defects, episodic swimming behavior, asynchrony during development and appear to accumulate more fat than wild type worms. Supported by the Natural Engineering and Science Research Council of Canada (NSERC)

688C  The Investigation of the Role of the TRPV Channel OSM-9 in Olfactory Long-term Memory Formation.  Kelli Benedetti, Fernando Muñoz-Lobato, Noelle L’Etoile  University of California, San Francisco, Department of Cell and Tissue Biology, San Francisco, CA.

Memory is one of the most important abilities of the brain. It is defined as an alteration in behavior from an experience. For example, the C. elegans nematode will downregulate its chemotactic response to an innately attractive odor if it is not paired with food1. This process is termed olfactory classical conditioning. Through spaced training with this odor, C. elegans will maintain this memory of the food-related odorant butanone for a prolonged period of time, akin to long-term memory formation. Although TRP channels are classically thought of as primary sensory receptors, intriguingly, it has been reported that the OSM-9 TRPV (vanilloid) channel protein is required for odor classical conditioning, and we discovered a new role for it in long-term memory formation (LTM). Nevertheless, the mechanisms that regulate TRPV-mediated LTM remain a mystery. Our goal is to uncover the mechanism of OSM-9/TRPV-mediated LTM in the olfactory sensory circuit of C. elegans. We hypothesize that OSM-9 functions in specific cells in the olfactory circuit by facilitating Ca\(^{2+}\) flux in the olfactory neurons to mediate odor long-term memory in adult C. elegans animals. TRP channels are known to be monovalent and divalent cation-permeable channels3. Previous research shows that OSM-9 and Ca\(^{2+}\) are the most downstream components in the conditioning-promoting pathway4,5. Thus, OSM-9 may cause an influx of Ca\(^{2+}\) into the olfactory sensory neurons or potentially other neurons within the circuit to promote LTM. We will test the spatiotemporal requirements for OSM-9-mediated LTM after long-term odor exposure and the mechanism of OSM-9-mediated Ca\(^{2+}\) dynamics in promoting olfactory LTM. These studies will be accomplished through the use of microscopy, functional imaging, behavioral analysis, and genetic techniques. TRP channels are well studied in their ability to
Receptors of this class (GABA, ACh, glycine) are commonly cited as alcohol targets in mammalian studies. We are pursuing the functional role any particular gene is given to the memory. This principle is demonstrated by showing that habituation to tap can be decomposed into various response dimensions (e.g., speed, frequency, distance) and across various timescales (e.g., initial, mutant traditionally thought to be deficient in short-term memory do, in fact, show memory but that this memory may be looking at only a single response, it bodes well to measure as many facets of it as possible. A more nuanced account of memory qualitatively (rather than simply quantitatively) different than that shown by N2. This suggests that even when researchers are looking at only a single response, it bodes well to measure as many facets of it as possible. A more nuanced account of memory will, in turn, allow a more nuanced understanding of how different genetic pathways contribute to different components of a memory. This work serves to highlight the advantages offered by high-resolution tracking of multiple behavioral facets and the complexity of studying something as simple as short-term memory for habituation.

690B A genetic screen for mediators of acute alcohol effects. Andrew G. Davies, GinaMari G. Blackwell, Laura D. Mathies, Jill C. Bettinger Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA USA.

The behavioral effects associated with the consumption of alcohol (ethanol) in humans reflect the summation of many interactions of alcohol with a variety of protein targets. We are using C. elegans to identify individual mechanisms of action of alcohol with the idea that molecular interactions between alcohol and a target protein are likely to be conserved across species. We have used a forward genetic approach to screen for new mutations that produce decreased sensitivity to the locomotor-depressing effects of alcohol. Using genetic mapping and whole-genome sequencing, we have identified two new alcohol resistance genes, nep-2 and lgc-31. nep-2 encodes a metallo-endopeptidase that appears to act by cleaving peptides, including the peptide encoded by the snet-1 gene (Yamada et al. 2010 Science 329:1647). Loss-of-function mutations in nep-2 result in alcohol resistance, which is consistent with the idea that one or more peptides cleaved by NEP-2 act via a neuropeptide receptor to counter an action of alcohol. A snet-1 mutation only partially suppresses the nep-2 alcohol resistance, demonstrating that SNET-1 is not the only relevant NEP-2 target peptide. We are screening neuropeptide receptor genes to identify the receptor that mediates this action with the assumption that a mutation in the receptor-encoding gene should suppress the nep-2-induced alcohol resistance. Loss-of-function mutations in the ligand-gated ion channel-encoding gene, lgc-31, produce an alcohol resistance phenotype. Receptors of this class (GABA, ACh, glycine) are commonly cited as alcohol targets in mammalian studies. We are pursuing the possibility that ethanol activates the LGC-31 channel directly, by altering specific amino acids in the 2nd transmembrane domain, which have been shown in related channels to alter ethanol-related activation properties. The ligand for LGC-31 is not known; we are testing possible ligands based on the orthology of lgc-31 to the human zinc-activated channel gene (ZACN) and the serotonin-gated ion channel gene (HTR3D).

Knowledge of the range of molecular actions of alcohol will allow for the identification of genetic factors that predispose individuals to develop alcohol use disorders.

691C SWI/SNF chromatin remodeling complexes are required for normal behavioral responses to acute ethanol exposure. Jill C. Bettinger, GinaMari G. Blackwell, Andrew G. Davies, Laura D. Mathies Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA, USA.

Fifteen million adults in the United States have an alcohol use disorder (AUD). There are very few effective pharmacological interventions for AUD, and one reason that has slowed the rational development of pharmacotherapies has been difficulty in identifying relevant targets of ethanol’s action. We use C. elegans to model the acute effects of ethanol on neuronal function to identify the molecular targets of ethanol and determine the mechanisms of the development of tolerance to those effects. A human genome-wide association study identified variation in two different members of the SWI/SNF chromatin remodeling complex as being associated with alcohol use disorder. We examined the role of the SWI/SNF complex in acute behavioral responses to ethanol in worms. There are different SWI/SNF complexes that share the core enzymatic subunits but differ in their accessory subunits. We found that different SWI/SNF complexes regulate different components of the acute response to ethanol: SWI/SNF BAF complexes are required for the initial depressive effects of ethanol, whereas SWI/SNF PBAF complexes are required for the development of acute ethanol tolerance. Although SWI/SNF complexes play important and diverse roles in many tissues, we found that they are required in adults and in neurons for normal ethanol responses. We predict that SWI/SNF complexes regulate the expression of genes that are important for determining the level of acute sensitivity to ethanol and the development of tolerance to ethanol’s effects. We have focused first on the development of tolerance to ethanol. We have localized a function of PBAF complexes in ethanol responses to GABAergic neurons. To identify the mediators of the response to ethanol, we have undertaken gene expression studies in which we are comparing several strains to identify PBAF-regulated genes specifically in adult neurons. We have found that among the differentially regulated
genes, there is an over representation of genes involved in lipid metabolism. We are testing a model in which lipid effects on membrane microarchitecture, and the proteins contained within, modulate the behavioral effects of ethanol, and SWI/SNF modifies ethanol responses through regulation of these lipid mediators.

692A Investigation of molecular mechanisms regulating MSI-1 activity during forgetting. C. Boglari, A. Arnold, F. Peter, A. Papassotopoulos, D. J.-F. de Quervain, A. Stetak 1) Transfaculty Research Platform Molecular and Cognitive Neurosciences, University of Basel, Switzerland; 2) Division of Molecular Neuroscience, Department of Psychology, University of Basel, Switzerland; 3) University Psychiatric Clinics, University of Basel, Switzerland; 4) Division of Cognitive Neuroscience, Department of Psychology, University of Basel, Switzerland.

We previously demonstrated that the C. elegans musashi homolog, MSI-1, promotes forgetting via the translational inhibition of the Arp2/3 complex (Hadziselimovic et al., 2014). We also found that MSI-1 acts downstream of the ionotropic glutamate receptor GLR-1. However, the signaling cascades and molecular mechanisms that regulate MSI-1 activity are currently unknown.

Here we present the first step to understand molecular mechanisms that regulate MSI-1 activity during forgetting. In order to find MSI-1 binding partners, we performed a yeast-2-hybrid screen and identified 97 proteins that interacted with the full length MSI-1. We classified the candidates by interaction strength and biological function and selected 14 MSI-1 binding partners for further analysis. We confirmed the physical interaction of the candidates with MSI-1 either with GST pull-down or co-immunoprecipitation. Using this approach, we identified elongation factors, the cell cycle regulator CDC-25.1, the Ca2+-binding protein CLIK-1, and the actin regulatory protein GSNL-1 as MSI-1 binding partners. In addition, we also identified MAT-1 as an MSI-1 interacting protein both in the Y2H screen and in vivo by co-immunoprecipitation. Interestingly, MAT-1 is a subunit of the anaphase-promoting complex (APC/C) and regulates the GLR-1 receptor levels in the ventral nerve chord (Juo & Kaplan, 2004). Therefore, we investigated the role of mat-1 loss in memory and found that the mat-1 mutants showed increased long-term memory retention, similar to that of msi-1 mutants. Thus, MSI-1 may regulate GLR-1 levels through the interaction with MAT-1 and the APC/C complex.

In a parallel approach we investigated the possible modulation of MSI-1 activity through phosphorylation. We demonstrated that a large fraction of the MSI-1 was phosphorylated and this phosphorylation was RNA-binding dependent. Next, using mass-spectrometry analysis we identified 3 phosphorylated amino acid residues; moreover, one of these amino acids is conserved between worm and human MSI-1. Finally, we modified with CRISPR/Cas9 the endogenous protein and deleted individually or simultaneously the phospho-sites in order to investigate the potential role of phosphorylation on MSI-1 activity. Using these approaches, we made the first step to understand in depth the molecular mechanisms that regulate MSI-1 activity during forgetting.

693B Superoxide dismutase SOD-1 modulates C. elegans pathogen avoidance behavior. Alexander Horspool, Howard Chang Dept. of Biological Sciences, Binghamton University, SUNY, Binghamton, NY.

The C. elegans nervous system mediates protective physiological and behavioral responses amid infection. However, it remains largely unknown how the nervous system responds to reactive oxygen species (ROS) activated by pathogenic microbes during infection. Here, we show superoxide dismutase-1 (SOD-1), an enzyme that converts superoxide into less toxic hydrogen peroxide and oxygen, functions in the gustatory neuron ASER to mediate C. elegans pathogen avoidance response. When C. elegans first encounters pathogenic bacteria P. aeruginosa, SOD-1 is induced in the ASER neuron. After prolonged P. aeruginosa exposure, ASER-specific SOD-1 expression is diminished. In turn, C. elegans starts to vacate the pathogenic bacteria lawn. Genetic knockdown experiments reveal that pathogen-induced ROS activate sod-1 dependent behavioral response non cell-autonomously. We postulate that the delayed aversive response to detrimental microbes may provide survival benefits by allowing C. elegans to temporarily utilize food that is tainted with pathogens as an additional energy source. Our data offer a mechanistic insight into how the nervous system mediates food-seeking behavior amid oxidative stress and suggest that the internal state of redox homeostasis could underlie the behavioral response to harmful microbial species.

694C Rictor limits temperature-dependent dauer formation by controlling intestine-neuron signaling. M.P. O’Donnell, P. Chao, J. Kammenga, P. Sengupta 1) Dept. of Biology, Brandeis University, Waltham, MA; 2) Laboratory of Nematology, Wageningen University, Droevendaalsesteeg 1, 6708PB Wageningen, The Netherlands.

Multicellular animals often exhibit plasticity in their developmental trajectories as a common strategy to cope with stressful environments. A striking example of such plasticity is the nematode alternative larval morph – the long-lived, stress-resistant dauer larva. In C. elegans, when food is limiting and population density is high, an instructive pheromone-derived signal results in decreased TGF-ß and insulin signaling that act in parallel to promote dauer formation. This process is highly temperature-dependent, and high temperatures can also be instructive in regulating dauer formation via largely unknown mechanisms. In addition to these sensory cues, it is likely that this developmental decision involves weighting of internal state signals, although the sources of these signals and pathways involved in this process are unknown. We find that mTOR signaling, via the mTORC2 component rict-1/ricTOR, limits temperature-dependent dauer formation likely via gut-brain signaling. RICT-1 acts in the intestine to regulate the level of TGF-ß signaling from the ASI sensory neurons. Temperature sensitivity in this context is encoded by the combined activity of TORC2-sensitive TGF-ß signaling, as well as a temperature-dependent reduction of insulin signaling via unidentified pathways. We show that intestinal TORC2 signaling also regulates foraging behaviors in adult animals via modulation of peptidergic signaling suggesting that this pathway conveys internal state information to affect both developmental and behavioral traits. We further identify multiple quantitative trait loci that contribute differentially to natural variation in both dauer formation and foraging behaviors. These data suggest that an intestine-neuron circuit, regulated by the
It is pivotal for animals’ fitness to discern a variety of environmental cues and utilize individual cues for efficient foraging.

K. Katae, M. Tomioka, Y. Iino  Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, 696B

We have reported that behaviors. However, little is known about the neural mechanisms that discriminate different cues and generate appropriate foraging basis of the observed sex-based differences in nociceptive sensory behavior.

Shared ASH neurons is modulated by its sexual state. We will present our continuing work to identify the cellular and molecular mechanisms which the state of the sex determination pathway is “flipped” in specific cell types. We have found that reversing the sex of the entire nervous system reverses the nociceptive behavior of the animal: hermaphrodites with a male nervous system became less sensitive to ASH-detected aversive stimuli, while males with a hermaphrodite nervous system became more sensitive. The same held true when only the sensory neurons of animals were sex-reversed, indicating that sex differences in sensory function may underlie differences in the response to aversive stimuli. One intriguing possibility is that the development or function of the shared ASH neurons is modulated by its sexual state. We will present our continuing work to identify the cellular and molecular basis of the observed sex-based differences in nociceptive sensory behavior.

It is pivotal for animals’ fitness to discern a variety of environmental cues and utilize individual cues for efficient foraging. However, little is known about the neural mechanisms that discriminate different cues and generate appropriate foraging behaviors.

We have reported that C. elegans learns to approach or avoid salt concentrations previously experienced in the presence or absence of food, respectively, and this learning relies upon the ASE gustatory neuron pair. Here, we show a new paradigm of learning, which we named sugar chemotaxis learning, where animals are attracted to monosaccharides, such as glucose and fructose, after exposure to the sugars during feeding, whereas they avoid those sugars after cultivation without sugars. The animals which experienced salt with food are not attracted to glucose and vice versa, manifesting that the animals discern two tastants, glucose and salt. Cell-specific functional rescue experiments revealed that a battery of sensory neurons are involved in glucose chemotaxis learning, among which ASEL is indispensable. Interestingly, whereas addition of salt activates ASEL, removal of glucose activates ASEL. This glucose response is augmented after prolonged exposure to glucose with food. Furthermore, photostimulation of ASEL augments animals’ forward motion after cultivation without glucose, whereas this behavioral response is abolished after cultivation with glucose.

Together, these results suggest that different patterns of ASEL response to salt and glucose may confer an ability to discriminate two tastants. Signaling pathways required for glucose chemotaxis learning will be also discussed.

C. elegans is known to sense and respond to dozens of odors through G-protein coupled receptors expressed in three pairs of olfactory sensory neurons - AWA, AWB and AWC. In addition, worms display many olfactory behaviors including dose-dependent odorotaxis, odor specificity, short and long-term odor memory, and odor-associative behaviors. One of these attractive odors, diacetyl, was shown to bind to its cognate receptor, ODR-10, by expressing the receptor in a heterologous cell line and demonstrating activity upon binding to diacetyl. However no other odor-receptor pairs have been definitively identified aside from ODR-10. In order to begin to identify new odor-GPCR matches in C. elegans, we conducted odorotaxis assays to a set of 192 odors. We found that worms are highly attracted to 14% of these odors, mildly attracted to 10%, and show aversion to 7% of odors. We plan to identify which olfactory sensory neuron C. elegans uses to sense each of the odors using mutant analysis. To identify odor receptor pairs for AWC-sensed odors, we plan to express AWC-expressed ORs (Hsueh et al., 2016) in HEK293 mammalian cells, and determine their odor ligand using a cell-based reporter gene assay. We expect that these results will not only identify new odor-receptor pairs to study C. elegans olfactory behavior, but will contribute to our understanding of the genomic structure and evolution of olfactory receptors in the animal kingdom.
**698A** Chemosensory GPCR SRI-14 are required for concentration dependent odor preference in *C. elegans*. W. Choi, Kyuhyung Kim Department of Brain & Cognitive Sciences, DGIST, Daegu, Daegue, KR.

Animals must recognize and discriminate among thousands of chemicals in order to generate the correct behavioral response. Understanding basic design of a sensory system in simple animals gives the opportunity to elucidate detailed molecular and neural mechanisms underlying sensory responses in higher animals. *C. elegans* detects a large number of odorants via three neurons pairs including the AWA, AWB, and AWC, which elicit a multitude of olfactory behaviors (Bargmann, 1993, Cell). Previous genetic and behavioral experiments have identified set of signaling genes including olfactory receptors (Sengupta et al., 1996), but the knowledge is still limited. Specifically, the mechanisms of how the same odorants can elicit either attractive or aversive responses depending on the chemical concentration are not known yet.

First, we are trying to construct a comprehensive map of odorants and their receptors in *C. elegans*. We screened 29 volatile chemicals that are not tested previously, and found that animals respond to 13 volatiles. We further identified that the AWC neurons are required for chemotactic responses to these chemicals. We then performed candidate gene searches and found that the chemosensory GPCR mutants sra-13 and str-2 which express in AWC exhibit specific defects in chemotactic responses to 2-Furyl methyl ketone, Ethyl pyruvate, or 1-propanol, respectively. Interestingly, we also found that chemosensory GPCR sri-14 are required for both the attraction to low concentration DMTS and aversion to high concentration DMTS. The defect of DMTS chemotaxis in sri-14 mutants were restored when we expressed the wild-type sri-14 gene to the AWC neurons. Furthermore, for DMTS avoidance, not sri-14 gene in AWC expression but sri-14 gene in ASH expression fully rescue the defect of avoidance to high concentration DMTS, suggesting that the SRI-14 is required for both attraction and avoidance to DMTS and acts in AWC and ASH, respectively. We next found that Ca\(^{2+}\) response of AWC to low concentration DMTS was decreased in sri-14 mutants. We are currently measuring the Ca\(^{2+}\) response of ASH to high concentration DMTS compared to the sri-14, and trying to investigate the neural circuitry mechanism how the SRI-14 regulates both aversive and chemotactic behaviors in *C. elegans*.


Animals must tune their physiology and behavior appropriately to the environment. How such adaptations are achieved remains largely unknown. Biogenic amines are conserved signaling molecules that link food cues to behavior and metabolism in a wide variety of organisms. The biogenic amines serotonin (5-HT) and octopamine regulate a number of food-related behaviors in *C. elegans*, including locomotion and feeding. We use long-term quantitative behavioral imaging in a multi-well device (WorMotel) to investigate the role of 5-HT and octopamine in food-dependent locomotor behavior states. We find that well-fed and fasting animals exhibit distinct patterns of locomotion characterized by differences in locomotion behaviors known as roaming, dwelling, and quiescence. Worms lacking endogenous 5-HT, such as tph-1 mutants, exhibit fasting-like locomotion even under fed conditions, whereas worms lacking octopamine, such as txb-1 mutants, exhibit feeding-like locomotion under fasting conditions. Therefore, we conclude that 5-HT and octopamine promote feeding-like and fasting-like locomotor patterns, respectively. We find that exogenous treatment of fasting worms with 5-HT produces a reduction of quiescent behavior and increase in dwelling. 5-HT produced by the NSM neurons acts on the MOD-1 receptor to promote low-amplitude locomotor behavior characteristic of well-fed animals. Interestingly, 5-HT produced by the ADF neurons acts through the SER-5 receptor to suppress quiescent behavior and promote roaming in fasting worms. Therefore, 5-HT can act both to promote high-amplitude and low-amplitude locomotion through the SER-5 and MOD-1 receptors, respectively. Octopamine, produced by the RIC neurons, acts through SER-3 and SER-6 receptors to promote roaming behaviors characteristic of fasting animals. Both the SER-3 and SER-6 receptors are required for behavioral responses to octopamine. Overall, we find that 5-HT signaling is required for worms to assume food-related locomotion behavior, whereas octopamine signaling is required for worms to assume fasting-related behaviors. The requirement for both neurotransmitters in both the feeding and fasting states enables increased behavioral adaptability. Our results define the molecular and neural pathways through which parallel biogenic amine signaling tunes behavior appropriately to nutrient conditions. Current experiments aim to further identify the specific tissues and neurons in which these receptors act to exert their effects.


Humans have over 70 potassium channel genes, but only some of these have been linked to disease. In this respect, the KCNQ family of potassium channels is exceptional: mutations in four out of five KCNQ genes underlie a range of diseases including cardiac arrhythmias, deafness, and epilepsy. For example, mutations in KCNQ1 are associated with heart arrhythmias such as long-QT syndrome (LQTS), in which the cardiac action potential is prolonged. Mutations in KCNQ2 and KCNQ3 are associated with benign neonatal epilepsy. Homologs of KCNQ genes are found in a wide range of model organisms, including flies and mice, where they often have functions analogous to those in humans, but less is known about the functions of these potassium channels in the nematode *C. elegans*. We investigated the effect of mutations in *C. elegans* KCNQ-like genes on the electrical excitability of the pharynx, a rhythmic muscular pump involved in feeding. One such gene, kqt-1, is orthologous to the subfamily of human KCNQ genes that comprises KCNQ2 to KCNQ5, and is mainly expressed in the muscles of the pharynx. kqt-3 is orthologous to the human gene KCNQ1. Although not expressed in the pharynx, kqt-3 is present in mechanosensory and chemosensory neurons, which can regulate feeding behavior. We hypothesized that mutations in kqt-1 and kqt-3 induce abnormalities in pharyngeal pumping that are reminiscent of cardiac arrhythmias. Taking advantage of a new microfluidic
activation of epidermal growth factor (EGF) of the single ALA neuron. (Hill et al, Curr. Biol '14). Following its activation by EGF, ALA releases neuropeptides that include FLP-13 neuropeptides (Nelson et al, Curr. Biol. '14; Nath et al, Curr. Biol. '16), which lead to SIS by silencing wake-promoting neurons. My current experiments are aimed at identifying the cells or cell types that are damaged by UV radiation and release EGF to trigger ALA activation and sleep.

To address this issue, we have defined the Worm Common Object Notation, a set of rules for how to write tracking data in the ubiquitous JSON format, so that it can be easily shared between labs. To facilitate easy adoption of WCON, we have further written software in a variety of languages that will read or write data in WCON format. So far, we have implementations in Python, Scala, Matlab, and Julia, and wrapper libraries for Octave, R, and Java to use one of the main implementations.

Additionally, the Tracker Commons project of which WCON is a part contains a small but rapidly growing set of pre-packaged analysis tools for routine manipulation of worm tracking data. We will also maintain a list of other WCON-compatible analysis tools as they become available.

If you are involved in worm tracking, we invite you to adopt WCON and help make C. elegans behavioral data widely accessible. WCON is developed under the open source Tracker Commons project of the OpenWorm Foundation. We invite contributions and improvements!

Development of a high-statistics, three dimensional imaging system for C. elegans under magnetic and chemical stimuli. Chris Dao1, Aaron Chow, Nitin Vincent, Charles Wang, Lina Li, Jasmine Alberto, Angela David, Angela Du, Joseph Thatcher, Katushi Arisaka, Elegant Mind Club University of California at Los Angeles, Los Angeles, CA.

In contrast to previous behavioral experiments limited to two dimensional surfaces, we extended the study of C. elegans into three dimensional environments to observe the resulting unconstrained motion. While research has been initiated towards single worm, high magnification tracking, this has limited practicality in terms of gathering a large number of statistics with bulk behavior of many worms simultaneously. Incorporating stimuli allows for a new, more thorough examination of motion and environmental interaction, extending inquiry beyond simple free motion. Previously performed, two dimensional results suggest hypotheses, which may serve as limiting-case projections of greater trends in three dimensional space. A novel, cost effective system was developed to image C elegans’ behavior over a period of 30 to 60 minutes using time lapse acquisition. The setup consists of three, perpendicularly oriented DSLR cameras, synchronized via the open source, MIT developed DigiCam control software and illuminated with lowly-invasive, conical, red LED light sources. Samples consisted of N2 type worms embedded in the center of 2% porcine gelatin (4 to 5 cm) quartz cuvettes. Each camera had an affixed aperture to elongate its depth of field, promoting focus throughout the volume of the cubes. In addition to free motion studies, three sets of Helmholtz coils (with axes coinciding with the cameras’ optical axes) were utilized to generate a uniform magnetic field at the location of the sample. NaCl concentration gradients throughout the gelatin were also simulated and created for three dimensional chemotaxis experiments. Numerous free motion trials have been conducted alongside magnetic field experiments of field magnitude ranging from 0 Gauss (cancelling the geomagnetic field) to 10 Gauss. Using this data, analysis software tools were developed in MATLAB in order to extract the center of mass trajectory of worms navigating the gelatin, enabling further quantification of motional trends such as the worms’ velocities and distributions of initial and final positions. While preliminary results for free motion (with chemical attractants) appear to reflect standard biased random motion, positive verification of magnetic field trials remains elusive.


Following exposures to environments that cause cellular damage, animals stop feeding and moving and go to sleep. I am studying the mechanism of C. elegans somnolence following ultraviolet exposure. Stress-induced sleep (SIS) is dependent on activation of epidermal growth factor (EGF) of the single ALA neuron. (Hill et al, Curr. Biol ‘14). Following its activation by EGF, ALA releases neuropeptides that include FLP-13 neuropeptides (Nelson et al, Curr. Biol. ‘14; Nath et al, Curr. Biol. ‘16), which lead to SIS by silencing wake-promoting neurons. My current experiments are aimed at identifying the cells or cell types that are damaged by UV radiation and release EGF to trigger ALA activation and sleep.
Our lab works with sterilizing doses of UVC radiation (254 nm), which directly induces DNA damage in the form of double stranded DNA breaks (DSBs), cyclobutane pyrimidine dimers (CPDs), and 6-4 photoproducts. DSBs have been shown to recruit histone 2A variant X (H2A.X). We found that in C. elegans too, H2A.X nuclear staining is observed after UV exposure. The recruitment to DSBs is not immediately measurable but is detectable 30 minutes after irradiation. Like DSB, we found that the levels of CPDs increase after UV. We are currently characterizing the time course of DNA damage following UV exposure in wild-type an SIS-defective mutants.

In addition to direct DNA damage, UVC radiation also produces reactive oxygen species (ROS), which could induce additional DNA and protein damage. To determine the location of UV radiation sensatiion, we are expressing the ROS-producing protein KillerRed under the control of tissue-specific promoters and measuring the level of SIS in the worms.

We will present an update on the above experiments.

704A Males need molecular promiscuity for mating: Extracellular matrix genes mec-1, mec-5, and mec-9 are not just for touch neurons. D.M. De Vore, M. Barr  Cell Biology/Genetics, Rutgers University, Paterson, NJ.

Extracellular matrix is made up of a network of interacting proteins that surround and support cells in functions such as mechanosensation, attachment, and signaling. ECM is dynamic and is necessary in tissue morphogenesis and activity from embryonic development through the lifespan of an organism. Alterations in ECM quality and quantity contribute to many human diseases and disorders. ECM proteins have been implicated in cilia formation and directionality in Drosophila and abnormal ECM is observed in ciliopathies such as polycystic kidney disease, Bardet-Biedl Syndrome, and nephronophthisis. Autosomal Dominant Polycystic Kidney Disease (ADPKD), the most common monogenic disorder, is caused by mutations in the polycystin-encoding genes PKD1 and PKD2. Polycystin ciliary localization is evolutionarily conserved from the nematode Caenorhabditis elegans to man. The C. elegans polycystins LOV-1 and PKD-2 localize to cilia of male-specific sensory neurons (CEMs in the head and HOB and RnB neurons in tail, respectively) where they are required for male mating behaviors. In a forward genetic screen for regulators of PKD-2::GFP ciliary localization, we isolated a mutation in the collagen gene mec-9 and discovered new functions for ECM genes previously implicated in the function of non-ciliated touch receptor neurons. We found that ECM encoding genes mec-1, mec-5, and mec-9 play multifaceted roles in ciliated sensory neurons. ECM components regulate polycystin localization and polycystin-mediated male mating behaviors, control ciliary and dendritic integrity, and modulate the release of ciliary extracellular vesicles. Intriguingly, mec-9 has cell-specific functions that are controlled by a short isoform that is differentially expressed in the male and that is not required for touch receptor mechanosensation. Our findings reveal the promiscuity of these ECM components and activity in ciliated and non-ciliated neurons of the worm. While the polycystins have been implicated in sensing and regulating collagen in zebrafish models, roles for ECM proteins in regulating ciliary integrity, ciliary polycystin localization, and ciliary function has not been previously appreciated.

705B Automated animal tracking and quantitative analysis of C. elegans social behavior. S.S. Ding1,2, L.J. Schumacher3,4, A.E. Javer1,2, R.G. Endres3,4, A.E.X. Brown1,2  1) MRC London Institute of Medical Sciences, Hammersmith Hospital Campus, Du Cane Road, London, GB; 2) Institute of Clinical Sciences, Imperial College London, Du Cane Road, London, GB; 3) Department of Life Sciences, Imperial College London, London, GB; 4) Centre for Integrative Systems Biology and Bioinformatics, Imperial College London, London, GB.

Social behavior is common in the animal kingdom, but studies are often limited to the observational level, as few systems allow for perturbation in a controlled environment. To this end, we use C. elegans to dissect the behavioral mechanisms of aggregation, a simple social behavior. C. elegans natural isolates aggregate into tight groups, whereas the laboratory strain forms looser groups or feeds alone; the difference is due in part to a mutation in the neuropeptide receptor gene npr-1 which arose during laboratory domestication. We quantified the behavior of N2, an npr-1 mutant and a natural isolate using fluorescence imaging and automated animal tracking, and built a chain-like agent-based mathematical model to identify the key differences.

706C Protophenotypes in C. elegans relevant for schizophrenia and major depression. Donard Dwyer1,2, Julie Dagenhardt2, Eric Aamodt3  1) Dept Psychiatry, LSU Health Sciences Ctr, Shreveport, LA; 2) Dept Pharmacology, Toxicology & Neuroscience. LSU Health Sciences Ctr, Shreveport, LA; 3) Dept Biochemistry & Molecular Biology, LSU Health Sciences Ctr, Shreveport, LA.

Efforts to deconvolute the complexity of genetic contributions to psychiatric illnesses such as schizophrenia and major depressive disorder (MDD) have led to the concept of endophenotypes – heritable traits that reflect intermediary pathological processes of a complex disorder. We have coined the term ‘protophenotypes’ to describe endophenotypes that have been conserved across species during evolution. The goal of our studies is to characterize the genetics and pharmacology of protophenotypes in C. elegans related to pre-pulse inhibition and startle defects in schizophrenia, social withdrawal (a prominent feature of both schizophrenia and MDD) and avolition/anhedonia, which is characteristic of various affective disorders in man. To model pre-pulse inhibition we evaluated touch suppression of pharyngeal pumping in various mutant strains and with pharmacological agents. To gain insight into social withdrawal, we studied aggregation in social feeding strains in the absence and presence of drugs. Lastly, we developed a model of avolition/anhedonia or diminished motivation based on defects in insulin/IGF-1 signaling, exposure to DMSO and food deprivation. In this model, we observed “suicidal” worms that failed to seek food or escape, but remained in place until they died, despite preservation of responsiveness to external stimuli. Genetic and pharmacological studies revealed that dopamine affects touch suppression via a primitive counter-circuit that presages the arrangement of dopaminergic pathways in man controlling emotions and logical thought. We found that serotonin regulated social feeding, which was inhibited by 5-HT2 receptor antagonists. This finding was consistent with serotonergic defects in MDD

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and schizophrenia. Studies of the third protophenotype related to diminished motivation revealed that defects in insulin receptor signaling (DAF-2) and proteins that regulate insulin secretion (UNC-64, but not UNC-31) produced rapid immobility in animals subjected to food deprivation in the presence of 1% DMSO. The immobility state in daf-2(II) mutants was maintained by elevated serotonergic and muscarinic cholinergic signaling. Immobility was reversed with antidepressants (e.g., amitriptyline, imipramine and amoxapine) and antipsychotics (e.g., clozapine and olanzapine, but not haloperidol) that decrease immobility in the forced swimming test – an established model of depression in man. These findings may help to explain the 2-3-fold increase in MDD in patients with diabetes. Finally, this work may establish mechanisms for how fundamental behaviors conserved through evolution contribute to the emergence of psychiatric illness in man.

707A Genome-wide temporal expression profiling of genes regulated during negative olfactory long-term memory in C. elegans. B.G. Fenyes1,2, V Freytag1, S Probst1, Cs Boglari1, N Hadziselimovic1, Y Hauser1, A Milnik1, V Vukojevic1, D de Quervain1, A Papasotropoulos1,3, A Stetak1,13 1) Transfaculty Research Platform Molecular and Cognitive Neurosciences, University of Basel, Basel, CH; 2) Semmelweis University, Budapest, HU; 3) University Psychiatric Clinics, University of Basel, Basel, CH; 4) Division of Cognitive Neuroscience, Department of Psychology, University of Basel, Basel, CH.

The identification of genes related to encoding, storage, and retrieval of memories is of major interest in neuroscience. In the present study, we performed a microarray-based time-course assay using neuronally enriched mRNA pool following negative olfactory associative conditioning in C. elegans, which results in the formation of long-term memory (LTAM). We identified a core set of 712 (538 up-regulated and 174 down-regulated) genes that follow three distinct temporal peaks demonstrating multiple gene regulation waves in LTAM. We observed an immediate gene activation wave, followed by a 4 hours post-training and a later, 8 hours gene activation. Two of these activation peaks (0h and 4h post-conditioning) coincide with the memory inhibitory effect of actinomycin D that shows a biphasic inhibitory effect of the negative LTAM. Similar to observations in other model systems, negative olfactory LTAM is CREB dependent. Temporal rescue of CREB mutants showed that the protein activity is required in the first two hours following training for normal long-term memory.

Comparing with a recently published set of positive olfactory LTAM genes, 50% of the here identified up-regulated genes overlap between the two studies, thus possibly represent stimulus-independent memory regulated genes. On the other hand, the remaining genes were not related to positive associative memory, and may specifically regulate negative LTAM. Deletion of a selected set of genes showed with high frequency long-term memory impairments without affecting learning. Our results provide evidence for the existence of a multistep gene activation process during the formation and retrieval of long-term memory and define general memory implicated genes as well as conditioning-type dependent gene sets.

708B Gonadal maturation changes chemotaxis behavior and neural processing in the olfactory circuit through the function of a guanylyl cyclase, GCY-28. M. Fujiwara, T. Ishihara Dept Biol, Kyushu Univ, Fukuoka, JP.

Many animal species change their behavior depending on their stage of development. However, the mechanisms involved in translating their developmental stage into the modifications of the neuronal circuits that underlie these behavioral changes remain unknown. In Caenorhabditis elegans, the olfactory preferences are changed over development, and the changes depend on germline cells which proliferate dramatically during the larval stages. We have shown that the germline cells affect the neuronal activities of a circuitry comprised of a small set of neurons including an olfactory neuron, AWA, and their downstream interneurons, AIA and AIB. Removal experiment of either one of these neurons suggested that these neurons are required for the regulation of olfactory preferences.

Here we show that gcy-28 mutants have a defect in the germline-dependent regulation of olfactory preferences; gcy-28 mutant did not exhibit the change in odor preferences regardless of whether germline cells proliferate normally. gcy-28 encodes a guanylyl cyclase with an extracellular ligand binding domain, and is expressed in many tissues including the nervous system. Cell-specific rescuing experiments suggested that GCY-28 acts in the AIA interneuron to mediate the olfactory regulation. It is possible that germline proliferation may change the neural processing through modifying GCY-28 function in AIA. However, the expression levels and the subcellular localization patterns of GCY-28, which was visualized by tagged GFP, in the AIA neurons did not exhibit the change in odor preferences regardless of whether germline cells proliferate normally.

709C The transcription factor fkh-8 is involved in sustaining CO2 function in C. elegans. Destane Garrett1, Bryan Cawthon2, Brian Nelms1 1) Fisk University, Nashville, TN; 2) Vanderbilt University, Nashville, TN.

Sensing and responding to changing levels of oxygen and carbon dioxide is an important function across the animal kingdom. In the model organism Caenorhabditis elegans, the inability to sense fluctuations in CO2 levels can potentially lead to death. The BAG neuron is one cell type that can function as a mediator of high CO2 avoidance and can sense decreases in O2 levels. Some transcription factors, such as ETS-5 and EGL-13, are already known to be required for BAG neuron CO2 and O2 sensing. We have observed through genetics and behavioral studies that the transcription factor FKH-8 likely also plays a role in CO2 sensing. We are more specifically studying the role of FKH-8 in regulating BAG neuron gene expression by examining expression decreases or increases in specific BAG neuron reporter genes in fkh-8 mutants. Through these experiments, we hope to identify which important signaling components in BAG neuron CO2-sensing are controlled by FKH-8.

710A The neuronal and chemical basis of bacterial food odor preference. S. Worthy2, G. Rojas3, J. Phan1, J. King1, N. Doan1, C. Taylor2, E. Glater1 1) Department of Neuroscience, Pomona College, Claremont, CA; 2) Department of Chemistry, Pomona College, Claremont, CA.
Food choice is critical for survival because organisms must choose food that is edible and nutritious and avoid pathogenic food. Odors are some of the most important cues that animals use to find and distinguish among foods. The nematode *Caenorhabditis elegans* uses chemosensation to distinguish among various species of bacteria, their major food source. Although the neurons required for the detection of specific food-odors have been well-defined, less is known about the sensory circuits underlying the discrimination among the mixtures of odors released by different kinds of bacteria. We are examining the sensory neurons underlying bacterial preference among a diverse set of bacterial species. Specifically, we are testing the food preferences of *C. elegans* for bacteria found in their natural habitats (kindly provided by Marie-Anne Felix, Institute of Biology of Ecole Normale Supérieure, Paris, France). We have found that *C. elegans* prefers the odors of most species tested over *E. coli*. We have identified that the olfactory neuron AWC is involved in many preferences. In addition, we have preliminary data identifying the attractive odorants released by these bacteria using solid-phase microextraction-gas chromatography/mass spectrometry (SPME-GC/MS). While it has long been known that *C. elegans* recognizes volatiles known to be released by bacteria in general, little is known about what specific volatiles *C. elegans* uses to discriminate among different species of bacteria.


711B  **Food deprivation suppresses sleep via AMP kinase and circumvents the lethal effect of sleep loss in *C. elegans***.  Desiree Goetting, Rony Soto, Cheryl Van Buskirk  California State University, Northridge, CA.

A major challenge in understanding the function and evolution of sleep lies in identifying the mechanisms that offset the vulnerabilities that come with it, such as the inability to forage or escape. The nematode *C. elegans* has recently been identified as a useful model for the dissection of sleep function and evolution, as animals experience a primitive sleep-like state that is triggered by conditions that cause cellular damage. In response to noxious environmental conditions such as extreme heat, animals enter a state of behavioral quiescence characterized by a reduction in sensory responsiveness and a cessation of feeding and locomotion. This recovery sleep, or RS, appears to be beneficial under certain conditions, as sleepless mutant animals are impaired for survival following noxious heat exposure. We wished to investigate how the decision to enter into RS may be influenced by additional environmental inputs that could potentially alter the physiological benefit to be gained from sleep. Here we show that food deprivation suppresses RS, and that this effect is exacerbated as population density increases. In addition to suppressing sleep drive, food deprivation protects against the lethality associated with sleep loss, suggesting that food-deprived animals have a reduced need for sleep. We show that suppression of sleep drive during periods of food deprivation requires AMP kinase. Additionally, we show that competence to engage in RS is dependent on the neuroendocrine signal DAF-7/TGF-β, activating a previously identified neural circuit that shifts several aspects of development and metabolism from conservation to utilization of energetic resources. These data suggest that recovery sleep in *C. elegans* is an energetically costly activity that can be suppressed when environmental conditions are unfavorable and animals are required to compete for resources.

712C  **Distinct *C. elegans* Behavioral States in a Microfluidic Environment**.  D.L. Gonzales1,2, J.T. Robinson1,2,3,4  1) Applied Physics, Rice University, Houston, TX; 2) Electrical and Computer Engineering, Rice University, Houston, TX; 3) Bioengineering, Rice University, Houston, TX; 4) Neuroscience, Baylor College of Medicine, Houston, TX.

The nematode *C. elegans* provides a unique opportunity to investigate with great specificity the molecular, genetic and neuronal basis of behavior. The last decade has seen the discovery of various quiescent behaviors, such as developmental and stress-induced sleep, that have the prospect to help reveal the fundamental basis and need for sleep. Here, we show what appears to be a novel sleep-like state in microfluidic chambers. We discovered that approximately 90% of animals exhibit at least one abrupt transition from an active to a quiescent state. Although a similar quiescent behavior in liquid media has been previously reported (Ghosh & Emmons, 2008), the behavior we observed appears to be unique to the microfluidic environment and differs in several ways. For example, the quiescent bouts begin within 1 hr of transfer to liquid media, do not appear to occur on a periodic timescale and last for short periods of only a few minutes before the active state resumes. We initially hypothesized that the quiescent state was due to the heavy mechanical load imposed on animals during immobilization in small microfluidic channels. However, we have found that even when worms freely thrash in 500 µm-wide channels, approximately 70% still exhibit at least one quiescent bout lasting several minutes. This suggests that other characteristics of the microfluidic environment, such as the pressure within the microchannel, may play a role in inducing this behavior. In addition to determining the environmental cue, we hypothesized that the observed behavior is a novel form of stress-induced sleep. Therefore, we also tested for quiescence states in mutant worms that are known to display less stress-related sleep.

While the precise environmental stimuli and biological mechanisms underlying this sleep-like state are still unknown, this quiescence state is particularly advantageous for using genetically-encoded Ca²⁺ sensors to correlate behavior with neuronal activity. Currently, imaging neuronal activity in behaving worms requires some form of movement restriction or sophisticated equipment for tracking and imaging freely-moving animals. The quiescence observed here, in addition to being robust and easy
to detect, occurs in animals that are already confined, making whole-brain imaging more accessible during changes in the behavioral state. We therefore devised a platform to detect quiescence in real-time while simultaneously imaging neuronal Ca$^{2+}$ activity.

Future work will probe the precise environmental cue and genetic pathways involved in producing the quiescent state. This knowledge, when combined with the ability to measure the activity of many neurons during behavioral state transitions, makes this novel quiescent behavior a powerful tool for studying state transitions and sleep in worms.


713A Feedback from network states influence odor perception in C. elegans. A. Gordus Biology, Johns Hopkins University, Baltimore, MD.

Our response to the world is not simply the sum of what our sensory organs detect, but also how our brain chooses to represent these detections, and act upon them. However, knowledge of how sensory inputs are interpreted by our brains to yield behavioral outputs is lacking. Unlike simple reflexes, the majority of our behaviors are governed by an internal representation of the external world. This internal state is not static; but shows dynamic activity during both awake and sleep periods. In short, we are always thinking. Even in sensory deprived contexts, nearly all regions of our brain remain active. Therefore, the central challenge with predicting behavioral output from sensory input is that our perception of the world is dynamic and highly variable, rendering many behaviors unpredictable. Understanding this process will provide a foundation for understanding the learning process, and the principles that govern how behavioral novelty arises and adapts to the environment.

To address how behavioral novelty is generated, we tested and implemented numerous genetic tools to manipulate and record neurons involved in chemotaxis toward isoamyl alcohol, an odor sensed by the AWC sensory neuron. Olfactory neurons respond to odor stimuli with rapid and reliable changes in activity, but downstream AIB interneurons respond with a probabilistic delay. The interneuron response to odor depends on the collective activity of multiple neurons — AIB, RIM, and AVA — when the odor stimulus arrives. These three neurons participate in an ongoing synchronously fluctuating internal state that has a very probabilistic response to odor. However, certain activity states that are less synchronous respond to odor more reliably.

Artificially generating these activity states by modifying neuronal activity increases the reliability of odor responses in interneurons and the reliability of the behavioral response to odor. The integration of sensory information with network state may represent a general mechanism for generating variability in behavior.

714B Cell-specific protein degradation regulates stress-induced serotonergic neuromodulation. Xicotencatl Gracida1,2, Gareth Harris3, Yun Zhang2,3,5, John A. Calarco4,5 1) FAS Center for Systems Biology; 2) Department of Organismal and Evolutionary Biology; 3) Center for Brain Science, Harvard University, Cambridge, MA, 02138; 4) Department of Cell and Systems Biology, University of Toronto, Toronto, Canada, M5S 3G5; 5) Co-senior authors.

Neuromodulatory cells transduce environmental information into lasting modulatory effects on physiological and behavioral processes. However, our understanding of how specific neuronal cell types regulate these modulations is limited by the complexity of the nervous system. To overcome this challenge, we adapted the Translating Ribosome Affinity Purification (TRAP) approach in C. elegans to profile ribosome-associated mRNAs from the neuromodulatory dopaminergic and serotonergic cells. From these profiles, we find that elc-2, an ortholog of the mammalian Elongin C, is specifically expressed in the ADF serotonergic sensory neurons, a neuronal class involved in stress response.

We show that elc-2 plays a critical role in mediating a heat-induced long-lasting change in the feeding behavior. We demonstrate a role for ELC-2 and the von Hippel-Lindau VHL-1 protein, both of which are components of a conserved Elongin-Cullin-SOCS-box (ECS) E3 ligase involved in ubiquitin-mediated protein degradation, in modulating feeding behavior after experiencing the heat stress. Disrupting serotonin production in the ADF neurons, mutating a metabotropic serotonin receptor, or inhibiting neumodulation recapitulates behavioral defects found in the ECS mutants. At the molecular level, the heat stress induces a transient redistribution of ELC-2 within the nucleus. Because the ECS components are expressed in many mammalian tissues, including the brain, our results raise the intriguing possibility that these factors play a similar role in the mammalian nervous system. Together, our results characterize the subcellular and dynamic regulation of an E3 ubiquitin ligase in specific neuromodulatory neurons and identify a novel function for an ECS-mediated protein degradation in integrating stress signals to regulate serotonergic neuromodulation of lasting behavioral states.

715C A single set of interneurons drives opposite behaviors in C. elegans. M. Guillermor, M. Carrillo, E. Hallem Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, Los Angeles, CA 90095, USA.

An organism’s ability to tailor its behavioral choices to changing conditions is essential for survival. As a result, many chemosensory stimuli evoke innate behavioral responses that can be either appetitive or aversive depending in the context. However, the circuit mechanisms that enable these context-triggered valence changes are poorly understood. Here, we show that C. elegans can alternate between attractive and aversive responses to carbon dioxide (CO$_2$) depending on its recently experienced CO$_2$ environment, and both responses are mediated by a single, highly flexible microcircuit. The valence of the CO$_2$ stimulus is determined by the coordinated responses of a set of valence-encoding interneurons that change their CO$_2$-evoked activity as a result of context-dependent modulation to promote opposite behavioral states. A distinct interneuron pair regulates behavioral sensitivity to CO$_2$ independent of valence. Glutamatergic and neuropeptidergic signaling mediate both CO$_2$ avoidance and attraction, and a combinatorial code of neuropeptides acts on the circuit to regulate valence and sensitivity.
precisely controlled chemicals in microfluidics behavioral arenas. Preliminary data suggest that behavioral state probability
changes in wild-type worms exposed to hexanol are distinct from those of worms exposed to isoamyl alcohol. Specifically, unlike
similar to those driven via weighting of antagonistic pathways, we characterized behavioral state changes in response to
this receptor and potential modulation by additional peptide ligands.

Here we described the function of a luqin-like RF-amide neuropeptides, that are potent activators of NPR-22 activation in cell-
based GPCR activation assays. In hermaphrodites, the luqin-like precursor gene is expressed in a single pharyngeal
motorneuron, M1, but in males, expression can be seen in several male-specific neurons.

Due to expression of the luqin-like peptide gene in M1 and evidence of npr-22 expression in pharyngeal muscle we sought to
observe the pumping rate of animals overexpressing the Luqin-like precursor gene as well as mutant animals containing
deletions in either the peptide gene or npr-22. Overexpression resulted in a reduced pharyngeal pumping rate, whereas peptide null mutants showed a slight but significant increase in pumping rate. There was no significant change in the pumping rate of npr-22 mutants compared to N2. This suggests a potential inhibitory role for luqin-like peptides in regulation of pharyngeal pumping and feeding behaviour. Concurrent with changes in pharyngeal pumping rate, single worm tracking data revealed that peptide-overexpressing animals are statistically shorter in length, had a reduced tracking distance and lower foraging rates than wild type worms.

These data suggest a novel role for luqin-like peptide ligands in control of pharyngeal pumping and feeding behaviours, which potentially require the NPR-22 receptor. In addition, given the expression of these peptides in male-specific neurons, including some cells in the male tail, we are currently investigating if luqin-like peptides are involved in male mating behaviour in C. elegans.

Luqin-like peptide ligands of NPR-22 are involved in pharyngeal pumping and feeding behaviours in C. elegans. Iris Hardege1, Isabel Beets2,3, Sven Zels4, Yee Lian Chew5, Liliane Schoofs6, William Schafer 1) Neurobiology, MRC Laboratory of Molecular Biology, Cambridge, UK; 2) Cell Biology Division, MRC Laboratory of Molecular Biology, Cambridge, UK; 3) Functional Genomics and Proteomics Group, Division of Animal Physiology and Neurobiology, KU Leuven, Belgium.

The C. elegans NPR-22 receptor has been recently linked to a number of RFamide neuropeptide ligands involved in
behavioural modulation, including the regulation of quiescence via FLP-11 and intestinal serotonin induced fat loss via FLP-7. However, the broader expression pattern of NPR-22, including expression in pharyngeal muscles, suggests a complex role for
this receptor and potential modulation by additional peptide ligands.

Modulation of antagonistic sensory pathways drives plasticity in olfactory behavior. A.H. Hartmann1, N.D. Dwyer2, P. Chao3, C.I. Bargmann4, P. Sengupta 1) Biology, Brandeis University, Waltham, MA; 2) University of Virginia, School of Medicine, Charlottesville, VA; 3) Howard Hughes Medical Institute, The Rockefeller University, New York, NY.

Animals must integrate sensory inputs with contextual information, such as internal state or external cues, to execute adaptive
behavioral responses. An important goal of neuroscience is to determine how this information is encoded within the nervous
system. Behavioral plasticity can stem from genetic, molecular, and circuit level changes in neural function, allowing the
organism to thrive in a dynamic environment. It is well-established that the response of C. elegans to olfactory stimuli can be
influenced by the environment. I aim to identify the genes, neurons and circuits that alter response valence to olfactory cues,
thereby describing how behavioral plasticity is encoded within the neural ensemble.

I have found that antagonistic sensory pathways converge to mediate the behavioral response of C. elegans to the volatile
odorant hexanol. I show that the AWC sensory neurons are required for worms to exhibit attraction to hexanol, while the
ASH/ADL nociceptive neurons appear to facilitate behavioral aversion to this chemical. Consistent with these observations,
AWC and ASH show calcium responses to removal and addition of hexanol, respectively. Under standard conditions, the AWC-
mediated attraction pathway predominates resulting in attraction behaviors. However, environmental conditions such as the
presence of saturating concentrations of isoamyl alcohol sensed by AWC, or genetic manipulations that alter AWC sensory
properties reveal the underlying ASH/ADL-dependent aversive pathway. Consequently, differential weighting of these
antagonistic pathways as a function of context can drive flexibility in the animal’s response to hexanol.

To determine whether behavioral strategies underlying attraction behaviors that are driven exclusively by a single pathway are
similar to those driven via weighting of antagonistic pathways, we characterized behavioral state changes in response to
precisely controlled chemicals in microfluidics behavioral arenas. Preliminary data suggest that behavioral state probability
changes in wild-type worms exposed to hexanol are distinct from those of worms exposed to isoamyl alcohol. Specifically, unlike
attraction to isoamyl alcohol, attraction to hexanol does not appear to be mediated via klinokinesis, suggesting that modulation of
as yet undefined states, or an alternative behavioral strategy, e.g. klinotaxis, may underlie hexanol attraction. These
observations support the hypothesis that the ability to drive similar endpoint behaviors via multiple neuronal and circuit
strategies allow animals to rapidly and flexibly alter their responses as a function of context and experience.

Analyses of the molecular mechanism regulating developmental change in odor preference of C. elegans. T. Hino1, M. Fujiwara2, T. Ishihara2 1) Graduate School of Systems Life Sciences, Kyushu University, Fukuoka, JP; 2) Faculty of Sciences, Department of Biology, Kyushu University, Fukuoka, JP.

Many animal species change their behavioral patterns according to developmental stages. The behavioral changes must be
important to appropriately behave at the each developmental stage. However, the molecular mechanisms which give rise to the
behavioral changes depending on the development are unclear.
We have shown that *C. elegans* larvae show a different odor preference from adults. While larval animals exhibit a weak chemotactic response to the food-related odorant, diacetyl, adult animals exhibit a strong response.

To analyze the mechanism that regulates the developmental changes in the odor preferences, first, we examined which olfactory neuron contributes to the change in odor preferences. In *C. elegans*, two classes of olfactory neurons, AWA and AWC, act to sense attractive odorants. Both the AWA-defective and the AWC-defective mutants showed reduced chemotaxis throughout the development. These mutants, however, still exhibited chemotactic enhancement to diacetyl at the adult stage, suggesting that both olfactory neurons and/or their downstream circuits are involved in the developmental change.

Second, to reveal the molecular mechanism, we screened for mutants, whose larvae show a strong attractive response to diacetyl, and isolated one mutant candidate. At the larval stage, this mutant exhibited a higher chemotactic response to diacetyl compared to wild-type larvae, while at the adult stage this mutant exhibited the similar response as wild-type adults. To identify the gene responsible for this phenotype, we mapped the mutation on LGIII. Whole genome sequencing revealed ~10 sequence variants predicted to affect protein-coding genes on LGIII. We are now trying to further narrow down the mapped region to identify the responsible mutation among them.

Furthermore, we found that cGMP-dependent protein kinase, EGL-4, is possibly involved in the developmental change of the odor preference. The egl-4 mutants at the adult stage exhibit low chemotaxis to diacetyl, compared to wild-type adults. On the other hand, egl-4 mutants at the larval stage exhibit comparable chemotaxis to diacetyl to wild-type larvae. Thus, EGL-4 may be required for chemotactic enhancement to diacetyl to occur at adult stage.

By analyzing the gene identified in our screening and egl-4, we hope to uncover the molecular mechanism which gives rise to the behavioral changes during development.


How animals move through their natural habitats is fundamental to understanding their biology. We possess considerable knowledge of *Caenorhabditis elegans* locomotion and movement behaviour from extensive investigations of these animals living on the 2D surface of an agar plate. However, *C. elegans* in the wild live in the complex-structured 3D environment of decaying vegetation, and we have very limited information about how *C. elegans* behaves in their natural habitats. This raises concerns that *C. elegans* locomotion data collected in 2D may be incomplete and unrepresentative, severely limiting our models and predictions about their neural control, biomechanics and movement.

One reason for the gap in our knowledge is the difficulty in recording accurate 3D movements of animals at the scale of *C. elegans* (~1 mm) with high spatial and temporal resolution. At the macro scale, conventional 3D imaging relies on multiple camera views with overlapping focus regions, but lenses do not have sufficient magnification to image at the *C. elegans* scale. Conventional microscopes have a very narrow depth of focus so cannot be used in a multiple camera system. Instead, they have to employ Z-stacking to image static bodies in 3D, making them unsuitable for imaging a freely moving body in real-time. To overcome these obstacles, we built a 3D imaging system using three telecentric lenses each with a 7-21 mm depth of focus (depending on the magnification) attached to three 4.2 megapixel cameras. This system allows us to image the worm moving freely through up to 9.2 cm$^3$. This volume is calibrated using photogrammetry to solve for the lens distortion and camera geometry. A novel image analysis algorithm produces accurate reconstructions of the 3D posture, in the form of a smooth midline, and location of the worm within the volume.

We recorded individual worms moving freely through a range of viscoelastic fluids corresponding to different concentrations of gelatine in M9 buffer solution. All recordings were between 25 and 45 Hz. Across a wide range of viscoelasticities, we found that the body postures of the worm are more commonly 3D than 2D. Specifically, we report novel 3D body postures exhibited by *C. elegans*, including a helicoid locomotion gait and a “lasso” turning posture. Furthermore, the trajectories of the worms are more commonly 3D than 2D. These kinematic and performance data inform a new integrated and 3D neuromechanical locomotion model.

720B  **Morphological and Functional Characterization of Age-Dependent Changes in *A. elegans* Thermosensory Neuron**.  Tzu-Ting Huang$^1$, Yuki Tsukada$^2$, Takashi Tamada$^2$, Ikue Mori$^2$, Chun-Liang Pan$^1$  1) Institute of Molecular Medicine, National Taiwan University, Taipei, Taiwan; 2) Group of Molecular Neurobiology, Division of Biological Science, Graduate School of Science, Nagoya University, Nagoya, Japan.

Widespread age-dependent changes could be found at multiple levels of neural architecture in *C. elegans*, including the neuronal soma, axon, dendrite or synapses. Whether age-related changes occur to *C. elegans* ciliated endings, microtubule-based signaling compartments in the sensory neurons, has not been characterized. Here we report age-dependent deterioration of sensory ending architecture in the AFD thermosensory neuron, including reduced microvilli and engorged cilia. Age-dependent changes of the AFD sensory ending were modified by *daf-16* or *daf-2* mutations in a way that was consistent with alteration of life span by these mutations. This suggests that insulin signaling, one of the most conserved regulatory mechanisms of life span, modulates age-dependent decline of ciliated endings. Age-dependent changes could also be found in ciliated endings of multiple amphid neuronal classes, indicating a global deterioration of primary sensory neurons during aging. To understand whether functions of the AFD neurons deteriorate during aging, we performed calcium imaging and thermotaxis assays. We found that compared to young neurons, aged AFD neurons showed precocious activity at temperature lower than the cultivation temperature ($T_c$), and they continued to respond at temperature higher than the $T_c$. Interestingly, mid-age wild-type animals were cryophilic in thermotaxis assay, consistent with our observations that high AFD activity induced turning behaviors. Our work provides a detailed account of the morphology, function and related behaviors of a *C. elegans*.
thermosensory neuron during aging and lays the foundation for future exploration of molecular underpinnings of such age-dependent changes in the sensory system. (supported by National Health Research Institutes, NHRI-EX106-10529NI and the Ministry of Science and Technology, MOST 104-2320-B-002-058-MY3)

721C Identification and analysis of multifunctional neurons in a C. elegans thermotaxis behavior. M. Ikeda, S. Nakano, A. Giles, I. Mori Neuroscience Institute and Group of Molecular Neurobiology, Graduate School of Science, Nagoya University, Nagoya.

Neurons in C. elegans are multifunctional and can encode multiple behavioral outputs. However, how a set of neurons in a circuitry regulate multiple outputs still remains poorly understood. To challenge this problem, targeting the whole functional neural circuits from controllable sensory input to behavioral outputs would be an essential approach, in which worms’ behaviors that are resulted as circuit outputs can be subdivided into appropriate and analyticable behavioral elements.

Here, we combined high-throughput behavior analysis and comprehensive genetic cell ablations to identify a set of neurons that regulates multiple outputs during thermotaxis behavior: the migration of worms toward their past cultivation temperature on a thermal gradient. By using the Multi-Worm Tracker with a custom-built program, we defined and automatically detected subdivided behavioral elements, such as “omega turns,” “reversal turns” and “curves.” We successfully identified several elements that were regulated during the thermotaxis. We then performed computer simulations and confirmed that the behavioral elements we identified were sufficient to reproduce the actual thermotaxis behavior. The simulations also demonstrated that “curves” and “reversal turns” drove worms to the cultivation temperature most powerfully among any other behavioral elements. To identify the neurons regulating the “curve” and the “reversal turn” behaviors, respectively, we genetically ablated individual neurons including thermosensory neurons such as AFD, AWC and ASI, and a series of interneurons, which are predicted to mediate information between these thermosensory neurons and motor neurons, which had been previously shown to regulate steering behaviors or backward locomotion. Our analyses showed that AFD, AIB and AIZ regulate both “curves” and “reversal turns”, and that each of these two behavioral elements recruits distinct sets of additional neurons for their regulation. Interestingly, different sets of neurons were employed to regulate even the same behavioral element, “curve,” depending on whether worms were located in above or below the cultivation temperature on the thermal gradient.

Currently, we are constructing mathematical models of the neural circuits, and searching the parameters of the models, which reproduce experimental data. We so far obtained the several optimized parameter sets, which are probably grasping the properties of actual neural circuits. To confirm the validity of the models, monitoring the actual neuronal activities of freely moving worms is underway. Quantitative analysis of the obtained models will shed light on the mechanisms how neurons and neural circuits generate multiple outputs.

722A A low cost bio-imaging system incorporating machine learning algorithms for automatic analysis of animal behavior. Adam Iliff, Apurva Virkud, Shawn Xu Life Sciences Institute, University of Michigan, Ann Arbor, MI.

The overall goal of this project is to develop an imaging system with machine learning capabilities to aid in the study of how genes and neural circuits give rise to animal behavior. Our secondary mission was to create a complete imaging system that was low enough in cost for labs to use many devices in parallel, or for high school and college classrooms to be able to conduct imaging-based biological experiments. Imaging equipment has increased in quality and decreased in cost to a point in which we were able to build an ultra-low-cost imaging system for recording animal behavior which could accomplish our objectives. Specifically, the system is optimized for recording locomotion of the genetic model organism C. elegans on a near-flat translucent surface. We utilized the free programming language Python with machine learning packages to incorporate automatic analysis of the recorded videos. Several machine learning algorithms for classifying and annotating animal behavior were tested against the performance of human experts, and the top performing algorithms are implemented in the final software. This system has the potential to save researchers time and money and allow them to quickly determine how manipulating genes and neural circuits alters animal behavior. Future plans include adapting the system for other organisms and more complex behaviors.

723B Mechanosensory experience determines nociceptive and proprioceptive behavioral plasticity. Sharon Inberg, Yael Iosilevskii, Benjamin Podbielwicz Department of Biology, Technion- Israel Institute of Technology, Haifa, IL.

We are interested in how animals adjust their innate behavioral outputs in response to sensory experience. C. elegans has the ability to undergo molecular, cellular and behavioral plasticity. Our sensory experience paradigm involves comparing isolated worms, to those that were grown in crowded conditions. We found that animals grown in isolation exhibit a change in the arborized dendritic structure of the PVD nociceptor neuron. To test behavioral consequences of mechanosensory isolation we used two assays to measure the response to high threshold mechanosensory stimulation and proprioception. We found that isolation induced a decrease in response to harsh touch that was gentle touch-independent, since mec-4 touch-insensitive worms show the same isolation-induced reduction in response to harsh touch. The reduction in the responsiveness was also demonstrated by optogenetic stimulation of the PVD, where isolated worms exhibit reduced responsiveness. These findings suggest that the presynaptic activities of the PVD are responsible for the experience induced behavioral plasticity. To reveal the mechanisms mediating these behavioral changes we used mutants for a family of voltage independent sodium channels (Degenerins). We tested mutations on different degenerins for isolation-induced behavioral plasticity and three were found to have reproducible effects: asc-1, mec-10, and degt-1. Most of the combinations of these three genes failed to induce the behavioral plasticity for the response to harsh touch following isolation, indicating that degenerins are important for the experience induced plasticity in response to harsh touch. In parallel to the nociceptive changes following isolation, a change in
the proprioception of the worms was observed. Specifically, isolation induced an increase in the wavelength and the amplitude of crawling worms, without any effect on the velocity or the total tracking length covered by the worms. As controls, we isolated worms with glass beads in their plates and we observed partial rescue of the proprioceptive phenotype, indicating that the isolation induced change is mediated by mechanosensory signals. Taken together, we propose that nociceptive and proprioceptive innate behavioral repertoires that are mediated by the PVD are plastic and dependent on the amount of mechanosensory experience and a combination of the activities of three degenerins.


Perlara PBC, is a public benefit company committed to discovering small molecule therapeutics for rare genetic diseases. Our approach consists of the use of simple, whole animal models such as yeast, worm, flies in drug discovery screens. Molecules that pass successfully through our screens are validated in patient cells before advancing into higher animal models of the disease. Niemann Pick Type C (NPC) is a devastating lysosomal storage disease with high mortality rates. The nematode gene, ncr-1, was engineered to generate a nematode model lacking functional ncr-1 protein. The resulting animal showed slowed development, small brood size and sensitivity to cholesterol deprivation. We capitalized on the growth defect observed in the absence of cholesterol to conduct a discovery screen. 50000 molecules were screened at an initial concentration of 25μM to identify candidates that rescued the growth defect. "Hits" resulting from this screen were validated in a secondary assay on worms as well as on patient-derived cells. Perl101 was identified as a lead candidate for a class of molecules that rescued the NPC phenotype in worms and changed the cellular fingerprint of NPC cells. RNA sequencing of nematodes exposed to incremental concentration of Perl101 also provided insight into its mechanism of action. Furthermore, as an unoptimized screening hit, Perl101 demonstrated high oral bioavailability, blood brain barrier permeability and minimal adverse effects in a 90 day mouse tolerability study. Additional studies to probe the mechanism of action of Perl101 and its efficacy in a mouse model of NPC are underway.

In summary, utilization of simple animal models such as nematodes, flies and yeast present an untapped opportunity to discover therapeutic candidates with greater translational potential for rare genetic diseases. A parallel, multi-model whole animal screening approach leverages shared evolutionary linkages and increases the probability of identifying potential therapeutics. This approach can lead to the discovery of treatments for a class of diseases with high unmet needs.

725A Integrated neuromechanical model demonstrates stretch-reception can generate and propagate wave responsible for forward locomotion on agar. Eduardo J. Izquierdo1,2, Randall D. Beer1,2 1) Cognitive Science Program, Indianaan University, Bloomington, IN; 2) School of Informatics and Computing, Indiana University, Bloomington, IN.

Behavior is grounded in the interaction between an organism’s brain, its body, and its environment. With only 302 neurons and a fully-reconstructed neural and muscle anatomy at the cellular level, C. elegans is an ideal candidate organism to study behavior with the help of computational models. Of particular interest is understanding the neuromechanical basis of locomotion, since nearly its entire behavioral repertoire is expressed through movement. How the rhythmic pattern is generated and propagated along the body is not yet well understood. We report on the development and analysis of a model of forward locomotion that integrates known neuroanatomy, neurophysiology and body mechanics of the worm. Our model is the first to consider recent experimental analysis of the structure of the ventral cord circuitry and the effect of local body curvature on nearby motoneurons. We develop a neuroanatomically-grounded neural model of the ventral nerve cord subcircuit, using a neural model capable of reproducing the full range of electrophysiological properties observed in C. elegans neurons. We integrated the neural model with a reconstruction of a biomechanical model of the worm’s body from published descriptions, with updated musculature and stretch receptors. Unknown parameters were evolved using a genetic algorithm to match the speed of the worm on agar. We performed 100 evolutionary runs and consistently found electrophysiological configurations that reproduced realistic control of forward movement. The ensemble of successful solutions reproduced key experimental observations that they were not designed to fit, including the curvature profile of the body’s movement, and the wavelength and frequency of the propagating wave. Analysis of the ensemble revealed forward locomotion is possible without intrinsic oscillations in either the head or the rest of the ventral nerve cord. Circuits were capable of initiating oscillations in the head using only stretch reception, providing a novel hypothesis. Similarly, circuits relied on stretch reception to propagate the dorsoventral oscillation, without the need for bistability in the motoneurons, as had been previously proposed, and with gap junctions across neural units playing only a minor role. Altogether, we provide an existence proof for forward locomotion through stretch-reception in an up-to-date neuromechanical model of the worm, as well as a series of testable hypotheses about its operation.

726B Genetic basis of vulnerability to polysubstance abuse and modulation of motivational state. L. René Garcia1, Changhoon Jee2 1) Dept. of Pharmacology, University of Tennessee Health Science Center, Memphis, TN; 2) Dept. of Biology, Texas A&M University, college station, TX.

Pathological neuroadaptation is a hallmark of drug dependence and addiction. Substance abuse causes numerous changes in brain chemistry that lead to drug addiction. The development of drug dependence depends on the progression of chemical tolerance, possibly contributing to increased chemical consumption. Consequently, withdrawal or abstinence from chemical exposure can induce motivated compulsion and promote vulnerability to relapse in drug-dependent animals.

To understand how drug dependence is developed and also to identify fundamental mechanisms of modulation of motivation, we hypothesized genetic variants that impact the neuromodulation of behavioral arousal such as motivational drive will
predispose the animal to substance dependence. It has been demonstrated by a C. elegans animal model of pathological neuroadaptation of brain stress system due to persistent activation of it that is known to control hypothalmo-pituitary axes. A seb-3, CRF receptor-like GPCR, was isolated as a key mediator in stress response and pivotal role in the development of drug dependence. For example, the seb-3 (gf) allele-animals display increased behavioral arousal, tolerance to ethanol and nicotine, and represent sexual dimorphic compulsive reproductive behaviors. Thus we screened additional genetic variations that altered behavioral arousal, possibly predisposed animals to substance dependence. Consequently, 22 additional genetic variants that displayed enhanced arousal were isolated. To further understand how polysubstance abuse differs from, or is similar to, those observed in single substance abuse, those genetic variants are being sorted via drug response such as nicotine and ethanol. In addition, we are also pursuing analyzing genetic variants that exhibited drug tolerance whether they represented potentiated sexual compulsion to further comprehend the basic mechanism of how motivational states are modulated.

727C  Caenorhabditis elegans exhibits an increase in the probability of pirouettes and head swing amplitude in the absence of the SAA class of neurons. E. Kalogeropoulou 1, I.A. Hope 1, N. Cohen 2 1) School of Computing, University of Leeds, Leeds LS2 9JT, UK; 2) School of Biology, University of Leeds, Leeds LS2 9JT, UK.

Many of the behaviours of C. elegans are mediated by the worm’s ability to orient itself towards chemical gradients or other sensory cues. Two mechanisms of orientation have been observed in the worm: pirouettes and steering. Past studies have identified a number of neuron classes that contribute to navigation by linking neuronal laser ablations and observations of mutants to changes in body postures and locomotion statistics 1,2. Recently, optogenetic manipulation has also shed light on the neuronal control of the worm’s chemotactic behaviour 3.

SMB motor neurons have been postulated to be part of the navigation circuit. We have generated a chromosomally-integrated transgenic line (UL4230) where the SMBs are genetically ablated in early larvae. The line demonstrates a loopy phenotype similar to that previously observed following laser-ablation of SMBs 1. We have also targeted another class of neuron, located close to and highly connected via synapses to the SMBs but not previously explored: the SAA interneurons.

We initially used expression of GFP to confirm that the selected promoters drove expression in the location originally described. A combination of lad-2 and unc-42 promoters was used to genetically ablate the SAAs by targeted expression of the worm’s caspase, as encoded by two distinct parts of the ced-3 gene, such that the intact enzyme is produced only in these neurons specifically 4. Absence of GFP expression confirmed the specific and targeted ablation of the SAAs.

The generated strains, with SMBs or SAAs ablated (UL4230 and UL4207), demonstrated a phenotype different to that of N2 in both spontaneous and evoked locomotion. Locomotion was evoked using a radial gradient of NH₄Cl. Initial results suggest that both SMBs and SAAs suppress the probability of pirouettes and decrease the amplitude of sinusoidal undulations. Additional experiments are underway to explore the contributions of SAA and SMB, separately and together, to undulations and steering, and to link those to the pirouette initiation pathway.


728A  Photo avoidance reflex influenced by C. elegans’ own motional state. Laura Rabichow, Arash Kardoust, Meera McAdam, Reule Yardley, Chaiti Bhagawat, Nihal Punjabi, Diane Lee, Joslyn Santana, Evan Ross, Tiffany Nguyen, Anika Mahavi, Kushal Mohnot, Daniel Fu, Javier Carmona, Steve Mendoza, Katsushi Arisaka, Elegant Mind Club UCLA, Los Angeles, CA.

Self-awareness is a critical aspect related to the effective navigation of a complex environment. For a given input stimulus to a sensory structure, behavioral output seems closely related to an organism’s own motion. An example may be drawn from a relativistic pressure applied to a C. elegans’ mechanosensory structure in time, based on two conditions. When considering a mechanical pressure driven by the worm’s own motion (as if a worm struck a stationary barrier) the response is dramatically different to one of similar magnitude unrelated to the worm’s motion (if a stationary worm was lightly touched with an eyebrow hair). Interestingly, these conditions apply the same force to a given sensory structure in time, but result in a much higher probability of avoidance response if the worm is stationary at the time of the input. To more closely evaluate this complex phenomenon, the well characterized, highly deterministic photo-avoidance behavior was implemented in a number of experimental wave speed conditions. We postulate that a lower avoidance response probability will be found in quickly moving C. elegans as compared to those with slow wave speeds. Gelatin concentration was altered from 1 - 4 % in order to shift the wave speed accordingly, while phototaxis stimulation acted as our input force; provided from a well-calibrated 405nm laser with a custom-built line confocal microscope supplied a triggering signal to the laser stimulus while recording worm position and orientation over experimental trials. Offline, a MatLab program was used to analyze the data and sinusoidally fit an analytic function to the worm’s body in time. Wave speed and center of mass velocity were extracted from this data, and avoidance probability was investigated as an independent variable. Preliminarily, observations suggest that lower gelatin concentrations...
Role of two-pore domain K⁺ channels in the regulation of movement in C. elegans. M. Kasap¹, E. Aamodt², D. Dwyer¹,³ ¹) Pharmacology, Toxicology and Neuroscience, LSUHSC Shreveport, Shreveport, LA; 2) Biochemistry and Molecular Biology, LSUHSC Shreveport, Shreveport, LA; 3) Psychiatry, LSUHSC Shreveport, Shreveport, LA.

 unc-58 encodes one of the 46 two-pore domain potassium channels (K2Ps) in C. elegans. Close human genetic counterparts of unc-58 are linked to Birk-Barel syndrome and migraine (Barel et al., Am J Hum Genet. 83(2):193-199, 2008; Lafrenière et al., Nat Med. 16(10):1157-1160, 2010). In C. elegans, a gain-of-function (gf) mutation in unc-58(e665) causes the worms to adopt a rigid posture with constant shaking. Our first aim was to investigate poorly studied unc-58(e665) phenotypes. Second, we wished to determine if pharmacological screening would reveal drugs that corrected these phenotypes. We classified the defects of the gf mutation in unc-58(e665) into four categories: development and aging, locomotion, feeding and egg-laying. We covered seeded plates with drugs, allowed them to dry, added worms and made periodic observations of them. We were able to identify drugs that can correct these deficits. Clozapine, the most effective antipsychotic drug used to treat schizophrenia, corrected the locomotion, egg-laying and feeding defects. Structurally similar antipsychotic risperidone also helped to alleviate the defects of unc-58(e665) gf animals. Lithium, the front-line drug used for bipolar disorder, corrected the egg-laying and developmental defects. Both clozapine and lithium activate the SGK signaling pathway in C. elegans (Weeks et al., J Neurosci Res 89:1658, 2011). However, it did not appear they were acting through SGK signaling. The time-course of the effects of clozapine and lithium suggested phosphorylation might be involved. Therefore, we tested whether Akt signaling mediated the drug actions. Among 10 antipsychotics that activate Akt signaling, only haloperidol corrected the locomotion defect. Loratadine, reported as a K2P blocker, corrected the locomotion defect rapidly, indicating that it directly blocked the channel. However, loratadine was the only K⁺ blocker that corrected the locomotion defect so far. Moreover, it has been shown that PKA down-regulates activity/expression of K2Ps (Cain et al., Mol Cell Neurosci. 37(1):32-39, 2007). Since our data also indicated phosphorylation, we hypothesized that regulating PKA activity can correct the gf in unc-58(e665). We were able to show that increasing cAMP levels rescued the defective unc-58(e665) locomotion. We want to investigate further these pathways that may provide new ideas about how to develop drugs aimed at K2Ps for the treatment of tremor, dystonia and other disorders.

The GCY-29 receptor guanylyl cyclase shapes thermosensory signaling in C. elegans. M. Khan, V. Hapiak, A. Takeiishi, P. Sengupta Brandeis University, Waltham, MA.

The ability to sense and respond to external cues in the context of past experiences and current conditions is crucial for animals to survive and maintain functional homeostasis. Temperature represents a critical environmental variable that regulates nearly all physiological processes. Consequently, animals have evolved mechanisms to detect and respond to small changes in both external and internal temperature. Unlike many organisms that prefer a constant environmental temperature, the preferred temperature of C. elegans is governed by a ‘memory’ of its cultivation temperature (Tc). This temperature preference is plastic, and resets in an experience-dependent manner. These behaviors are mediated primarily by the AFD sensory neurons, which respond to temperature variations above a Tc-determined threshold. Thermotransduction in AFD is mediated by a subset of three receptor guanylyl cyclases (rGCs). We have recently shown that the AFD-expressed rGCs GCY-8, -18, and -23 are necessary and sufficient for thermosensation (Takeiishi, Yu, et al., 2016). In addition to these rGCs, the AFD neurons also express the GCY-29 rGC. Similar to the thermosensory rGCs, GCY-29 is also localized to the AFD sensory endings. The goal of this work is to explore the role of GCY-29 in shaping AFD-mediated thermosensory signaling. Using calcium imaging and thermotaxis navigation behavioral analyses, we have found that GCY-29 contributes to the detection and tracking of temperature stimuli, as well as shaping the Tc-determined response threshold of AFD. Our findings suggest that GCY-29 may modulate the functions of the thermosensory rGCs to more precisely coordinate AFD response ranges and shape their thermosensory response profiles.

The Role of Post-Translational Modifications in the Regulation of Serotonin Signaling. Andrew Olson, Santosh Kumar, Michael Koelle Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, United States.

C. elegans uses serotonin as a neurotransmitter to slow locomotion, and we have used this model system to discover that post-translational modifications appear to regulate serotonin signaling. Through large-scale genetic screens for mutants that fail to paralyze in response to exogenous serotonin, we found that C. elegans mutants for either of two subunits of the ELPC Elongator Protein Complex are defective for serotonin signaling. Conversely, transgenic animals overexpressing ELPC are hypersensitive to the effects of exogenous serotonin. ELPC is conserved from C. elegans to humans and functions as a cytoplasmic lysine acetylase to reversibly modify other proteins. This is the first time that ELPC or lysine acetylation has been implicated in regulating serotonin signaling. We used two-dimensional gel electrophoresis to show that in C. elegans lysates, Gα₉, a neural G protein encoded by the goa-1 gene through which serotonin signals to slow locomotion, exists as a complex series of species of differing charge. Acetylation eliminates the positive charge on a lysine side chain, and differential acetylation on several Lys could produce the complex pattern of Gα₉ species we see. Our preliminary results suggest that the series of differently charged Gα₉ species in wild-type lysates shifts to a less complex and more positively charged set of Gα₉ species in lysates of ELPC mutants, as would be predicted if Gα₉ is acetylated by ELPC. We isolated Gα₉ from both mouse brain and C. elegans lysates and analyzed the purified proteins for post-translational modifications using mass spectrometry. In both species, Gα₉ is acetylated on several conserved Lys residues near the N-terminus. We note that the signaling defects in ELPC mutants are much more restricted than those of Gα₉ null mutants. Both ELPC and Gα₉ mutants are defective for response to exogenous serotonin, but Gα₉ null mutants have additional defects not seen in ELPC mutants, including defects in response to exogenous dopamine as well as in many behavioral assays. Thus we hypothesize that ELPC may reversibly acetylate Gα₉ to specifically
regulate its ability to be activated by serotonin receptors, while not strongly affecting its ability to mediate signaling by other receptors. We are using CRISPR-Cas9 technology to mutate the acetylated Lys residues of Gaọ to the similar but non-acetylatable amino acid arginine to determine if acetylation at specific positions is responsible for regulating serotonin signaling.

732B TAX-4, Cyclic nucleotide-gated channel α subunit, regulates nictation. B. Lee1, D. Lee2, N. Kim3, J. Lee3 1) Department of Biophysics and Chemical Biology, Seoul National University, Seoul, Korea; 2) Department of Molecular Bioscience, Northwestern University, Evanston, Illinois, USA; 3) School of Biological Science, Seoul National University, Seoul, Korea.

One of the dispersal strategies of C. elegans dauers to escape harsh conditions is nictation, in which dauers wave their heads in order to attach to carriers like fruit flies and isopods to move to other environments. Our previous study showed that TGF-β signaling regulates nictation behavior. DAF-7, TGF-β homologue of C. elegans, is released from ASI to bind DAF-1 and DAF-4 on RIM/RIC, allowing dauers to nictate. In this study, we found another regulator of nictation, tax-4, through forward genetics. TAX-4 is a cyclic nucleotide-gated (CNG) channel α subunit that can make homotetramer or heterotetramer with β subunit. While tax-4(ok3125) dauers showed very low nictation ratio, tax-4(ok3125);tax-4(snu28, snu29, ks11 or p678) showed high nictation ratio. We also found that tax-2, a CNG channel β subunit, is also involved in nictation regulation. tax-4(ok3125);tax-2(p671 or ks31) showed high nictation ratio. Next, we tested daf-11, a guanylyl cyclase that acts upstream of CNG channels in cyclic GMP signaling pathway. daf-11(m47) dauers showed high nictation ratio and suppressed low nictation ratio of tax-4(ok3125). These data suggest that cyclic GMP signaling regulates nictation behavior through suppression of TGF-β signaling or TGF-β independent pathway. Cell type specific rescue experiments showed that tax-4 is not necessary in ASI, ASJ or AWC, suggesting that tax-4 in other cells are required for regulation of nictation.


Although some mechanisms of molecular and circuit plasticity that underlie learning and memory have been identified, much about the neural circuits involved in memory formation and recall remains unknown. For example, the temporal dynamics of various learning and memory processes are still under active investigation.

C. elegans associates paired stimuli such as odors, temperature and food, and can execute learned preferences through locomotory action. Because the timing of presentation and removal of such stimuli cannot be controlled precisely, temporal dynamics underlying the learning are obscured. Furthermore, the complete neural circuits involved in the learning often span many neurons, making it difficult to pinpoint the exact sites of plasticity when learning occurs.

Previous studies in our laboratory described neural circuits in the pharynx that inhibit pharyngeal pumping and initiate spitting in response to noxious stimuli such as short-wavelength light or hydrogen peroxide. The temporal precision of light administration and the short latency of the pharyngeal response makes light-induced pumping inhibition a good system with which to study learning. Furthermore, if the learning occurs within the pharyngeal nervous system, the relatively simple circuitry that is distinct from the rest of the somatic system should allow easier investigation into the learning mechanisms.

We have demonstrated classical conditioning of C. elegans by pairing light (as the unconditioned stimulus) with a neutral odor, Isoamyl alcohol (IAA) (as the conditioning stimulus), and scoring inhibition of pharyngeal pumps as a quantitative readout for learning. Worms learned to inhibit pumping when exposed to IAA after they were trained to associate the IAA stimulus with noxious light. The simultaneous presence of both IAA and light was necessary, as training with either stimulus alone did not produce learning. The learning was also dependent upon the relative timing of stimulus presentation: worms failed to learn when the light preceded the odor.

Many neurons can respond endogenously to light, potentially complicating our efforts to specify the circuit components involved in our learning paradigm. Thus, we are also seeking individual cell types that when optogenetically activated will inhibit pumping and might be used as an alternative to our current unconditioned light response. To this end, we have demonstrated that channelrhodopsin-based activation of the ASH neurons is sufficient to cause pumping inhibition.

We expect that the temporal precision of the training stimuli and simplicity of the pharyngeal circuit that underlies this learning paradigm will greatly facilitate analyses to uncover the circuitry underlying learning and memory formation at the level of single cells and small networks.


Central pattern generators, or CPGs, are intrinsic neural rhythms generated in the absence of sensory information. The CPG underlies several diverse and essential behaviors, functionally streamlined and localized for their non-divorceable utility. Traditionally, the CPG has been understood unjustly in the confines of rhythmicity and motor processes. Beginning and framing the C. elegans as a foundational model for the incredible versatility of the basic CPG, we delve into the CPGs unconventional essentiality in sensorimotor integration, substantiation of the physical principles of causality and locality, and its novelty in the context of understanding more complex behaviors, such as human saccadic eye movement. We then discuss the global trends and emergent properties of such rhythms across several organisms that characterize the manifestation of a meaningful patterned neural activity, premised again, in the lens of the highly elucidative neural system of the C. elegans. Penultimately, we demarcate the morphological evolution of the neural information code and neuromodulatory vehicles that biological pressures have enforced on the increasingly complex design of organisms, stalling the C. elegans, once again, as the basis and transitional prism for the evolution of this information code from an amplitude based phenomena (graded potential code) to a frequency based phenomena (action potential code). Ultimately, the thematic motif of this article demonstrates the immense utility in understanding the overlooked 302-neuron nervous system of the C. elegans towards unveiling powerful truths in the
evolutionary ladder and trending homologies in more complex organisms- of particular interest here, pattern generated behaviors. Our analyses incept and extends from a compilation of personal lab discoveries and extrinsic literary interpretations of the idiosyncrasies of the C. elegans nervous system, and has been protracted homologies across various Animalia and processes, including the aforementioned saccades in human oculomotor codes.

735B Observing the pattern of C. elegans egg-laying behaviors via 2D and 3D environment.  Tong Young Lee, Kyoung-hye Yoon, Jin II Lee Division of Biological Science and Technology, Yonsei University, Wonju, Gangwon-do, KR.

The nematode C. elegans is one of the premier experimental model organisms today. In the laboratory, they display characteristic development, fertility, and behaviors in a 2D habitat. In nature, however, C. elegans is found in three dimensional environments such as rotting fruit. To investigate the biology of C. elegans in a 3D controlled environment, we designed a nematode cultivation habitat which we term the nematode growth tube or NGT-3D. Growth, brood size, and lifespan in NGT-3D are comparable with 2D NGM cultivation. Interestingly, we observe differences in patterns of feeding and egg-laying behaviors in 3D compared to 2D. We wonder if these differences affect the progeny in any way and are analyzing brood size and behavior patterns in the next generation. Additionally, we are screening through candidate mutants to identify any genes that are important for survival or fitness in 3D environments. Characteristic patterns of egg-laying and feeding behaviors observed in 3D might indicate a maternal behavior to benefit the survival and fitness of young in natural environments, and thus we plan to analyze the relationship between 3D egg-laying behaviors and another possible maternal behavior, internal hatching or worm bagging, that occurs in harsh environments. Overall, observing patterns of behaviors in a more natural 3D environment allows us to broaden our understanding of nematode biology and ecology.

736C Bacterial endotoxin lipopolysaccharide (LPS) stimulates egg-laying, a serotonin-dependent behavior.  A.C-Y. Leung1, J.T. Gunderson2, C.C.N. Hughes2, G.I. Reed2, M. Lee1,2 1) Institute of Biomedical Studies, Baylor University, Waco, TX; 2) Department of Biology, Baylor University, Waco, TX.

Lipopolysaccharide (LPS) is a Gram-negative cell wall component and septic shock-causing endotoxin. LPS triggers innate immune responses in mammals, as well as in invertebrates including insects and nematodes. In humans, LPS is a known ligand for one of the toll-like receptors (TLRs), which are involved in innate immune responses that are conserved across the animal kingdom. Here we report that LPS stimulates the well-studied behavior of egg-laying in the nematode C. elegans, which is controlled by a serotonin signaling pathway, a conserved pathway found also in human to control mood and cognitive functions. This finding suggests that bacterial pathogens can affect a reproductive behavior. In C. elegans, the putative receptor of LPS and the sole TLR in the species, TOL-1, has been shown to regulate pathogen avoidance. Our second finding is that the stimulatory effect on egg-laying of LPS is TOL-1-dependent, as tol-1 (nr2033) mutant shows significantly reduced response to LPS, suggesting that LPS acts on the nematode by binding to TOL-1. Furthermore, the tol-1 mutant is found to be insensitive also to serotonin stimulation, supporting our hypothesis that TOL-1, activated by LPS, is a novel regulator of the serotonin-controlled egg-laying behavior. The fact that such host-pathogen interaction plays a role in the host’s serotonin-controlled behavior supports the idea about bacterial pathogens’ influence on mood and cognitive functions in their host. Provided that egg-laying is stimulated by serotonin and other drugs used for treating depression in humans, it would be interesting to further investigate the mechanism and the significance of LPS behaving like an anti-depressant in the nematode and possibly in humans.

737A Developing intersectional strategies with the cGAL bipartite system to perform cell-specific genetic analysis of pharyngeal pumping in C. elegans.  J. Liu1,2, H. Wang1,2, P. W. Sternberg1,2 1) Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA; 2) Howard Hughes Medical Institute.

Rhythmic behaviors such as locomotion, feeding, circulation, and respiration are prevalent in the animal kingdom, but the genetics of how such behaviors are governed is not well understood. Pharyngeal pumping in C. elegans is one such rhythmic behavior, performed by a compact system of 20 muscle cells and 20 neurons. However, genetic analysis of the pharyngeal nervous system is currently not amenable due to a lack of precise genetic access. This can be overcome using bipartite expression systems, which allow for complex control of gene expression in genetically tractable organisms. Unlike traditional promoter:gene fusions, bipartite systems couple the promoter to a foreign transcriptional activator; the activator’s associated recognition site is then coupled to the gene. Cells in which the promoter is active then produce the desired gene product. A more advanced strategy to achieve fine tissue expression patterns involves intersectional ‘split’ strategies. Rather than using a single promoter to express the transcriptional activator, the activator is instead split into two fragments, each driven by a different promoter. Each fragment by itself is non-functional; only in tissues where both promoters are active, does the full transcriptional activator reconstitute and drive gene expression. Controlling gene expression with two promoters can provide the cell specificity necessary to study the pharyngeal nervous system. Previously we developed cGAL, an optimized GAL4-UAS bipartite system for C. elegans, by modifying the activation domain, the UAS copy number, and using a DNA-binding domain from a GAL4 gene of the cryophilic yeast species S. kudriavzevii. Now we describe a split strategy for cGAL, using intein domains, to reconstitute the full transcriptional activator. Inteins, when attached to separate protein partners, catalyze an auto-splicing reaction and covalently join their partners together. Using this split cGAL system, we obtained single cell type driver access to various motor and interneurons in the pharynx- most important of which are the MC neurons which laser ablation has shown to be the major drivers of pharyngeal pumping. With this degree of genetic control, we can silence the MC neurons and perform forward genetics to discover genes important to rhythmic pumping. We can also perturb or record upstream neurons to decipher how the network influences the major controller of a rhythmic behavior.
738B  Sensorimotor integration regulates rapid decisions during chemotactic steering.  *H. Liu*, Wenxing Yang, Taihong Wu, Yun Zhang  Department of Organismic and Evolutionary Biology, Center for Brain Science, Harvard University, Cambridge, MA 02138, USA.

During active exploration, the sensory cue perceived by an animal is constantly patterned by the animal’s own movement. Thus, to track the sensory target rapid decisions on locomotory outputs are often generated by integrating the sensory information with the ongoing motor state. However, the neuronal mechanisms that govern sensorimotor integration remains largely unknown.

When *C. elegans* steers towards an attractive odorant, the repeated head undulations allow the nematode to sample the odorant gradient within the space spanned by the head swings. We find that one half head undulation that swings between the most dorsal and the most ventral head positions is the smallest unit to generate the maximum performance on steering. Thus, we characterize how the sensorimotor integration during the half head undulations generates the steering decision. Our previous studies show that the axon of an interneuron RIA exhibits two activity patterns, the compartmentalized activity that encodes and regulates dorsal-ventral head undulations and the synchronized activity that is evoked by sensory stimulations [Hendricks et al., 2012]. We now find that disrupting RIA function alters the rapid steering decisions during chemotaxis. By imaging the calcium transients in the RIA axons in restrained and freely moving animals, we find that the two activity patterns quantitatively encode the concentration of the stimulating odorant versus the amplitude of head undulations and that sensory-evoked synchronized activity suppresses the motor-encoding compartmentalized activity during chemotactic steering. This axonal sensorimotor integration transduces the spatial gradient of the odorant into the asymmetry of the RIA axonal activities. We further show that asymmetric axonal output of RIA biases the steering during movements towards the attractant. In addition, we show that experience modulates chemotactic steering by regulating the sensorimotor integration in RIA. Together, our results characterize how sensorimotor integration gives rise to rapid steering decisions during chemotactic movements.

739C  Subjective value guides rational foraging decisions in *C. elegans*.  A. Katzen1, W. Harbaugh2, P. Glimcher3, S. Lockery 1) Institute of Neuroscience, University of Oregon, Eugene, OR; 2) Department of Economics, University of Oregon, Eugene, OR; 3) Center for Neural Science, NYU, New York, NY.

Value-based decision making - choices driven by subjective assessments of utility - is a central function of the brain and the focus of intensive study in mammals. Until now, evidence that nematodes are capable of value-based decision making has mainly been suggestive. However, economists have developed formal procedures for determining whether a consumer’s decisions are based on subjective value as opposed to random or capricious impulses. We recently developed microfluidic devices that enable such tests to be performed on nematodes for the first time. The worm is held at the confluence of contiguous streams of high and low quality bacterial food leaving its head free to move. Bacteria concentrations are adjusted by the experimenter to change the relative “prices” of the two foods in terms of number of bacteria consumed per pharyngeal pump. Food concentrations can also be adjusted in tandem to increase or decrease the worm’s overall consumption possibilities, i.e. “budget.” Consumption is measured by counting pharyngeal pumps recorded electrically.

Worms typically fed in both streams, consuming a mixture of high and low quality food that was unique for each combination of price and budget. We found that worms make globally rational choices in that they obey transitivity. That is, for all sets of food mixtures A, B, and C, if A is preferred to B, and B to C, then A is preferred to C. As transitivity is the necessary and sufficient condition for value maximization, these data provide formal evidence that *C. elegans* exhibits value-based decision making.

Further, we found that the olfactory neuron AWC, known to be activated by the sudden absence of food, is required for intact food choice behavior. Surprisingly, however, we found that AWC is also activated by the switch from high quality food to low quality food, even when the two foods are at the same concentration (price). Thus, subjective value may be represented at the level of individual olfactory neurons.

Our behavioral and neuronal data are consistent with a model in which olfactory neurons represent the subjective value of the local environment to direct behavior toward preferable mixtures of particular foods. To our knowledge, this is the first formal demonstration of value-based decision making in a genetically tractable model organism with a simple nervous system, opening the door to the discovery of conserved genes and neural circuits for rational decision making.

740A  The SIK3 homologue KIN-29 regulates sleep.  L.E. Lopes1, J. Grubbs2, M.N. Nessel1, A.M. van der Linden2, D. M. Raizen1  1) Neurology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 2) Biology, University of Nevada, Reno, NV.

*Caenorhabditis elegans* (*C. elegans*) displays two sleep states- following cellular stress (SIS) and during development (DTS) - whose mechanisms appear to be at least partially distinct (Trojanowski et al, Journal of Neuroscience 2015; Turek et al, Curr. Biol. 2013; Hill et al, Curr. Biol. 2014). It also displays quiescent behavior under conditions of satiety (You et al, Cell Metab, 2008). Following exposure to conditions that cause cellular stress, epidermal growth factor (EGF) activates the ALA interneuron (Hill et al, Curr. Biol. 2014). KIN-29, a serine/threonine kinase, has been previously described to be involved in the regulation of both satiety quiescence (Van der Linden et al, Genetics 2008) and developmentally timed sleep in *C. elegans* (Funato et al, Nature 2016). In mice and *Drosophila*, a gain of function mutation in *kin-29* homologues, leads to an increased need for sleep (Funato et al, Nature 2016). To get at the mechanism by which SIK3/KIN-29 promotes sleep, we here study the role of *kin-29* in the regulation of developmentally timed sleep (DTS) and stress induced sleep (SIS) in *C. elegans*. Animals with a *kin-29* null mutation display defects in both DTS and SIS. Our preliminary data suggests that *kin-29*’s effect on sleep is mediated by the histone deacetylase HDA-4, since an *hda-4* mutation suppresses the defect in SIS and DTS seen in *kin-29* mutants. *kin-29* mutants display normal quiescence following EGF over-expression, suggesting that KIN-29 acts upstream of ALA activation in the SIS pathway. We are currently trying to identify the cells in which *kin-29* is acting to regulate sleep. To determine if KIN-29 activity is sufficient to induce sleep in *C. elegans*, as previously shown in mice and *Drosophila*, we are making transgenic
animals with a kin-29 PKA site mutated to make KIN-29 constitutively active. Finally, to understand when KIN-29 signaling is necessary we will use a conditional CRISPR/Cas9 system to knock out KIN-29 in animals following development. These studies aim to elucidate the conserved role of KIN-29/SIK3 in the regulation of sleep.

741B Genetic sex acts through the DM gene mab-3 to promote sex differences in C. elegans pheromone attraction. J. Luo1, K. Fagan1, F. Schroder2, D. Portman 1 1) Department of Biomedical Genetics, University of Rochester, Rochester, NY; 2) Boyce Thompson Institute, Cornell University, Ithaca, New York.

Sex differences in behavior provide an opportunity to understand how a single genetic cue modulates neural circuit development, function, and behavior. In C. elegans, males are potently attracted to some ascaroside pheromones, particularly ascr#3 (C9), while hermaphrodites are repelled. Using neuron-specific genetic sex reversal, we screened sensory neuron classes that might be involved in male pheromone attraction. We found that the sexual state of the shared sensory neuron ADF determines the behavioral response to ascr#3: masculinization of ADF generates robust ascr#3 attraction in hermaphrodites, while feminization of ADF abolishes attraction in males. The C. elegans sex determination pathway acts through the master regulator tra-1, whose activity specifies the hermaphrodite state. To ask how tra-1 regulates ADF’s properties, we focused on the DM-domain transcription factor mab-3, which is known to be male-specifically expressed in ADF. We found that mab-3(+/f) mutants were moderately defective in ascr#3 attraction, while hermaphrodites were unaffected. mab-3 function requires ADF, as mab-3(+/f) had no effect in ADF-ablated males. Moreover, ADF-specific expression of mab-3 rescued the mab-3 mutant phenotype. Thus mab-3 likely acts downstream of tra-1 to promote ADF’s male-specific properties. Consistent with this, we found that mab-3 is partially required for the ascr#3 attraction seen in ADF-feminized hermaphrodites. However, because ADF-dependent sex differences still exist in mab-3 mutants, tra-1 likely has additional targets in this cell. We are currently working to understand how mab-3 controls sex differences in ADF function and to identify factors responsible for the mab-3-independent sex differences in this cell.


Research has shown that Caenorhabditis elegans demonstrates a highly deterministic behavioral response under an electric field; Under such conditions, worms migrate to the negative pole of the field, coupled with an angular offset proportional to the absolute strength of the field. Under relatively large applied voltages (12 V/cm) worms migrate to the field’s negative pole with a larger opening angle, as compared to a more modest 5 V/cm. While this relatively broad observation is interesting in itself, a more comprehensive motional attractor analysis is yet to be conducted on C. elegans demonstrating electrotactic responses. To address this, we experimentally observed the motion of ten C. elegans on 2% agarose under electric fields varying from 5 - 12 V/cm. A custom built worm-tracking microscope was implemented for these purposes in order to accurately record the worm’s body configuration and absolute position during trials. Matlab fitting software was written and used to analytically describe the worm’s motion under experimental and controlled conditions. In the absence of an electric field, the motion of C. elegans may be well-modeled as a propagating sinusoid, fit along the entirety of the worm’s body. However, under the application of an electric field, the sinusoidal fit requires a damping term to fully represent an apparent partial paralyzation of the worm’s posterior half. This result suggests the possibility that the presence of an electric field likely inhibits specific motor neurons responsible for the muscular control of the posterior regions of the worm’s body.

743A The nematode Caenorhabditis elegans displays a chemotaxis behavior to tuberculosis-specific odorants. J. Marsili2, M.F. Neto1, Q.H. Nguyen2, S. Aldakeel2, S.M. McFall1, C. Voisine2 1) Center for Innovation in Global Health Technologies (CIGHT), Department of Biomedical Engineering, Northwestern University, Evanston, IL 60208, USA; 2) Department of Biology, Northeastern Illinois University, Chicago, IL 60625, USA.

The World Health Organization’s Millennium Development Goal of halting and reversing the tuberculosis (TB) epidemic by 2015 has been met; on average, incidence of TB has fallen 1.5% per year and is now 18% lower than in 2000. Despite these gains, TB remains one of the leading causes of morbidity and mortality globally—in 2014, 9.6 million people fell ill with TB and 1.5 million died from the disease. Each year, approximately one third of the TB infected population remain undiagnosed, equating to roughly 3 million people. A simple, affordable diagnostic test for pulmonary tuberculosis (TB) is urgently needed to improve detection of active Mycobacterium tuberculosis.

Recently, it has been suggested that animal behavior can be used as a biosensor to signal the presence of human disease. For example, the giant African pouched rats can detect tuberculosis by sniffing sputum specimens while honeybees respond to three of the four tuberculosis-specific volatile organic compounds (TB-VOCs) detected in the breath of TB positive patients by proboscis extension. However, both rats and honeybees require animal housing facilities and professional trainers, where few laboratories in low-income, high-burden TB settings possess the needed infrastructure. Rather than using trained animals, we propose exploiting the innate sensing capabilities of the nematode Caenorhabditis elegans, which has demonstrated reproducible, behavioral responses to over 100 VOCs.

Our data shows that the innate olfactory behavioral response of C. elegans can be used to detect the TB-specific VOCs methyl p-anisate, methyl nicotinate, methyl phenylacetate and o-phenylalanisole, in chemotaxis assays. It is important to note, the breath of a TB patient contains a heterogeneous mixture of TB-VOCs, although the proportionality of this blend remains unknown. To further evaluate the efficacy of C. elegans as a potential TB biosensor, we also investigated C. elegans’ behavioral response to combinations of TB-VOCs, both pairwise and collectively. We found that C. elegans detects a mixture of all TB-VOCs, exhibiting a significant avoidance behavior. In addition, dauer larvae, a long-lived stress resistant alternative development state of C.
elegans in which the animals can survive for extended periods of time in dry conditions with no food, were also demonstrated to detect the VOCs. Taken together, we propose a potential work-flow for a noninvasive diagnostic test for TB that incorporates collecting VOCs from the breath of the patient, exposing dauer larvae housed in a microfluidic device to the VOCs, and recording their response. These results showcase the potential for C. elegans to be incorporated into a new and affordable diagnostic test device for TB and, perhaps, other disease states, capitalizing upon its innate olfactory responses.

744B  Neural cell adhesion molecule 1 (NCAM-1) is associated to long-term memory in nematodes and humans.  Vanja Vukojevic 1, Pavlina Mastrandreas 1, Iris-Tatjana Kolassa 1, Thomas Elbert 2, Sarah Wilker 4, Virginie Freytag 1, Fabian Peter 1, Philippe Demougin 1, Dominique J.-F. de Quervain 2,3, Andreas Papassotiropoulos 1,3, Attila Stetak 1,3  1) University of Basel, Department of Psychology, Division of Molecular Neuroscience, Birmannsgasse 8, 4055 Basel, Switzerland; 2) University of Basel, Department of Psychology, Division of Cognitive Neuroscience, Birmannsgasse 8, 4055 Basel, Switzerland; 3) University of Basel, University Psychiatric Clinics, Wilhelm Klein-Strasse 27, 4055 Basel, Switzerland; 4) Clinical and Biological Psychology, Institute for Psychology and Education, University of Ulm, 89069 Ulm, Germany; 5) Clinical Psychology and Neuropsychology, University of Konstanz, 78457 Konstanz, Germany.

The neural cell adhesion molecule 1 (NCAM-1) has been implicated in several brain-related biological processes, including neuronal migration, axonal branching, fasciculation, and synaptogenesis, with a pivotal role in synaptic plasticity. Here, using an interdisciplinary approach we investigated the evolutionary conserved role of neural cell adhesion molecule 1 (ncam-1) in learning and memory. We conducted a large-scale transcriptomic study examining the temporal expression profile of different genes during C. elegans long-term memory, and observed sustained changes in ncam-1 expression up to 24 hours upon aversive olfactory conditioning. Loss of ncam-1 function selectively impaired long-term memory in a negative but not in positive olfactory associative memory paradigm, without causing acquisition, sensory, motor or short-term memory deficits. Finally, both C. elegans and human NCAM-1 expression in nematodes rescued loss of C. elegans ncam-1 gene function. Considering the conserved role of NCAM-1 in high order complex organisms, we next expanded our C. elegans findings in human, using expression and methylation data across three independent samples. In two samples of European descent, we showed a negative correlation between DNA methylation of the NCAM1 promoter and aversive memory (i.e., recognition of negative pictures) as well as a strong positive correlation between NCAM1 expression and the same recognition performance. In the second sample we could also show a strong correlation between NCAM1 expression and long-delayed recall of negative pictures. Finally, in a population of genocide survivors of African descent, DNA methylation at an alternative promoter of the NCAM1 gene was inversely correlated with symptoms of posttraumatic stress disorder (PTSD), including traumatic aversive memory.

Together, our results support a conserved role of NCAM1 in negative associative memory from nematodes to humans and might, ultimately, prove to be helpful in elucidating novel mechanisms of memory-related disorders.

745C  A quantitative, multi-worm microfluidic assay for gentle and harsh touch response behavior.  Patrick McClanahan, Joyce Xu, Christopher Fang-Yen  Department of Bioengineering, University of Pennsylvania, Philadelphia, PA.

C. elegans is an important model for understanding the genetics and physiology of the sense of touch. Traditional assays for touch in C. elegans include manually touching the animal with a fine hair (gentle touch) or wire pick (harsh touch) and observing its behavioral response. These assays are limited by their low throughput and qualitative nature. To address these limitations, we developed a microfluidic system that uses an array of pressure-driven microvalves to deliver continuously variable, spatially localized mechanical stimuli to up to 60 freely behaving worms at once. The microfluidic device has two layers. The ‘worm layer’ comprises an array of 64 sinusoidal channels in which the worms can crawl, and the ‘control layer’ comprises 15 straight channels separated from the worm layer by a flexible membrane. When pressurized, the control channels inflate and impinge on the worm layer, delivering a mechanical stimulus to the worms inside. The control channel spacing is similar to the length of the animals, so that each worm is likely to experience a single mechanical stimulus during each pressurization. Machine vision is used to track the worms throughout an experiment. By delivering a series of stimuli of increasing amplitude, we use our single assay to quantify the 50% response thresholds to gentle and harsh touch in terms of worm channel ceiling deflection. We found response thresholds of 8.8 ± 2.6 µm for WT and 46.9 ± 3.3 µm for mec-4 animals, suggesting that the threshold for harsh (mec-4-independent) touch is about 5 times larger than for gentle (mec-4-dependent) touch. We then take advantage of the localized nature of the stimuli to quantify changes in the receptive field resulting from defects in the development of the posterior touch receptor neurons in egl-5 mutants. We find that while WT worms respond to mid-posterior touch with forward movement, egl-5 mutants respond with reversals, suggesting that the anterior touch cells are sensitive to posterior touch. Finally, we use the behavioral history of the worms to investigate how the gentle touch response depends on movement prior to the stimulus and the location of previous stimuli. We find that the movement of the animals immediately prior to the stimulus does not affect the type of response, even for touches to the mid section of the body, which can result in either forward or reverse movement, suggesting that touch stimulus can "reset" the locomotory control circuit. However, the location of the previous touch does affect touch response, with an anterior touch biasing responses in the forward direction, and a posterior touch biasing responses in the reverse direction, suggesting location-dependence of touch habituation. The ability to deliver localized gentle and harsh touch stimuli to a large number of animals and record their behavioral output will facilitate further studies of C. elegans touch sensation, sensory adaptation, and nociception.

746A  A humanized test system: Using pharyngeal pumping phenotypes as a readout for the serotonergic signaling pathway in C. elegans.  Adela Chicas-Cruz 1, Terra Hieber 1, Yoanne Clovis 1, Kathryn McCormick 1, Chris Hopkins 2, Trisha Brock 2  1) NemaMetrix, Inc., Eugene, OR; 2) Knudra Transgenics, Salt Lake City, UT.

The neurotransmitter, serotonin (5HT) has widespread regulatory effects in humans, from contractions of the gut to sensory
perception in the brain. Likewise, it is a widespread and important neurotransmitter in *C. elegans*, where it regulates pharyngeal pumping and feeding behavior. Here, we model this biochemical pathway using pharyngeal pumping phenotypes and provide a reliable readout for serotonergic signaling. We obtained and analyzed electrophorography (EPG) data using a multi-axial device and associated software for *tpf-1* and *mod-5* loss-of-function mutants as well as *mod-5* gain-of-function mutants. First, we support previous findings and demonstrate a reduction in pumping rates for *tpf-1* mutants, which fail to synthesize 5HT. Next, we present new data for worms that have reduced reuptake of 5HT by the *mod-5 5HT reuptake transporter*. *mod-5* null mutants display significantly higher pumping rates than control worms with no exogenous pumping stimulus. Intriguingly, preliminary data using the SSRI fluoxetine (Prozac) exhibits similar results to these *mod-5* nulls by blocking the reuptake by the transporter. The *mod-5* reuptake transporter is orthologous to the human 5HT transporter SLC6A4. We created a gain-of-function mutant by ectopically expressing human SLC6A4 in the pharynx (mod-5p::hSLC6A4::ttb-2UTR). As expected, the humanized animals retained a pumping defect even when stimulated by food. Only with hyper stimulation of the humanized line by exogenous addition of 10 mM 5HT did the levels of pumping restore to wild type levels. Experiments are underway to move the humanized line into the null background and determine if full rescue of *mod-5* function is possible with a human transgene. Combined, these experiments have demonstrated that monitoring pharyngeal pumping is an easy to use platform for exploring the 5HT pathway in *C. elegans*.

**747B High-throughput behavioural characterization and precise structure-function analysis of genes and gene variants associated with Autism Spectrum Disorder.**

T.A. McDiarmid, P. Pavlidis, K.Z. Haas, C.H. Rankin 1,2

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A primary challenge in studying genes associated with Autism Spectrum Disorder (ASD) is the lack of an in vivo system in which to rapidly functionally validate and characterize the large number of candidate risk genes. The sheer number of mutations associated with ASD and the time and money constraints associated with modeling ASD in mammals necessitates an alternative approach. Here, we use the high-throughput capacity of Caenorhabditis elegans as an in vivo platform to functionally validate and characterize ASD-associated genes and their variants. We used our machine vision system, The Multi-Worm Tracker, to characterize morphology, locomotion, and habituation phenotypes of 99 strains of *C. elegans* covering orthologs of 87 ASD-associated genes. In parallel, we have used CRISPR-Cas9 triggered homologous recombination to replace daf-18 with human PTEN as well as transgenic strains each expressing a different ASD-associated de novo missense mutation in human PTEN in order to validate and assess the functional affects of these putatively pathogenic amino acid substitutions in vivo. This research has generated a large number of novel genotype to phenotype relationships that range from severe developmental delays and uncoordinated movement to subtle deficits in sensory and learning behaviours, as well as detailed structure-function information indicating which ASD-associated PTEN variants are strong function altering mutations. This data will be a powerful in vivo tool to inform future targeted in vivo studies in higher organisms and holds the potential of identifying novel therapeutic targets for ameliorating the effects of ASD.

**748C Diet-dependent modulation of chemoreceptor gene expression.**

A. McDonagh, T. Ngo, J. Grubbs, A.M. van der Linden, Biology Department, University of Nevada, Reno, NV 89557, USA.

Chemosensory plasticity in response to dietary signals and nutritional need has been observed in a wide range of animals, which allows them to identify suitable food sources and avoid harmful environments. However, the mechanisms behind how dietary signals alter the properties of chemosensory neurons is poorly understood. Dynamic changes in the expression levels of individual chemoreceptor genes could provide a strategy by which *C. elegans* rapidly modifies its behavior according to its diet. We previously identified an individual chemoreceptor gene, *srh-234*, in the ADL sensory neuron type of *C. elegans*, which is downregulated upon starvation (Gruner et al, PLoS Genetics, 2014). This downregulation is dependent on cell- and non-cell-autonomous mechanisms regulated by insulin signaling and bHLH and MEF2 transcription factors (Gruner et al, PLoS Genetics, 2016). We now show that a standard *E. coli* OP50 diet relative to the good *Comamonas* DA1877 diet induces dramatic changes in *srh-234* expression levels in ADL neurons similar to a starvation response, i.e. expression of a *srh-234p::gfp* reporter is high when animals are fed a *E. coli* diet, but low when animals are fed a *Comamonas* diet. When we mixed *Comamonas* with *E. coli* in different dilutions (1:1 and 1:9 DA1877:OP50), we found that *srh-234p::gfp* expression is strongly reduced similar to a *Comamonas* diet alone. These mixed diet experiments coupled to the observation that *Comamonas* is considered not to be nutrient poor diet (Watson et al, Cell, 2014), suggest that the *Comamonas*-induced reduction in *srh-234p::gfp* expression in ADL is likely not due to a starvation response. These results lead to the hypothesis that specific bacterial signals (e.g. secondary metabolites) produced by *Comamonas* may directly via ADL neurons or indirectly through food ingestion regulate *srh-234* expression. We preliminary show that *srh-234p::gfp* expression is not reduced when animals smell (but not touch) *Comamonas* when fed on a *E. coli* diet, implying that perhaps ingestion of a *Comamonas*-generated signal is required to influence *srh-234* expression in ADL in a cell-nonautonomous manner. We are currently investigating the pathways by which previously known bacterial-derived signals produced by *Comamonas* (Watson et al, Cell, 2014) regulate *srh-234* expression levels in ADL neurons. Taken together, we propose that turning chemoreceptors on and off at the level of transcription in chemosensory neurons may help *C. elegans* to optimize their chemosensory responses to relevant microbial molecules in their environment.

**749A A two-pore potassium channel regulates *C. elegans* locomotor behaviors.**

J. Meng, W. Hung, S. Mouridi, M. Mercier, T. Boulin, M. Zhen 1,2

1) Department of Physiology, University of Toronto, Toronto, ON, Canada; 2) Lunenfeld-
Most Animals can memorize various environmental conditions and alter behaviors according to past experiences. Although transmission of the signal from the receptor to the nucleus for gene expression is conserved through evolution, we find that the specific genes that mediate behavioral responses vary. We have tested whether the DAF-16/FOXO transcription factor regulates taste avoidance learning. The DAF-16/FOXO transcription factor is a key regulator of lifespan and stress resistance in C. elegans. Mutants of the DAF-16/FOXO transcription factor have altered behavioral responses to chemical stimuli.

Previously, the lab of Tomioka (T. Nagashima, Y. Iino, Biological Sciences, The University of Tokyo, Tokyo, JP.) has shown that Taste avoidance learning involves the expression of the neuropeptide PDF and the MCM interneurons. We have found that mutants without MCMs and mutants in the neuropeptide PDF neuropeptide fail to undergo sexual conditioning.

We are now investigating the circuit for chemotaxis learning to identify the neurons within it that are modulated by the MCMs and PDF neuropeptide.

**Reference**

**750B  Sexy learning in C. elegans.** L. Molina-Garcia, L. Lin, S. Rashid, A. Barrios Department of Cell and Developmental Biology, University College London, London, GB.

We learn from experience. When an animal repeatedly encounters a signal coupled with either a punishment or a reward, it eventually learns to expect both to occur together in a process called associative learning. A central goal in neuroscience is to understand how neural circuits integrate conflicting (rewarding and aversive) experiences that need to be behaviourally resolved during learning.

To shed light into this process at the molecular and cellular level, we are dissecting a neural circuit for associative learning in the C. elegans male.

Previously, the lab of Sakai (et al., 2013) and our lab (Sammut et al., 2015) have shown that C. elegans males undergo sexual conditioning, a form of associative learning by which a rewarding experience with mates overrides the behavioural consequences of an aversive association with starvation. Sexual conditioning leads to a switch in behavioural responses to an environmental stimulus from avoidance to attraction.

We have also identified the MCM interneurons and PDF neuromodulation as regulators of the sexually conditioned behavioural switch (Sammut et al., 2015). These studies showed conditioned responses to salt.

Here we demonstrate that other stimuli such as benzaldehyde or diacetyl can also be sexually conditioned. In addition, we show that mutants without MCMs and mutants in the neuropeptide pdf-1, which is expressed in the MCMs, fail to undergo sexual conditioning also to odors. These results indicate that sexual conditioning is a general, broadly employed strategy of the male to effectively search for and locate mates during navigation. Furthermore, the MCM neurons and PDF neuromodulation are common mechanisms underlying sexual conditioning to distinct stimuli.

We are now interrogating the circuit for chemotaxis learning to identify the neurons within it that are modulated by the MCMs and PDF neuropeptide.

**751C  Novel associative learning phenotype in a purine biosynthesis mutant.** C.A. Moro, L. Franklin, W. Hanna-Rose The Pennsylvania State University, University Park, PA.

Inborn errors of purine metabolism are rare genetic disorders that affect the efficacy of enzymes in the purine biosynthesis pathway. Adenylosuccinate lyase (ADSL) catalyzes two steps in the *de novo* pathway of purine metabolism. A complete deletion of ADSL is likely embryonic lethal in humans; however, decreased enzymatic activity is permissive for most patients to reach early childhood. Clinical presentations of ADSL deficiency include cognitive impairments, behavioral abnormalities, seizures, and degenerative motor deficits. Currently, there are no effective treatments other than seizure prevention. We sought to specifically model behavioral aspects of ADSL deficiency in C. elegans in order to elucidate the molecular mechanisms underlying the disorder and to generate ideas for therapies.

We used associative learning assays to test neurological function of *adsl-1*(RNAi) animals. Naive young adult C. elegans chemotax to attractants such as the gustatory cue sodium chloride. However, when exposed to a learning paradigm consisting of food deprivation in the presence of the chemoattractant, post-treated worms no longer show a preference to the chemoattractant. We found that *adsl-1*(RNAi) animals have normal chemotaxis and are attracted to sodium chloride. However, after pairing sodium chloride with starvation, their post treatment response is altered relative to controls. *adsl-1*(RNAi) animals are strongly repelled by the chemoattractant sodium chloride after the learning paradigm. We conclude that *adsl-1* animals may be more susceptible to associating the attractant to starvation, and we are investigating this model.

**752A  Multiple isoforms of the DAF-16/FOXO transcription factor regulate taste avoidance learning.** T. Nagashima, M. Tomioka, Y. Iino Biological Sciences, The University of Tokyo, Tokyo, JP.

Most Animals can memorize various environmental conditions and alter behaviors according to past experiences. Although signaling pathways underlying learning and memory have been extensively studied using various organisms such as *Drosophila* and mice, it has been challenging to understand the functional role of signaling pathways in defined neurons. We focus on taste...
avoidance learning in C. elegans, in which animals learn to avoid salt concentrations encountered under starvation conditions. We have shown that the local action of the insulin-like signaling at the axon of the ASER gustatory neuron regulates taste avoidance learning (Ohno et al., 2014). The insulin-like signaling regulates longevity, development and several other phenomena through control of DAF-16, the FOXO transcription factor homolog. On the other hand, it remains unclear how DAF-16 functions in taste avoidance learning. To clarify the role of DAF-16 in learning and memory, we have analyzed the functional role of DAF-16 in taste avoidance learning.

We found thatdaf-16 loss-of-function mutants show defects in taste avoidance learning; they showed defects in avoidance of high or low salt concentrations after starvation conditioning with high or low salt concentrations, respectively. It was previously reported that DAF-16 has several isoforms with different functions. Behavioral phenotypes of isoform-specific mutants and isoform-specific rescue experiments suggested that at least four DAF-16 isoforms, a-, b-, d- and f-isoforms, were functional in high-salt avoidance, while only the a-isoform of DAF-16 can support low-salt avoidance. Moreover, cell-specific rescue experiments showed that expression of DAF-16 (a-isoform) in the ASER neuron rescued the defect in high-salt avoidance but not low-salt avoidance. These results imply that the functional roles of DAF-16 might vary according to the salt concentrations. We are trying to clarify the molecular mechanism downstream of DAF-16 in taste avoidance learning.

753B  Tachykinin neuropeptides promote sleep.  Ravi Nath, Paul Sternberg  HHMI and Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA.

Sleep is a complicated state where multiple behaviors must be shut down. In C. elegans this means that the animal must stop eating, defecating, moving, and responding to stimuli. Our goal is to understand how these physiological processes are shut down. We found that ALA, a single neurosecretory cell, coordinates the inhibition of multiple physiological processes with neuropeptides. Of the many ALA-transcribed-neuropeptide genes we found that three genes (flp-13, nlp-8, flp-24) each regulate a specific set of sleep-associated behaviors. Here we focus on the nlp-8 gene, which is the only annotated instance of tachykinin in the C. elegans genome. We used an overexpression strategy to determine the biologically active neuropeptides encoded by nlp-8. We discovered that tachykinin neuropeptides encoded by nlp-8 inhibit behavior. The tachykinin neuropeptide family is found throughout bilaterians, and regulates many processes and behaviors: the immune system, the gastrointestinal tract, inflammation, aggression, and pain. The tachykinin family of neuropeptides is divided into two classes: invertebrate and vertebrate, with only rare instances of vertebrate-class tachykinin found within invertebrates. The C. elegans tachykinin gene is unique in that it encodes both the vertebrate and invertebrate classes of tachykinin peptides. We found that both classes of tachykinin neuropeptides were sufficient to inhibit locomotion in C. elegans. The C. elegans genome also encodes three tachykinin receptors (tkr-1, tkr-2, tkr-3), which are homologous to vertebrate (human) tachykinin receptors. We tested if the tachykinin receptors (tkr-1, tkr-2) were able to suppress the nlp-8 overexpression phenotype, and found a partial suppression of nlp-8 inhibition of locomotion in the tkr-1; tkr-2 double mutant. These results support the hypothesis that C. elegans stress-induced sleep represents a form of sickness sleep, given tachykinins' broad role in inflammation and sickness in animals. Taken together, these results raise the possibility that tachykinin has an ancestral role in sleep-regulation.

754C  The DVA neuron promotes wakefulness via a cAMP-mediated mechanism.  Francis Janton, Ryan Vance, Nicole Sullivan, Amelia Brown, Mary Szurgot, Matthew Nelson  Biology, Saint Joseph's University, Philadelphia, PA.

Caenorhabditis elegans display unique forms of sleep: developmentally timed sleep (DTS) and stress-induced sleep (SIS), which are regulated by overlapping and distinct mechanisms (Trojanowski et al., 2015). The cyclic adenosine monophosphate (cAMP)/Protein Kinase A (PKA) pathway promotes arousal in invertebrates and vertebrates (Crocker and Sehgal, 2010). This signaling pathway is downregulated during DTS in C. elegans (Belfer et al., 2013) and we show the same is true for SIS. To determine where cAMP/PKA functions during sleep we have been expressing and activating the near infrared light-activated adenylyl cyclase called Ilac, which converts ATP into cAMP in the presence of red light, in candidate neurons (Ryu et al., 2014). We have chosen this tool because, unlike blue light, red light does not wake worms up during sleep. We find that pan-neuronal activation of Ilac reduces both DTS and SIS. Induction of cAMP in either the motor neurons, command interneurons or the sleep-regulating interneuron RIA is not sufficient to reduce DTS or SIS. However, activation of Ilac in the DVA interneuron, by expressing it from the full twk-16 promoter or using a DVA-specific enhancer from the twk-16 promoter (Puckett Robinson et al., 2013), significantly reduces SIS, DTS and non-physiological quiescence induced by neuropeptide over-expression (Nelson et al. 2013 and 2014). This suggests that DVA may be a common wake-promoting neuron downstream of distinct sleep promoting circuits. To confirm DVA’s place in the known circuitry, we have expressed a CAMP biosensor, Epac1-camps (Shafer et al., 2008) in DVA and are currently measuring CAMP levels during sleep. What lies downstream of the CAMP/PKA pathway during DTS and SIS is still unclear. The CAMP response element binding protein (CREB) is a transcription factor known to play a role in the CREB homologue; and crh-1 loss-of-function mutants display increased amounts of DTS (Singh et al., 2014), which we have observed as well. We show evidence that CRH-1 functions downstream of CAMP/PKA in the nervous system during DTS, and are currently testing if DVA is the wake-promoting site of action. Surprisingly, we find that crh-1 mutants have reduced SIS, thus CAMP/PKA promotes arousal after SIS in DVA via distinct mechanisms.

755A  Identifying Novel Genes Required for Synaptic Function Among Human 21st Chromosome Orthologs.  S. Nordquist, J.T. Pierce-Shimomura  Department of Neuroscience, University of Texas at Austin, Austin, TX.

Down syndrome is one of the most common genetic causes of cognitive impairment. Though trisomy of the 21st chromosome has long been known to underlie Down syndrome, the contributing role of individual genes to Down syndrome phenotypes is not fully determined. To gain insight into the in vivo roles of these genes, we studied loss-of-function phenotypes of all HSA21 orthologs in the nematode Caenorhabditis elegans. Excluding the expansion of keratin genes, 52% of HSA21 genes have a

Animals can properly respond to environmental stimuli. Such behavior is controlled by central nervous system (CNS). However, the mechanism of information processing in CNS controlling behavior remains unclear. Recent technical innovation enables us to visualize neuronal activities by Ca²⁺ imaging, so that whole information processing can be analyzed by measuring the whole neuronal activities at the cellular level. We analyzed whole neural activities in the CNS of worms as a model of simple brain to elucidate the mechanism of information processing controlling behavior.

To measure the whole neural activities, we have developed the imaging system which enables time-lapse 3D imaging in three channels by using the Ca²⁺ probes, YC2.60 or GCaMP6f. By the imaging analyses, we can measure whole neural activities in unanesthetized animals at the cellular level. By using this imaging system, we have measured spontaneous neural activities in the CNS of worms and found synchronized activities. We also found that such spontaneous activities were correlated to movement of animal.

To analyze the behavioral control in CNS, we analyzed the activities responding to olfactory stimuli. It is known that worms show positive chemotaxis to diacetyl and avoid Cu²⁺. When worms sense both stimuli simultaneously, worms have to control their behavior by processing the opposing stimuli. Therefore, we analyzed the whole neural activities during stimulating the worms with diacetyl and/or Cu²⁺. Our results suggested that the neurons functions distinctly depending on the combination of the stimuli.

In addition, we try to identify each neuron to analyze the activities of the specific neurons by the expression of the fluorescent proteins in the specific neurons and by the neuronal annotation by machine learning. By combining the whole neural activities and the identification of neurons, we will be able to elucidate the mechanisms of information processing in CNS.

757C Diacylglycerol encodes differences between past and current stimulus intensity. Hayao Ohno, Naoko Sakai, Takeshi Adachi, Yuichi Iino 1) Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, JP; 2) Department of Biological Sciences, Faculty of Science, Kanagawa University, Yokohama, JP.

C. elegans can memorize external salt concentrations and is attracted to the salt concentration at which it has been fed, whereas it avoids the previous salt concentration if it has been starved (Kunitomo et al., Nat. Commun., 2013; Ohno et al., Science, 2014). An important question is how worms compare the intensity of the past and current sensory stimuli and execute appropriate chemotactic behaviors.

The plasticity of salt chemotaxis is regulated by the diacylglycerol (DAG)/protein kinase C (PKC) pathway and the insulin/PI3K pathway, both of which act in the ASER taste receptor neuron. Genetic manipulations that activate or inactivate the DAG/PKC pathway result in worm migration to higher or lower salt concentrations, respectively. The insulin/PI3K pathway is essential for the avoidance of salt concentrations associated with starvation.

We have monitored the abundance of DAG in the ASER presynaptic regions, where the nPKC-epsilon/eta ortholog TTX-4 (a.k.a. PKC-1) is localized. When the external salt concentration is increased or decreased from the concentration that has been experienced with food, DAG level is reduced or elevated, respectively, in a manner dependent on the TAX-4 CNG channel subunit and the EGL-8 phospholipase Cβ. These DAG responses are largely diminished after starvation. An unbiased genetic screen and mutant analyses show that the DAG pathway acts downstream of the insulin/PI3K/Akt pathway. Our results suggest that the abundance of DAG in the ASER presynaptic region can encode differences between past and current salt concentrations and the insulin/PI3K pathway regulates the change of salt concentration preferences through modulation of the DAG dynamics.


Since nearly its entire behavioral repertoire is expressed through movement, understanding the neural basis of locomotion is especially critical as a foundation upon which analyses of all other behaviors must build. C. elegans locomotes in an undulatory fashion, generating thrust by propagating dorso-ventral bends along its body. How the rhythmic patterns are generated is not yet understood. While central pattern generators are involved in animal locomotion from leech to humans, their presence in C. elegans has been questioned, mainly because there has been no evident neural motif that supports intrinsic oscillations. With a fully reconstructed connectome, the question of whether it is possible to have a CPG in the ventral cord of C. elegans can be...
answered through computational models. We modeled the ventral cord circuit based on the most up-to-date connectome data and segmentation analysis. In order to explore the possibility of intrinsic oscillations, we used an evolutionary algorithm to determine the unknown physiological parameters of each neuron so as to match the features of the neural traces of the worm during forward and backward locomotion. We performed 1000 evolutionary runs and consistently found circuits that produced oscillations matching the main characteristic observed in experimental recordings. While most of the successful solutions shared common mechanisms, the polarity and weight of the synapses were not always the same. Each of the different solutions represents a configuration capable of generating locomotion-like intrinsic oscillations. This insight can be used to propose experiments on the living organism that test the hypotheses generated by the different solutions. Analysis of successful networks suggested roles for the known motorneurons that aligned well with experimental evidence. It also suggested a role for the AS-class, which has been almost entirely ignored in the study of C. elegans locomotion. In the majority of evolved circuits, motorneuron AS was fundamental to both the generation of oscillations and to the dorsoventral antiphase coordination. We also found that in most of the solutions the AS-class was bistable, while the B- and A-class were not. This is different from what previous models of locomotion had proposed thus far. In addition to providing an existence proof for the possibility of intrinsic oscillations in the ventral cord, we suggest a series of testable hypotheses about its operation. More generally, we show the feasibility and fruitfulness of a methodology to study behavior from a connectome, while neurophysiological properties are still missing.

759B  Roles of the CLC chloride channel clh-1 in food-associated salt chemotaxis learning of Caenorhabditis elegans.  Chanhyun Park, Yuki Sakurai, Yuichi Iino, Hirofumi Kunitomo  Department of Biological Sciences, School of Science, The University of Tokyo, Bunkyo-ku, Tokyo.

Animals modulate their chemotaxis behavior to seek preferred conditions by memorizing experienced environmental conditions and subsequently modifying navigation strategies. For instance, they show an experience-dependent behavioral plasticity in NaCl chemotaxis in which preference of NaCl is regulated by food availability. Worms are attracted to the salt concentration at which they have been fed, avoiding it if they have been starved. The insulin/Pi3-kinase signaling in ASER salt-sensing neuron regulates starvation-induced behavioral changes (Tomioka et al. 2006, Ohno et al. 2014). However, Pi3-kinase pathway mutants showed no discernible defect in the food-associated salt preference behavior, suggesting that the mechanism of food-associated learning is different from that of starvation-induced learning (Kunitomo et al. 2013). Through a genetic screen for mutants that show defects in the food-associated learning but not in the starvation-induced learning, we obtained two mutants JN572 and JN577. Further, we found that each of the two mutants has a point mutation in clh-1, which encodes a CLC chloride channel. CLH-1 is known to function as a HCO₃⁻ transporter in pH homeostasis of the amphid sheath glia (Grant et al. 2015). Four out of 9 CLC proteins in human are implicated in neurologic disorders. Interestingly, clh-1 deletion mutants that are expected to eliminate clh-1 functions, did not show behavioral defects in the food-associated learning. This result suggested that the mutations were neomorphic. Tissue-specific rescue of the clh-1 mutants indicated that CLH-1 acts in the salt sensing neuron ASER in the food-associated learning. In addition, measurement of ciliary length of ASER rescued olfactory defects will be conducted in specific sensory neurons, namely the ASH and the AWA neurons.

760C  The Effect of a Familial Alzheimer’s Disease-Associated Mutation on the Pathogenesis of Chemosensory Deficits in Alzheimer’s.  M. Parvand, T. Bozorgmehr, C. Rankin  1) Department of Experimental Medicine, University of British Columbia, Vancouver, Canada; 2) Department of Psychology, University of British Columbia, Vancouver, Canada; 3) Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, Canada.

Although olfactory dysfunction is one of the hallmark symptoms in Alzheimer’s disease (AD) prior to the onset of cognitive impairments, little is known about the causes of this dysfunction. The nematode Caenorhabditis elegans is an ideal model for system-level genetic understanding of sensory neural circuits and behavior. Many cases of familial AD are linked to mutations of the presenilin (PS) genes. These genes are orthologous with sel-12 genes in C. elegans. The purpose of this project was to examine the association between PS1 and olfactory deficits in order to investigate the cellular mechanism of these dementi linked deficits. To gain a better understanding of the relationship between presenilin 1 (PS1) mutations in AD and olfactory deficits, chemotaxis experiments (with the attractant diacetyl, and the aversive octanol) were conducted on worms with a mutation in sel-12. We found that adult sel-12 mutants had significantly decreased sensitivity to both odorants compared to wild-type worms. Extrachromosomal array expression of human wild-type PS1 into C. elegans rescued olfactory defects, confirming functional homology between the C. elegans and human gene. However, a PS1 mutant from an Alzheimer’s family was unable to rescue olfactory deficits. Moreover, C. elegans sel-12 mutants presented olfactory deficits throughout their lifespan, and the deficit increased with age, similar to the neurodegenerative progression of AD. Based on these data we concluded that mutations in C. elegans orthologue of PS1 is associated with decreased olfactory function, and this deficit was rescued by wild-type human PS1 gene. We suggest that the altered functioning of the Notch pathway may be involved in these chemosensory deficits. Additionally, to localize the region(s) where wild-type sel-12 function is required for normal olfaction, sel-12 and PS1 rescues will be conducted in specific sensory neurons, namely the ASH and the AWA neurons. Further, an examination of the morphology of these neurons will allow us to evaluate whether structural dysfunctions are associated with the observed behavioral deficits.

761A  The food of our mothers.  A.I. Pereira, K. Kagias, X. Gracida, Y. Zhang  Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.

A growing body of evidence in different organisms demonstrates that parental life experiences profoundly impact the
physiology and behaviour of the progeny. However, how the maternal environment to which embryos are exposed during development produces long-lasting effects on the next generation remains largely unknown. To address this question, we study the food choice in *C. elegans*, a behaviour critical for the survival of the animal. Previous studies show that *C. elegans* cultivated on the *E. coli* strain OP50 under the standard condition prefers the smell of a pathogenic bacteria, *P. aeruginosa* PA14, over the smell of OP50 and that exposure to PA14 significantly reduces the preference towards the smell of the pathogen. Here, we train hermaphrodite mothers with PA14 and test the adult progeny that are cultivated under the standard condition for their olfactory preference towards PA14. Our results uncover a strong correlation between the acquired olfactory aversion of PA14 in the trained mothers and the food choice of their offspring. Namely, the F1 progenies of the trained mothers that display a strong aversion to PA14 show a decreased preference towards the smell of PA14 in comparison with the control F1 progeny from untrained mothers. A detailed behavioral analysis reveals that both the olfactory decision and the locomotory trajectories towards the preferred food are modulated by the food consumed by the mothers. In order to understand the molecular basis of this effect, we are currently analyzing the gene expression profiles of the progenies from mothers that were exposed to the pathogenic or the non-pathogenic bacteria. We hope to discover potential mechanisms by which the maternal environment affects the physiology and behavior of the offspring and to provide novel insights into our fundamental understanding of nature versus nurture.

**762B** A new biomechanical model links material properties with locomotion behaviors and predicts that gait modulation is a form of speed optimisation in *C. elegans*. T. Ranner, N. Cohen  School of Computing, University of Leeds, West Yorkshire, GB.

An organism's ability to move freely is a fundamental behaviour across the animal kingdom. We present a biomechanical model of *C. elegans* locomotion together with a novel computational approach. We model the body as a flexible elastic shell, subject to muscle forcing along the body walls of the body. All parameters are grounded in behavioral, anatomical or physiological data. Our numerical approach allows us to solve for arbitrary body shapes. Our model replicates behaviours across a wide range of environments. We use it to study forward locomotion undulation gait, linking between the animal's material properties and its performance across a range of viscoelastic environments. The model makes strong predictions on the viable range of the worm's Young's modulus and suggests that animals can control speed via the known mechanism of gait modulation that is observed across different media.

**763C** An integrated neuro-mechanical model of *C. elegans* forward locomotion. J. Denham, T. Ranner, N. Cohen  School of Computing, University of Leeds, Leeds, GB.

Behavioural responses in *C. elegans* can be observed through changes in locomotive patterns. It is therefore important to consider the role of the physics in computational models of the neural circuit for motor behaviours. We present a dynamical systems model of *C. elegans* forward locomotion in which the neural circuit is divided into a series of repeating identical units, coupled via posterior stretch receptor feedback. Each unit includes cholinergic, bistable B-type motor neurons (modelled after bistable RMD neurons, Mellem et al. 2008, Boyle et al. 2012), implicit GABAergic D-type motor neurons (Boyle et al. 2012) and nonlinear viscoelastic muscle forcing along the body (Boyle et al. 2012). The neural model incorporates proprioceptive feedback as the mechanism for producing sustained oscillations. Integrating the neural model with a recent continuum mechanical body model (Cohen and Ranner 2017) provides this feedback and is also the method for incorporating environmental drag from the surrounding fluid, closing the neuronal-environmental loop. Biophysically realistic parameters are used to obtain sustained travelling waves in muscle activation which respond to changes in environmental viscoelasticity. To explore the pattern generation mechanism, we present results from bifurcation analysis performed in the isolated neural framework and in the fully integrated neuro-mechanical model. We show how these results are modulated by changes in the external drag and internal material properties of the passive and active body.

References


**764A** A Screen for Contributors to Dopamine Signaling Perturbations in Swimming-Induced Paralysis (Swip). O.M. Refai1, J. A. Hardaway2, C.L. Snarrenberg1,2, S. L. Hardie2, P. Freeman3, R. B. Blakely1,2 1) FAU Brain Institute, Florida Atlantic University, Jupiter, FL; 2) Department of Pharmacology, Vanderbilt University, Nashville, TN ; 3) Department of Biology, Fisk University, Nashville, TN.

The monoamine neurotransmitter dopamine (DA) regulates a wide variety of complex behaviors across phylogeny. The presynaptic DA transporter (DAT) is critical for restricting DA actions in space and time by limiting DA availability at synaptic and extra-synaptic DA receptors. Mutations impacting DAT protein expression or structure have been identified in subjects with multiple brain disorders in humans, including attention-deficit hyperactivity disorder (ADHD), juvenile Parkinsonism/dystonia, bipolar disorder and autism. In the nematode *C. elegans*, loss of function mutations in *dat-1* gene result in a hyperdopaminergic state that causes animals to paralyze in water, a phenotype termed Swimming-induced paralysis (Swip). This phenotype can be
Duchenne muscular dystrophy (DMD) is a lethal degenerative disease that affects 1 in 3,500 males. DMD is caused by mutations in the dystrophin gene, expressed in muscles and nervous tissue. Patients show developmental delays and other neurological phenotypes in addition to muscular decay that eventually leads to death. Much remains unknown about this disease, such as whether any type of muscular exertion might be beneficial for its treatment, or how absence of dystrophin leads to observed neurological phenotypes. We are presently using Caenorhabditis elegans to model DMD to gain insight into these and other questions. To determine if exercise has protective effects on dystrophic muscles, we subjected dystrophic (nlp-14) worms to swimming and burrowing regiments in order to mimic strength or endurance treatments respectively. We measured animal longevity. We find that both exercise treatments negatively affected the health of dystrophic muscle. To determine if NLP-14 induces quiescence, we have conducted a forward genetic screen in which nlp-14 over-expressing worms were randomly mutagenized with EMS, an approach we have taken in the past (lannacone et al., 2017). The mutated F2 progeny were screened and rare suppressor mutants, that did not become quiescent upon heat-induced over-expression of nlp-14, were isolated. We are currently isolating genomic DNA, which will be followed by whole genome sequencing to identify the causative alleles.

**765B** The orcokinin neuropeptide NLP-14 regulates sleep. **Niknaz Riazati**, Gerald Orlando, Natalie Barrett, Jaqueline Boran, Matthew Nelson Biology, Saint Joseph’s University, Philadelphia.

Caenorhabditis elegans sleeps during a life stage called lethargus (Raizen et al., 2008). Lethargus is timed by a transcription factor called LIN-42, homologous to the protein PERIOD, a circadian clock protein which regulates sleep timing in both Drosophila melanogaster and mammals (Jeon et al., 1999; Monsalve et al., 2011). However, the mechanisms by which LIN-42 controls sleep are largely unknown. However, rhythmic secretion of neuropeptides may play a central role (Nelson et al., 2013; Turek et al., 2016). The neuropeptide encoding genes, nlp-14 and nlp-15, show higher expression levels during lethargus (George-Raizen et al., 2014). NLP-14 and -15 share sequence similarities (~69%) with a class of neuropeptides in arthropods called orcokins (Nathoo et al., 2001). Orcokinins appear to be highly conserved within the Ecdysozoa group. Interestingly, in cockroaches, injection of exogenous orcokinin into the accessory medulla, a circadian pacemaker in the brain, results in a circadian phase shift (Hofer and Homberg, 2006). Taken together, this led us to hypothesize that NLP-14 and -15 regulate sleep in C. elegans. Our data show that nlp-14 but not nlp-15 over-expression induces behavioral quiescence in active adult animals. Specifically, when nlp-14 over-expression is induced under the control of a heat shock promoter, animals become quiescent for locomotion and show prolonged defecation cycles. To understand how NLP-14 induces quiescence, we have conducted a forward genetic screen in which nlp-14 over-expressing worms were randomly mutagenized with EMS, an approach we have taken in the past. The mutated F2 progeny were screened and rare suppressor mutants, that did not become quiescent upon heat-induced over-expression of nlp-14, were isolated. We are currently isolating genomic DNA, which will be followed by whole genome sequencing to identify the causative alleles.


Duchenne muscular dystrophy (DMD) is a lethal degenerative disease that affects 1 in 3,500 males. DMD is caused by mutations in the dystrophin gene, expressed in muscles and nervous tissue. Patients show developmental delays and other neurological phenotypes in addition to muscular decay that eventually leads to death. Much remains unknown about this disease, such as whether any type of muscular exertion might be beneficial for its treatment, or how absence of dystrophin leads to observed neurological phenotypes. We are presently using C. elegans to model DMD to gain insight into these and other questions. To determine if exercise has protective effects on dystrophic muscles, we subjected dystrophic (dys-1) worms to swimming and burrowing regiments in order to mimic strength or endurance treatments respectively. We measured animal locomotion, muscular output (through calcium ratiometry), muscular integrity (through immunohistochemistry), and animal longevity. We find that both exercise treatments negatively affected the health of dystrophic muscle. To determine if C. elegans can be used to model the neurological aspects of DMD, we ran dystrophic worms through a battery of tests and found that while dystrophic worms orient normally to low (0.1%) concentrations of diacetyl, they fail to be repelled by high (99%) concentrations. C. elegans is unique among animals used in DMD research in its ability to model not only the genetic insult, but also the behavioral and cellular phenotypes observed in patients. Future work using this system will shed light on the molecular mechanisms involved in the etiology of DMD, and point to new avenues for its potential treatment.

**767A** MicroRNA regulation of nicotine-dependent behavior in C. elegans. **M. Rauthan**1, J. Gong1,4, E.A. Ronan1,2, J. Liu1,4, S. Wescott1,3, J. Liu4, X.Z.S. Xu1,2,3 1) Life Sciences Institute, University of Michigan, Ann Arbor, MI; 2) Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI; 3) Neuroscience Graduate Program, University of Michigan, Ann Arbor, MI; 4) College of Life Science and Technology and Collaborative Innovation Center for Brain Science, Huazhong University of Science and Technology, Wuhan, Hubei.

Tobacco smoking is the leading cause of preventable death in developed nations. Nicotine is the principle addictive substance in cigarettes. Chronic exposure to nicotine up-regulates nAChRs and is thought to play a critical role in the primary steps of nicotine dependence, but the underlying mechanisms are not well understood. We have previously developed a C. elegans model of nicotine dependent behavior, and shown that the nAChR gene acr-15 is required for acute response to nicotine (Feng et al., 2006). Here we identify a key role for microRNA in regulating nicotine-dependent behavior by modulating nAChR expression in C. elegans. Specifically, we show that chronic nicotine treatment down-regulates microRNA machinery, leading to up-regulation of another nAChR gene that is specifically required for nicotine withdrawal behavior. This effect is mediated by a microRNA that recognizes the 3'UTR of nAChR transcripts. These observations uncover an interesting phenomenon that different nAChRs mediate distinct aspects of nicotine dependence in C. elegans. Our results reveal a functional link between nicotine, microRNA, nAChRs, and nicotine-dependent behavior.

Reference(s):
Changes in external environment or internal metabolic state affect animal behaviors. For example, food availability and nutritional status are critical determinants of this behavioral flexibility. However, the molecular mechanisms by which feeding and nutritional conditions regulate behaviors remain undefined. C. elegans hermaphrodites exhibit avoidance behaviors to acute exposure of a pheromone (referred as ascr#3 or C9). We here show that the ascr#3 avoidance behavior is modulated by nutritional conditions via the regulation of an insulin signaling pathway. We first found that ascr#3 avoidance was decreased in daf-2 insulin-like receptor mutants and mutations of daf-16 FOXO transcription factor suppressed defects of ascr#3 avoidance in daf-2 mutants. Cell-specific expression of daf-2 or daf-16 cDNA in the ADL ascr#3-sensing neurons fully rescued daf-2 or daf-16 mutants respectively. We next found that the ADL Ca^{2+} activity upon ascr#3 exposure was not altered in daf-2 mutants whereas a major ADL post-synaptic target interneuron AVA of daf-2 mutants exhibited decreased Ca^{2+} response to ascr#3 exposure. This altered neuronal activity of AVA in daf-2 mutants appeared to be resulted from decreased levels of synaptic vesicle protein SNB-1 synaptobrevin in ADL. We next screened which insulin-like peptides have roles in ascr#3 avoidance and found that ins-f18 acted in the intestine to mediate ascr#3 avoidance by antagonizing daf-2 signaling. Since the insulin signaling reflects nutritional conditions of an animal, we next tested whether starvation affects ascr#3 avoidance. Interestingly, we found that when animals were starved for 6-12 hours, the ascr#3 avoidance was increased, which was also suppressed by daf-2 mutations. We also found that secretion of the intestinal INS-18 was decreased after starvation. Taken together, we conclude that nutritional changes modulate ascr#3 avoidance via regulation of the insulin signaling pathway in the intestine-sensory neuron axis.

**769C The Analysis of function of TORC2 Signaling Pathway in Associative Learning in Caenorhabditis elegans.** N. Sakai, H. Ohno, M. Tomioka, Y. Iino  Dept. of Biological Sciences., Grad Sch. of Sci.,, Tokyo, JP.

Target of rapamycin (TOR) is a serine/threonine kinase conserved from yeast to humans. Multifunctionality and requirement for survival makes it difficult to understand the in vivo roles of TOR signaling. Here we show that TOR complex 2 (TORC2) contributes to associative learning between salt and food availability in Caenorhabditis elegans. The mutants of TORC2 components, rict-1 and sinh-1, show defects in migration to high salt levels and odor associative learning. We also found the mutants of TORC2 substrates, sgk-1 and pkc-2 show abnormal chemotaxis. Cell specific rescue experiments show that pkc-2 functions in neurons and sgk-1 functions in intestine to regulate associative learning, suggesting that TORC2/PKC-2 signaling and TORC2/SKG-1 signaling function in different tissues to control chemotaxis.

**770A Olfactory memory traces in the C. elegans nervous system.** Vimbai Samukange  Okinawa Institute of Science and Technology Graduate University.

In comparison to the naive worms (control), worms conditioned in distilled water, 1-propanol and HCL were still largely attracted to 1-propanol, however those simultaneously conditioned with 1-propanol and HCL were aversive to 1-propanol. These findings seem to suggest associative learning. Mutant varieties nmr-1 and crh-1 defective of learning and memory were also examined, as expected the worms learning and memory abilities were significantly reduced in comparison to that of the wild type. Factoring past results on the effect of transcription inhibitors on spaced and massed training, it is safe for us to conclude that nmr-1 defective mutants failed to form both LTM and STM/MTM whilst mutants defective in the crh-1 genes failed to form LTM. The nmr-1 gene is expressed in only six neurons, in our next set of experiments we will rescue this gene in each of the individual neurons. Our aim is to identify the neuron/s that is/are most critical for learning and memory formation in C. elegans. The forgetting curve that hypothesizes the decline of memory retention in time or simply put that shows how information is lost over time when there is no attempt to retain it, shows that the rate of memory retention of information learnt through massed training is much more reduced than that learnt through spaced training. Rescuing of the nmr-1 gene in the AVG, PVC, and RIM neurons significantly restored the worm’s memory forming abilities, thus tentatively speaking AVG, PVC, and RIM neurons are most likely to be the critical neurons in the formation of short term aversive associative memory between 1-propanol and acid. We speculate that AVG acts as sensory neuron, whilst PVC acts as an interneuron and RIM acts as effector/motor neuron. The data for the number of GFP + worms on the chemotaxis plates was 10% AVA, 57% AVD, 72% AVE, 30% AVG, 30% PVC, 30% 

**771B A gustatory neural circuit for experience-dependent behavioral plasticity.** H. Sato1, H. Kunitomo1, X. Fei2, K. Hashimoto2, Y. Iino 1) Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo JP; 2) Department of System Information Sciences, Graduate School of Information Sciences, Tohoku University, Sendai JP.

Most animals show experience-dependent behavioral plasticity in response to taste cues. C. elegans is known to memorize the particular concentration of salt (sodium chloride) at which it is cultivated and keeps approaching the memorized concentration. In this study, we searched for the neural circuit required for the memory of salt concentration.

We investigated the relationship between the neural response and locomotion of worms. We used a tracking-imaging system with microfluidic arena that allowed worms to crawl in a controlled liquid environment, and simultaneously recorded neural responses and locomotion of worms. First, we focused on the salt-sensing chemosensory neuron ASER and its downstream neurons AIB and RIM. Worms showed reversal behavior only when the salt concentration changed away from the cultivation concentration. On the other hand, the responses of ASER always correlated with the sensory input, and the responses of AIB
We observe that short fasting (1h) transiently increases chemotactic behaviors and locomotor activity, suggesting increased
food availability promotes wakefulness but sustained insulin signaling after removal from food scarcity thus pose a challenge to neural function. We measure chemotactic behaviors to the food-indicating cue oxygen to investigate behavioral and brain-wide neural activity adaptations under conditions of both short- and long-term food deprivation.

In summary, we propose that quiescence is a default state of neural networks in *C. elegans* and that neural population dynamics are indispensable for nervous system function, even under conditions of energy deprivation. Insulin serves as an arousal cue to maintain these dynamics during periods of fasting. When insulin signaling decreases the energetic cost of neural processing is balanced by the introduction of sleep-like quiescence periods.

**773A** Characterising behavioural defects of the *C. elegans* model of CMT2A. M.S. Soh, J.J. Byrne, G.K. Chandhok, G. Whitbread-Phee, N.M. Yapa, B. Neumann Department of Anatomy and Developmental Biology, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, AU 3800.

Charcot-Marie-Tooth disease (CMT) is characterised by progressive motor and sensory neuropathy, resulting in muscle weakness and mobility impairments. The most common axonal form of CMT, CMT2A, is caused by mutations in the Mitofusin 2 (Mfn2) protein, which is a large GTPase critical for optimal mitochondrial functioning. Despite mutations of Mfn2 first being described as causative for CMT2A more than a decade ago, we still lack a precise understanding of how Mfn2 mutations cause the disease, and thus have a complete lack of effective therapeutics. We aim to model CMT2A in *C. elegans* by targeting the ortholog of Mfn2 in this species (fzo-1) in order to better understand the disease pathophysiology and to discover novel drugs that can modulate the disease. Using CRISPR-Cas9 methods, we have generated a novel allele of *fzo-1* with the entire genomic region deleted (*fzo-1(tm1133)* allele). We have used this allele, as well as the *fzo-1*(*tm1133)* allele, to assess the consequences of *fzo-1* loss-of-function on animal behaviour, with a focus on locomotion and pharyngeal pumping. Notably, the absence of FZO-1 resulted in a significant reduction in the rate of locomotion compared to wild-type (WT) across all age groups tested, as demonstrated in both thrash and body bending assays. These observations mirror the mobility impairments endured by CMT2A patients.

Furthermore, the two *fzo-1* mutant strains displayed progressive defects in pharyngeal pumping compared to WT, with irregular and slower pumping rates observed. As the rate of pharyngeal pumping depends on the cycle of contraction and relaxation of the pharyngeal muscles, which in turn is determined by the pharyngeal muscle action potential, a deficit in pharyngeal pumping rate implies a corresponding defect in the neuronal control of the pharyngeal muscle contraction/relaxation. Finally, we have exploited the locomotor dysfunction of *fzo-1* mutants as a platform for high-throughput screening of small compounds, in order to discover novel modulators that can reverse the movement defects in *fzo-1* mutant animals. Our results help to define the normal biological role of Mfn-2/FZO-1, how this relates to CMT2A, and will also identify effective modulators of the disease phenotypes.

**774B** UNC-103/ERG potassium channel contributes to locomotor quiescence during sleep. Rony Sato 1, Cheryl Van Buskirk1, Paul W. Sternberg2 1) California State University Northridge, Northridge CA; 2) California Institute of Technology, Pasadena CA.

In recent years the field of sleep research has been bolstered by studies in non-mammalian model organisms like *C. elegans*, promising to shed light on the long-standing mystery of the core function of sleep. *C. elegans* has been found to experience two distinct types of behavioral quiescence that each fulfill all of the behavioral criteria for sleep. The first to be discovered was developmentally-timed sleep (DTS), which occurs at the end of each of the four larval stages (Raizen et al., 2008). The other
sleep state can be triggered at any time by exposure to damaging conditions such as noxious heat, tissue damage, and UV exposure, and is referred to as stress-induced sleep (SIS) or recovery sleep (RS) (Hill et al., 2014; Iannacone et al., 2016). We have shown that recovery sleep in *C. elegans* is dependent on EGF signaling, and that robust sleep can be induced at any time, in an EGFR-dependent manner, via forced expression of the EGF ligand LIN-3 (Van Buskirk and Sternberg, 2007). We have taken advantage of this ‘forced-sleep’ assay to uncover genes required for sleep regulation. We are particularly interested in determining which potassium channel genes, if any, contribute to *C. elegans* sleep, as K+ channel mutations produce short sleepers in *Drosophila* and zebrafish. An RNAi screen of K+ channel genes and their regulators identified UNC-103, an ERG-type potassium channel, as required for EGF-induced sleep. Here we show that UNC-103 is required for recovery sleep (RS) and contributes to locomotor quiescence during recovery sleep (RS) and contributes to locomotor quiescence during lethargus (DTS) as well, indicating that these two types of sleep share common downstream effectors. We present evidence for developmental compensation by other K+ channels in the unc-103 null mutant, as short exposure unc-103 RNAi produces a greater sleep disruption than long exposure. We find that the application of ERG-blockers disrupts sleep in an UNC-103-dependent manner. Last, we present the results of our current site of action analyses aimed at determining in which neurons the widely expressed UNC-103 is required during sleep.

**775C  Role of MPS-2 in age-dependent memory decline.** C. Haab1, C. Boglari1, B.G. Fenyves1, P. Mastandreas1, F. Peter1,2, A. Papassotiropoulos1,2,3, A. Stetak1,3,1) Molecular Neuroscience, University of Basel, Basel, Switzerland; 2) Life Sciences Training Facility, Biozentrum, University of Basel, Basel, Switzerland; 3) University Psychiatric Clinics, University of Basel, Basel, Switzerland.

With the increased life expectancy in the modern societies, understanding the molecular background of age-dependent cognitive decline gain more and more relevance. Mink-related peptides (MIRPs or KCNEs) are conserved single-pass transmembrane proteins that associate with voltage-gated pore forming potassium channels (KCs). These KCs, channels are heteromeric complexes that are active alone, however ancillary subunits of the MIRP/KCNE gene family modulate their fundamental properties. Both the Kc channels and the MIRP/KCNE family proteins show a broad expression including the nervous system.

The *C. elegans* genome encodes 4 MIRP homolog proteins (mps-1, -2, -3, -4). Previous studies showed that mps-2 or mps-3 is linked to increased sensitivity to Na+ ions. In addition, MPS-1, -2, -3 proteins were found to interact with the K+ ion-channel KVS-1 in the ADF neuron of *C. elegans* and influence the current characteristics of the ion-channel.

In this study, we investigated the role of MIRP proteins in olfactory associative memory. We identified mps-2, as the only member of the *C. elegans* homolog of the MIRP protein family that alters long-term memory in young adult worms without influencing the learning (acquisition) and short-term memory processes. The role of mps-2 is general; deletion of the gene causes similar phenotype in different olfactory long-term memory assays. Furthermore, we established a long-term salt memory assay and demonstrated that loss of mps-2 impairs gustatory memory. We found that mps-2 expression is up-regulated during long-term memory and in addition, in wild type worms the expression of the gene gradually decreases with age. In order to make a link between mps-2 and age-dependent memory decline, we ectopically expressed mps-2 in aged worms, which restored the impaired memory back to young animals’ performance. Finally, genetic epistasis and qPCR results show that mps-2 acts downstream of the CREB homolog crh-1 and upstream of voltage-gated potassium channels. Thus, MPS-2 may represent a novel component of long-term memory formation and may be responsible for age-dependent memory decline. Due to the functional conservation at the molecular level between *C. elegans* and vertebrates, our results will likely lead to fundamental advances on understanding molecular mechanisms of the age-dependent cognitive decline.

**776A The analysis of neurons and molecules required for the response to multiple sensory inputs using two kinds of odorants.** Y. Suehiro, S. Mitani Department of Physiology, Tokyo Women's Medical University, Shinjuku, Tokyo, JP.

*Caenorhabditis elegans* chooses behavioral patterns appropriately when the worms receive multiple sensory inputs. To investigate the molecules involved in the behavioral choice, we performed screening using 1498 mutant strains by the following assay. We presented two kinds of odorants diacetyl (DAc) and benzaldehyde (Bz), both of which elicit positive chemotaxis by activating different sensory neurons, and counted the number of worms near the source of DAc or Bz. As a result, we found that a mutant of metabotropic glutamate receptor mgl-1 showed excessive choice of the chemotaxis toward DAc. The behavioral phenotype of mgl-1 was rescued by injection of a construct, which contains partial genomic sequences including the mgl-1 coding region and the txt-3 promoter driving gene expression in AY neurons. Additionally, the extent of rescue was significantly changed according to the copy number of mgl-1 in extra-chromosomal arrays. Furthermore, we tested a mutant of HEN-1, which is secreted from AY neurons and is involved in another behavioral choice assay. In our behavioral assay, hen-1 worms showed strong choice of the chemotaxis toward Bz, contrary to mgl-1 worms. Furthermore, the phenotype of hen-1;mgl-1 was similar to that of hen-1 suggesting that MGL-1 works upstream of HEN-1. These data suggest that the behavioral phenotype of the mutant was due to the function of MGL-1 in AY.

Next, to investigate the role of AY neurons in response to complex odorant stimuli, we performed calcium imaging of AY using microfluidic PDMS chips to fix worms. Because AWC and AWA sensory neurons, which are upstream of AY, are reported to respond to the removal of Bz and the increase of DAc, we changed the odorant buffers from Bz to DAc buffer to mimic the situation under which the worms receive two kinds of odorant inputs. In wild-type worms, we found that the switching of the odors caused the temporary inhibition and the following activation of AY, while the AY are inhibited or activated by the excitation of AWC or AWA, suggesting that AY received the inputs from both of AWA and AWC. Therefore, we investigated the response of AY in mgl-1 after the switching of odors and we found that AY tended to be more excitable just after the switching.
neurons morphology during development (Christensen et al., Development, 2011), Neuroligins are evolutionarily conserved post-synaptic cell adhesion molecules that form trans-synaptic complexes with plasticity. Potentially CMK-1-dependent gene expression changes to modulate thermotaxis behavior. Current experiments are aimed at function of B. infantis, one of the well-known probiotic bacteria, extend the lifespan of Caenorhabditis elegans (Ikeda et al., Appl. Environ. Microbiol., 2007). In this study, we found that C. elegans exhibited low preference to B. infantis when compared with a standard food E. coli. Genetic screens identified tol-1 (a sole homolog of mammalian Toll-like receptors) and daf-16/FoxO as positive regulators of low preference to B. infantis. Worms with mutations in canonical TLR signaling molecules (pmk-3, mom-4 and ikb-1) that are employed in avoidance behavior to a pathogenic bacterium Serratia marcescens (Brand et al., Curr. Biol., 2015) showed a normal behavior to B. infantis, suggesting alternative mechanisms. Genetic analyses revealed that the function of daf-16 isoform b in AIY neurons is responsible for the behavior to B. infantis. Because daf-16 b regulates the AIY neurons morphology during development (Christensen et al., Development, 2011), daf-16 b may play development roles in AIY development to organize low preference to B. infantis.

Neural and molecular mechanisms underlying food preference have been poorly understood. We previously showed that Bifidobacterium infantis, one of the well-known probiotic bacteria, extend the lifespan of Caenorhabditis elegans (Ikeda et al., Appl. Environ. Microbiol., 2007). In this study, we found that C. elegans exhibited low preference to B. infantis when compared with a standard food E. coli. Genetic screens identified tol-1 (a sole homolog of mammalian Toll-like receptors) and daf-16/FoxO as positive regulators of low preference to B. infantis. Worms with mutations in canonical TLR signaling molecules (pmk-3, mom-4 and ikb-1) that are employed in avoidance behavior to a pathogenic bacterium Serratia marcescens (Brand et al., Curr. Biol., 2015) showed a normal behavior to B. infantis, suggesting alternative mechanisms. Genetic analyses revealed that the function of daf-16 isoform b in AIY neurons is responsible for the behavior to B. infantis. Because daf-16 b regulates the AIY neurons morphology during development (Christensen et al., Development, 2011), daf-16 b may play development roles in AIY development to organize low preference to B. infantis.

Animals change their behaviors in response to a wide variety of stimuli from the environment for their survival. Neuronal plasticity underlying behavioral change and molecular mechanisms in which neurotransmitters modulate the efficiencies of synaptic transmissions have been extensively studied. However, little is known about molecular mechanisms of forgetting including modulation by neurotransmitters. Olfactory adaptation, in which animals pre-exposed to odorant show reduced chemotaxation to the same odorant, is a useful model to reveal forgetting mechanisms. Olfactory adaptation is reversible, and animals recover from adaptation within a few hours when they are incubated in the absence of the odorant. Our previous studies revealed that tir-1 mutants exhibit prolonged retention of olfactory adaptation through non-cell autonomous mechanism. Interestingly, tir-1 mutants exhibit longer retention of olfactory adaptation only when they are recovered in the presence of food; when they are incubated without food, they recover from adaptation just as the wild type animals. This result suggests that the retention of olfactory adaptation is regulated by food. To reveal how environmental cues like food status regulate forgetting of olfactory adaptation, we analyzed the recovery from olfactory adaptation in monoamine mutants under two different conditions: with food and without food. Mutants defective in synthesis of monoamine neurotransmitters did not show defects in the retention of olfactory adaptation in the presence or the absence of food. However, the chemotactic analyses of the double mutants tdc-1; tir-1 and tir-1; tbh-1 suggest that octopamine mediates food signals in the regulation of retention of olfactory adaptation.

Dynamic regulation of behaviors by past experience allows animals to exhibit responses that are most optimal for their reproduction and survival. Consequently, the same stimulus can elicit different behaviors in different individuals based on the individual’s experience, internal state, and immediate context. The presence of food and feeding status are critical variables that modulate multiple behavioral strategies in C. elegans, including their navigation behaviors on spatial thermal gradients. While well-fed worms migrate to colder temperatures (negative thermotaxis) when they encounter temperatures higher than their cultivation temperature, starvation for =30 min suppresses this behavior. Thus, feeding state-dependent regulation of thermosensory behaviors in C. elegans provides an attractive model system in which to study how internal state (starvation) is integrated with environmental cues (temperature) to alter neuronal circuit functions. Temperature-evoked responses in the AFD thermosensory neurons are largely unaffected upon starvation suggesting that the starvation signal is integrated elsewhere in the thermotaxis circuit to alter behavior. We find that the AWC and ASI sensory neurons play a role in mediating this starvation-dependent behavioral plasticity. Although ablation of ASI alone does not affect this plasticity, ablation of AWC alone, or AWC and ASI together, partially or fully abolish starvation-dependent suppression of negative thermotaxis, respectively. Interestingly, we observe that the subcellular localization of the CMK-1 CaMKI (calcium/calcmodulin-dependent protein kinase I) enzyme we previously implicated in the feeding state-dependent dauer behavioral decision (Neal et al., 2015) is altered in AWC asymmetrically upon starvation. The INS-1 insulin pathway has previously been shown to contribute to starvation-dependent plasticity in thermotaxis behaviors (Kodama et al. 2006). We find that subcellular localization of CMK-1 is also altered in ins-1 mutants. Moreover, we find that basal activity of AWC is increased upon prolonged starvation, and in ins-1 and cmk-1 mutants.

Taken together, we propose that feeding status is integrated into the thermotaxis circuit via regulation of AWC activity and potentially CMK-1-dependent gene expression changes to modulate thermotaxis behavior. Current experiments are aimed at examining the contribution of asymmetry in starvation-dependent changes in AWC function in mediating thermotaxis behavioral plasticity.

Dissecting the role of neurexin and neuroligin in C. elegans chemosensory behaviors. M. L. Tames, Z. Liu, S. Chalasani Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, CA.

Neuroligins are evolutionarily conserved post-synaptic cell adhesion molecules that form trans-synaptic complexes with odors in mgl-1 than wild-type worms. From these data, it is hypothesized that MGL-1 modulates AIY during the behavioral choice depending on its expression level.
presynaptic neurexins. Importantly, mutations in these proteins are associated with devastating human conditions including autism, schizophrenia and others. Mammals have four Neuroligins (NL1-NL4) and three Neurexins (NRXN1-3). Both are type I membrane proteins found at most excitatory and inhibitory synapses. Although it is clear that this is an important pair of signaling molecules, less is known about the mechanisms that contribute to human brain disorders.

The *C. elegans* genome includes two genes, *nrx-1* and *nlg-1*, that encode single-pass transmembrane proteins that are structurally like vertebrate Neurexins and Neuroligins, respectively. While these proteins localize to most *C. elegans* synapses, animals with deletion mutations in *nrx-1* or *nlg-1* are viable and show normal locomotion, suggesting that these genes are not required for general synaptic function. However, others and we have identified specific and robust chemosensory behavioral defects in these mutants. We are using genetic and pharmacological methods to probe the role of *nrx-1* and *nlg-1* in modifying behavior.

### 781C Thermotaxis navigational modeling and virtual reality investigation


Caenorhabditis elegans exhibit a well-characterized host of thermosensory behaviors necessary for efficient navigation, foraging, and survival in the natural environment. Many of the neural structures responsible for temperature sensing have evolved to exceptional sensitivity over time, giving rise to cognitively complex, deterministic behaviors such as bias orientation and 90 degree turning. In order to better comprehend the underlying neural circuitry responsible for such behaviors, multiple PID-controlled hardware systems have been constructed to generate and control various thermal gradient conditions within 0.05 degrees C, over durations of ~30 minutes. Additionally, an IR laser-based thermosensory system has been fabricated, to provide spatiotemporally controlled thermal stimulus at a highly localized region of the worm’s body, enabling the establishment of a virtual thermal environment on which the worm can behave. Using these tools, we have investigated several intricacies of *C. elegans*’ response to temperature, including the mechanism governing 90 degree turns and biased orientation during negative thermotaxis. Making use of line confocal calcium imaging microscopy methods, the dynamics of the AFD sensory neuron has been observed, yielding multiple noteworthy datasets. Custom-written MatLab image processing tools based on Goodam and NEMO were utilized to conduct a systematic motional analysis in a variety of experimental thermal conditions. This poster aims to outline the some of these recent advancements in thermotaxis investigation in the elegant Mind Club, along with preliminary supporting datasets, on a case-by-case basis.

### 782A Calcium dynamics regulates the timing of decision-making in worms

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The brain processes sensory information to generate various physiological responses with different timing (*i.e.*, with different response latencies). In decision-making, for example, animals choose one from multiple behavioral options based on environmental sensory information, with a temporal delay associated with the certainty of sensory information. The neural mechanism of timing, however, is largely unclear. We report the cellular and molecular mechanisms underlying the timing of decision-making during olfactory navigation in worms. Based on subtle changes in concentrations of the repulsive odor 2-nonanone, worms efficiently choose the appropriate migratory direction after multiple trials as a form of behavioral decision-making, which is different from the typical biased random walk. From simultaneous monitoring of behavior and neural activity in virtual odor gradients, we found that two pairs of sensory neurons regulate this behavioral response in an opposing manner with different temporal dynamics. ASH nociceptive neurons exhibit a time-differential response to an increase in the 2-nonanone concentration, which leads to an immediate turning response similar to a "reflex." In contrast, AWB olfactory neurons exhibit a time-integral response to a decrease in the odor concentration, which leads to turn suppression with a temporal delay resembling "deliberation." We further found that the AWB response is independent of synaptic connections and is mediated by a gradual calcium influx, mainly via L-type voltage-gated calcium channel (VGCC) EGL-19, whereas the ASH response is mediated by rapid calcium influx via multiple types of calcium channels. Thus, the timing of neuronal responses, such as deliberate decision-making or rapid reflex, is determined by cell type-dependent involvement of calcium channels. Interestingly, such time-integral neural responses have also been observed in decision-making by primates and rodents, and are considered to be mediated by recurrent neural circuits, although intracellular mechanisms have also been proposed. We suggest that a single-cell temporal integrator with L-type VGCCs, such as the AWB neuron, may be the evolutionarily conserved molecular basis for decision-making.

### 783B MS-based neuropeptidomics in *C. elegans*

**Sven Van Bael,** Samantha Louise Edwards, Kurt Boonen, Wouter De Haes, Liliane Schoofs, Liesbet Temmerman Biology, University of Leuven (KU Leuven), Leuven, BE.

Neuropeptides constitute a functionally diverse family of neurochemical signaling molecules. *C. elegans* is well-suited for the study of neuropeptide biochemistry and function, since numerous peptides are known to contribute to many behavioral regimens, yet deleting peptides - or their processing enzymes altogether - does not affect *C. elegans* viability. We have developed an optimized protocol that enables the detection of about 120 individual peptides per sample (under
reference conditions; treatment may increase or decrease this number), as such vastly increasing the detection potential of C. elegans peptides. As a proof of concept, we applied this protocol to characterize the C. elegans enzymes required for the last step in the production of many bioactive peptides – the carboxyterminal amidation reaction – via mutant analysis, and faulty carboxyterminal amidation indeed results in a severely altered neuropeptide profile. Overall, about half of the more than 250 predicted peptides have been detected with our current method under standard conditions, and we are working on expanding the potential even further.

We estimate the number of currently detectable peptides to be somewhat larger, as it can be expected that several peptides would be produced under specific conditions only, yet some low-abundant peptides that are expressed in only a few cells still escape detection in whole-mount peptidomics. This problem should be solved by our efforts in establishing single-cell peptidomics approaches, which will increase the signal-to-noise ratio drastically.

784C Analyses in forgetting of an olfactory memory in C. elegans. J.H. Teo1, T. Kitazono1, T. Ishihara1,2 1) Graduate School of System Life Sciences, Kyushu University, Fukuoka, Fukuoka, JP; 2) Department of Biology, Faculty of Sciences, Kyushu University, Fukuoka, Fukuoka, JP.

Animals learn and forget information acquired from the environment to strive for survival in changing environment. However, working mechanisms in learning and forgetting remain unclear. Caenorhabditis elegans (C. elegans), which are attracted to odorants such as diacetyl and isoamylalcohol, display weak chemoattraction after prolonged exposure to odorant. Adapted wild type animals recover chemoattraction toward conditioned odorant after 4 hours of normal cultivation on food. This behavioral change is regarded as forgetting. Our previous studies showed that TIR-1/JNK-1 pathway in AWC neurons acts in forgetting the adaptation memory retained in AWA neurons. However, neuronal network between AWC and AWA functions in the forgetting process is not known. To identify neurons that are engaged in this pathway, we investigated interneurons which work downstream of AWC neuron in olfactory circuit. By examined the olfactory behavior of interneuron ablated animals, we discovered that AIB and AIY ablated animals showed normal adaptation and recovery processes after conditioning to odorants. On the other hand, animals lacking functional AIA showed normal adaptation but displayed defect in forgetting of olfactory adaptation to both diacetyl and isoamylalcohol. This suggests that AIA function is important for forgetting. In order to elucidate the forgetting mechanism in which AIA are associated, we inspected interaction of AIA interneurons in TIR-1 pathway by examine tir-1(gf); AIA malfunction double mutant animals. The double mutant animal suppressed adaptation defective phenotype in tir-1(gf), indicates AIA work downstream of TIR-1 pathway in forgetting. In addition, a mutation in egl-21 gene, which encodes carboxypeptidase E (CPE) protein required for neuropeptides processing, caused forgetting defective in olfactory memory. This indicates that neuropeptides release involves in regulating forgetting. Further analyses will be able to provide another insight in neuron networking and molecular mechanisms of forgetting.


Extensive advances have been made in understanding the behavior of C. elegans in two dimensional environments. However, they impose substantial constraints on the worm’s motion and ultimately restrict the set of possible natural behavioral states it can demonstrate. Addressing limitations encountered by previous efforts in three dimensional imaging, we designed and built a microscope capable of tracking the motion of C. elegans via a set of motorized stages while navigating freely within a sample volume. The variation of gelatin concentration (1% - 4%) and the utilization of temporally controlled ultraviolet photo-stimulation (405 nm) were also incorporated into the system. The addition of a refractive index mismatch correction chamber and fluorescence detection enable novel opportunities for observation and categorization of motion. Preliminary data of photoavoidance response in three dimensions was acquired and demonstrates the added complexity present in an unconstrained response. A novel use of fluorescence enables the identification of C. elegans’ absolute orientation with respect to the ventral nerve cord. A model of motion based on sinusoidal wave propagation was applied to C. elegans’ forward locomotion, thereby categorizing a set of three dimensional body states inhabited. From this analysis, we have identified three distinct motional states: one of which is sinusoidal in the worm’s ventrodorsal plane, another which is sinusoidal in their lateral plane, and a final state that is helical in shape. Fitting this parametric model allows the extraction of a variety of wave-based parameters including wavelength, frequency, wave speed and phase difference which may then be correlated with other dynamic quantities and gelatin concentrations. Namely, the phase difference acts as a direct indicator of the degree to which the worm’s posture is planar or helical, allowing the ability to parameterize its general motional form with a single number. Furthermore, from pre-existing, established data of the C. elegans’ connectome, we hypothesize a neuronal mechanism for rhythmic signal generation based on the SMD motor neurons which predicts the motional states observed.

786B Degeneration of dopamine neurons affects memory formation in Caenorhabditis elegans. Vishnu Raj, Agrima Nair, Aswathy Mangalath, Rashita Santhosh, Bejoy Vijayan, Anoopkumar Thekkuveettil Division of Molecular Medicine, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum, IN.

Degeneration of dopamine neurons often found to develop dementia as a comorbidity. It is not deciphered, however, the involvement of the dopamine connectome in memory formation as well as in dementia. Caenorhabditis elegans is an excellent model system to study the connectome variations because of its elementary and well-mapped nervous system. The four CEP neurons in the head region of the organism use dopamine as their neurotransmitter and play a critical role in the movement. To understand the role of dopamine circuit in learning, we used adaptive learning paradigm of Caenorhabditis elegans to olfactory cues. Significantly low associative learning towards butanone was observed in mutant DAT-1::ICE, which develops dopamine neuron degeneration in their late larval stages. These results were comparable to that of mutants of str-2, a G-protein coupled...
receptor in AWC neurons, having a significantly low associative learning. A similar pattern of learning defects were observed in UA-44 strain with age-associated dopamine neurodegeneration. However, Cat-2 and Tph-1 mutants, which are defective in dopamine and serotonin synthesis respectively, showed no significant changes in learning pattern. These findings suggest dopamine connectome have a critical role in memory formation.

787C  **Imprinting enhances learning and memory in *C. elegans***.  Aswathy Mangalath, Vishnu Raj, Agrima Nair, Rasitha Santhosh, *Anoopkumar Thekkuveettil*  Molecular Medicine, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum, Kerala, IN.

Imprinted memory, thought to be an epigenetic phenomenon, develops during a critical period in the early development. This form of memory is an unconditional learning and can last even in adulthood. The role of imprinting in normal learning and memory process is however not known. We addressed this question by inducing imprinted olfactory memory in *C. elegans* and assayed for its impact on olfactory learning and memory. Imprinted wild type N2 worms showed a better learning and memory compared to naive. Sra-11 mutants, a known imprinting defective strain, however, showed significantly better learning. However, cima-1 mutants, defective in A1Y presynaptic G-protein coupled receptor showed both imprinting and learning defect. These results suggested that though both imprinting and learning share same neuronal circuits, the molecular pathways could be unique in both. Besides, results suggest that imprinting involves both sra-11 and cima-1 receptors localized at post- and pre-synaptic terminal of A1Y interneuron, respectively, connecting AWC and RIA.

788A  **Insulin pathway differentially alters learning and memory in *Caenorhabditis elegans***.  Rasitha Santhosh, Agrima Nair, Aswathy Mangalath, Vishnu Raj, Swathy Suresh, *Anoopkumar Thekkuveettil*  Molecular Medicine, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum, Kerala, IN.

Dauer constitutive (Daf-c) mutants of *C. elegans* have been extensively studied as a model to understand the regulatory molecules involved in organismal development and longevity. Recently one of the longevity extending factor, insulin/IGF-1 receptor mutant Daf-2 have been reported to have enhanced learning and memory. To elucidate this further, we tested butanone-associated appetitive learning in insulin mutant ins-1 and found enhanced learning in this strain similar to that of Daf-2. However, daf-16, akt-1 and age-1 mutants did not show a significant reduction in learning, suggesting the limited involvement of Daf-2 downstream pathways in learning and memory. Surprisingly, associative-learning in post-dauer N2 strains showed a significant reduction in learning and memory paradigm compared to the control wild-type adults. Furthermore, 24-hour starved worms also showed significantly low learning and memory. These results suggest that the learning deficiency could be linked to the environment or epigenetic factors during early development of an organism. Besides, AWC sensory neuron, which is known to express the insulin receptor Daf-2 might have a regulatory role in integrating various cues to determine the learning and memory capabilities of the organism.

789B  **Connectome specific storage of short-term and long-term memory in *Caenorhabditis elegans***.  Agrima Nair, Rasitha Santhosh, Vishnu Raj, Aswathy Mangalath, Amal Wilson, *Anoopkumar Thekkuveettil*  Molecular Medicine, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum, Kerala, IN.

Molecular pathways involved in memory formation both long-term and short-term are still unknown, while the involvement of synaptic plasticity in these pathways has been documented. *C. elegans* show distinct short-term and long-term associated memory (STAM and LTAM) towards butanone. Here we use this two forms of memory and assay the connectomes involved in the pathways. Blocking translation using actinomycin-D affected the STAM and LTAM, while blocking transcription using cyclohexamide blocked LTAM and maintained STAM. The role of NMDA receptors was probed using nmr-1 and -2 mutants as well as using AP-5, a NMDA receptor antagonist. All these mutants and the AP-5 treated N2 worms showed a selective loss of LTAM. AMPA receptor glr-2 mutants, however, showed significantly low STAM and LTAM as compared to the control. Interestingly AMPA receptor glr-1 mutants showed STAM. This suggests the existence of selective neuronal circuits in *C. elegans* for short term and long term memory formation. To test this possibility, we assayed sra-11 mutant (AIY neuron-specific protein) and tdc-1 mutant (RIM neuron-specific protein). LTAM showed significant impairment in both these strains while STAM was similar to that of the controls, confirming the involvement of connectome in memory storage.

790C  **A versatile insulin-like signaling regulates taste avoidance learning**.  *M. Tomioka, Y. Inoue* Dept of Biol Sci, Univ of Tokyo, Tokyo, JP.

During life, animals experience a myriad of life-threatening events and cope with them by physiological and behavioral responses. We have found that an alternative splicing (AS) event in the insulin receptor gene, *daf-2*, has strong impacts on several biological processes such as development, lifespan and behavior in *C. elegans*. DAF-2a, which is produced by skipping of a cassette exon mainly resides in the cell body to control transcription of genes related to morphogenesis and stress responses, whereas a cassette exon-inclusion isoform, DAF-2c, is translocated to neuronal axons and regulates learning of the salt concentrations experienced under starvation, which we call taste avoidance learning. This AS event appears to balance physiological and behavioral responses to cope with a variety of life-threatening conditions. Among 40 insulin-like peptides in *C. elegans*, an insulin-like peptide, INS-1, plays an important role in taste avoidance learning. Mutants of INS-1 show a marked defect in taste avoidance learning. INS-1 is secreted from sensory neurons and interneurons that have synaptic inputs to the gustatory neuron ASER and acts on the axonal DAF-2 isoform. DAF-2c, in ASER in the learning. We demonstrated that the INS-1-to-DAF-2c signal during salt chemotaxis, but not during starvation conditioning, is required for starvation-induced salt avoidance. On the other hand, the cell body isoform DAF-2a may control learning during salt conditioning through FOXO transcription factor DAF-16. Our study proposes a model in which the versatile insulin-like signaling acting at different subcellular sites regulates taste avoidance learning in *C. elegans*.  

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791A A forward genetic screen for mutants defective in recovery sleep. Cheryl Van Buskirk, Students of CSUN
BIOL447 F.I.R.E. lab Biology, California State University Northridge, Northridge.

In response to damaging conditions such as noxious heat or UV exposure, C. elegans enters a period of behavioral quiescence known as stress-induced sleep or recovery sleep (RS), during which sensory responses are dampened and feeding and movement cease. Recovery sleep is mediated by activation of EGF receptors on the peptidergic ALA interneuron and subsequent release of a collection of neuropeptides. At this time it is not known how cellular damage leads to the initiation of EGF signaling, and gaps remain in our understanding of signal transduction events within ALA as well as in the target tissues affected by ALA peptides. In order to uncover genes required for recovery sleep we have initiated an EMS screen for sleepless F2 animals, using the pore-forming toxin Cry5B as our damage-inducing agent. Progeny of sleepless candidates are tested for responses to other known sleep-inducing stressors, such as heat and UV light. Mutants that are found to be generally defective in recovery sleep are kept for SNP mapping, complementation as needed, and eventual whole-genome sequencing. We will present our detailed methods, some obstacles that have been overcome in our mapping of sleep mutants, and information on candidate identity if available. We also describe how this project has been implemented within the context of an undergraduate laboratory course called BIOL447 FIRE: Full Immersion Research Experience.


Many behavioral responses of the nematode C. elegans show a degree of variation that suggests stochasticity in the underlying molecular pathways. We use chemotaxis to NaCl to investigate this process. C. elegans shows attraction to NaCl concentrations between 0.1-200 mM and avoidance of higher concentrations. This response can be analyzed behaviorally with quadrant assays in which worms are presented with a choice between buffered agar with or without NaCl. When animals are given a choice between 0 and 100 mM NaCl, WT worms move to the salt containing quadrants. However, a small fraction of (genetically identical) animals will end up on the quadrants without salt. We aim to explain the variability in C. elegans behavior by examining stochasticity at a molecular and cellular level. Perception of NaCl probably occurs by receptors and channels in the sensory cilia of the ASE neurons, including the guanylate cyclase subunits gcy-22 and gcy-14 and the epithelial sodium channel (ENaC) subunits del-2, del-4 and del-6. Stochasticity could be caused by variability in the localization of these proteins, their in- and export into and from the cilia, and by variability in intraflagellar transport (IFT). We use CRISPR/Cas9 technology to tag proteins of interest with full length or split GFP (spGFP) to visualize their transport dynamics and localization, and correlate this with the cellular or behavioral response to NaCl.

Thus far, we have generated del-6::spGFP, del-2::spGFP and gcy-22::spGFP worm strains. We found that GCY-22::spGFP localizes at the tip of the cillum and at the base of the cillum in the peri ciliary membrane compartment (PCMC) in ASE(R), similar to the published localization pattern of GCY-22::GFP fusion construct and the previously reported localization of GCY-14::GFP in ASE(L). Thus far, we were unable to show active transport of GCY-22::spGFP between the PCMC and the ciliary tip using live imaging and kymography. FRAP experiments suggest that GCY-22::spGFP fluorescence at the tip or PCMC recovers very slowly. We are currently performing FRAP experiments to determine the motility of the protein within these regions. DEL-6::spGFP localizes to the body wall muscles and hypodermis, but neuronal expression remains unclear. DEL-2::spGFP, on the other hand, shows clear localization at the base of the cillum in several head neurons, including ASE.

In future experiments, we will examine the variability in transport and localization of NaCl-receptor/channel proteins in ASE neurons and its cilia and test if this variability correlates with variation in gene expression, intracellular transport and/or IFT. Finally, we will analyze if this variability correlates with stochasticity of the response to NaCl.

793C Role of a MEC-4-actin interaction in the gentle touch neurons of Caenorhabditis elegans. D.S. Walker, W.R. Schafer MRC Laboratory of Molecular Biology, Cambridge, GB.

The response to gentle body touch requires the touch receptor neurons (TRNs); and components of the presumed mechanotransduction apparatus have been identified via mutant screens. MEC-4 and MEC-10 are members of the DEG/ENaC family of amiloride sensitive sodium channels. Proper, punctate localisation of MEC-4 along the length of the TRNs requires 15 protofilament microtubules (MEC-7 and MEC-12), and extracellular matrix components (MEC-1, MEC-5 and MEC-9). These were thought to act together to anchor the channel and thus allow mechanical gating, but MEC-4 does not localise to the ends of microtubules, so their precise role is unclear. While MEC-4 is essential for gentle touch, MEC-10 has a more limited, spatially confined role, is differently localised, and is additionally required for the response to harsh touch. We are interested in understanding the cellular basis of differential MEC-4 and MEC-10 localisation and how their distinct functions are determined. We describe the identification of a novel interaction between the C-terminal cytoplasmic domain of MEC-4 and actin. We show that the C-terminal domain of MEC-4 determines its localisation; and that actin is required for correct localisation of MEC-4. Expression of a competing peptide indicates that the interaction is required for the gentle touch response. We are investigating...
whether actin colocalises with MEC-4 and thus could perform an anchoring role in gating and whether the interaction is a target for regulation.

794A  The roles of glial xenobiotic metabolizing enzymes (XMEs) in chemosensation.  S.W. Wallace, S. Shaham Laboratory of Developmental Genetics, The Rockefeller University, New York, NY10065.

Animals sense their environment using sense organs, consisting of sensory neurons and associated glial cells. Chemosensory signal transduction often takes place at ciliated receptive-endings of sensory neurons, where odorant molecules bind to odorant receptors. These cilia associate with sense-organ glia, and are bathed in a glia-secreted extracellular matrix. While most studies on odor perception and associated behavioral responses have focused on information processing downstream of sensory neuron activation, there is increasing evidence for modulation of sensory responses at the periphery, including regulation of odorant-receptor interactions.

Xenobiotic metabolizing enzymes (XMEs) play key roles in modifying xenobiotic compounds, including drugs and toxins. XME substrates are hydrophobic compounds that are sequentially modified by phase I enzymes, including cytochrome P450 monooxygenases (CYPs) and carboxylesterases (CEs), to create functional groups, and phase II enzymes, including UDP-glucuronosyltransferases (UGTs) and glutathione s-transferases (GSTs), which add hydrophilic conjugates. These modifications make hydrophobic substrates more soluble, facilitating their excretion from cells through membrane transporters.

In mammals XMEs are expressed in the liver, where xenobiotic detoxification takes place, and to varying degrees in other tissues. Highest extra-hepatic expression is in the olfactory epithelium. XME proteins localize to the apical region of sustentacular glial cells, in close proximity to cilia of olfactory receptor neurons. Many XMEs contain signal peptides, suggesting they may be secreted into the olfactory mucus, and enzymatic activity has been observed in nasal mucus extracts. It has therefore been proposed that XMEs modify odorant molecules to regulate local odorant levels at sites of sensory signal transduction. This idea is supported by in vitro data showing that odorant molecules are modified by reconstituted XMEs, and that modified odorants elicit altered sensory responses. However the role of XMEs in odor sensation in vivo remains unclear.

We use the C. elegans amphid sensory neurons and their associated AMsh glia as a model to study glial functions in the nervous system. Through RNaseq gene expression analysis, we identified homologs of both phase I and phase II XMEs enriched in AMsh glia. We are using genetic and pharmacological manipulation of XMEs to assess whether glial XME activity regulates neuronal responses to odorants. Preliminary results suggest that XME expression in AMsh glia is regulated by experience, raising the intriguing possibility that modification of odorant molecules by glial XMEs may contribute to experience-dependent changes in behavior.

795B  Molecular basis of the nematode volatile sex pheromone perception.  Xuan Wan, King L. Chow  Division of Life Science, Hong Kong Univ Sci & Technol, Hong Kong, HK.

Both sexually matured C. remanei virgin females and self-sperm exhausted C. elegans hermaphrodites release a long-range volatile sex pheromone to attract adult males from afar. Previously, we reported that this chemotaxis behavior requires CEM, AWA and AIZ neurons. In AWA, a GPCR SRD-1 has been demonstrated as the receptor of this pheromone. Ectopic expression of srd-1 in AWB in srd-1 mutant in AWA male elicited distinct repulsive behavior towards pheromone; this result indicated the sufficiency of the SRD-1 receptor acting in AWA. From calcium imaging, we further confirmed the receptor was necessary for the excitation of AWA upon pheromone induction.

Male C. elegans is the least attracted to pheromone in comparison to C. briggsae, C. remanei, and C. brenneri. Endogenous srd-1 expression level is lower than that of its orthologue, cre-srd-1, in male C. remanei. Transformation rescue of the male srd-1 mutant in C. elegans by cre-srd-1 cDNA was shown to be more responsive to pheromone than those rescued by srd-1 cDNA. Based on protein structure analysis and protein sequence alignment, SRD-1 receptors across four nematode species were highly conserved in terms of their sequences and structure, except their C-terminal region (CT). Six amino acid sequences are polymorphic in C. elegans SRD-1 extracellular loop regions (ECL). The ECL polymorphisms substituted versions of SRD-1 cDNA cannot elicit a higher responsiveness to this pheromone. However, CT truncated cre-srd-1 and srd-1 cDNA are unable to rescue srd-1 chemotaxis mutant phenotype, suggesting the CT region to be critical for SRD-1 function. Specifically, after the CT domain was swapped between Cre-SRD-1 and SRD-1, the transgenic line carrying cre-srd-1-CT-swapped-in-srd-1 cDNA is more attracted to this pheromone than the one carrying srd-1-CT-swapped-in-cre-srd-1 cDNA. Since the CT region in GPCR is usually related to the intracellular signal transduction, we envision that both the expression level of gene product and intracellular signal transduction relay component may account for the differential response of different species in sex pheromone perception. (The study is supported by Research Grants Council, Hong Kong.)

796C  Robust and sensitive cGMP sensor for real time imaging of intact C. elegans.  Sarah Woldemariam1, Jatin Nagpal2, Yanxun Yu3, Joy Li4, Raakhee Shankar5, Benjamin Barsi-Rhyne5, Alan Tran6, Mary Futey1, Michelle Krzyzanowski5, Denise Ferkey5, Miri VanHoven4, Piali Sengupta3, Alexander Gottschalk5, Noelle L’Etoile1 1) UCSF, San Francisco, CA; 2) Goethe University, Frankfurt, Germany; 3) Brandeis University, Waltham, MA; 4) San Jose State University, San Jose, CA; 5) SUNY Buffalo, Buffalo, NY.

cGMP is a ubiquitous second messenger implicated in many important biological processes. In neurons, cGMP dynamics can regulate the function of ion channels and kinases, resulting in physiological changes. In order to understand how cGMP dynamics in neurons are modulated, we need a tool to visualize cGMP. To this end, we are characterizing a EGFP based cGMP
sensor codon optimized for use in *C. elegans* that will allow us to image cGMP dynamics in neurons in the living behaving animal. We measured its kinetics by coexpressing the sensor with the optogenetic guanylyl cyclase BeCyclOp in body wall muscle and measured its dynamics in response to numerous stimuli, including changing ion concentrations, noxious stimuli and temperature.

797A Phenotypic profiling of missense variants implicated in autism spectrum disorders. WR. S. Wong1, K. Brugman1, S. Maher1, S. Gharib1, J.Y. Oh1, M. Kato1, P. W. Sternberg1,2 1) Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA; 2) Howard Hughes Medical Institute.

Autism spectrum disorder (ASD) is a neurodevelopmental disorder with a strong genetic component. Human genetic studies have identified thousands of ASD-associated variants in over 850 genes. Understanding the physiological consequences of these variants will help characterize the essential components of ASD-associated genes. Here, we use *C. elegans* as genetic model to prioritize autism candidate alleles from the Simons Foundation Autism Research Initiative. We established an analysis pipeline. First, we use bioinformatics to filter human ASD-associated missense variants conserved in *C. elegans* protein sequence. Second, we construct the missense mutants using CRISPR/Cas9 and homology-directed repair knock-in technique. To analyze potential causal effects of autism-associated alleles, we then compare the phenotype of autism-associated alleles to the wild-type and the known loss-of-function allele of the same gene. For example, Leu1220Pro and Arg2624Gln missense mutations in *chd-7*, worm orthologs of human *CHD7/CHD8*, displayed decreased brood size compared to wild-type. Val369Met and Lys371Ser mutants of *egl-19*, worm ortholog of human *CACNA1D*, displayed subtle locomotion changes revealed by a quantitative locomotor tracking system. Our approach will help characterize novel missense alleles with human disease relevance and filter functional important alleles. This information will allow interpretation of these variants in genetic studies, and offer clear priorities for detailed studies in vertebrate models or human cells.

798B Investigating the role and mechanism of UBR-1, the *C. elegans* homolog of the Johanson-Blizzard Syndrome causative E3 ligase. M. Wu1,2, W. Hung3, V. Susoy3, J. Meng3,4, Y. Wang2, J. Chitturi1,2, M. Zhen1,2,4 1) Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada; 2) Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; 3) Center for Brain Science, Harvard University, Cambridge, MA; 4) Department of Physiology, University of Toronto, Toronto, ON, Canada.

Johanson-Blizzard syndrome (JBS) is a recessive systemic disorder, characterized by a combination of congenital abnormalities including defects in the nervous system and pancreas. Loss of function mutations in an E3 ligase UBR1 are responsible for the disorder, but the underlying mechanisms of how the loss of UBR1 activity gives rise to the pleiotropic symptoms of JBS remains elusive. Previously, our lab isolated several loss-of-function alleles of the *C. elegans* UBR-1, and found it to exhibit an altered bending pattern during reversal locomotion (Chitturi et al., manuscript in preparation). Here I present results that investigate the circuit defects that underlie this defect. Using CRISPR-Cas9-mediated genomic editing, I generated an allele of *ubr-1* where the RING domain, critical for the E3 ligase activities, is removed. I found that the motor defects of this and other alleles of *ubr-1* mutants are likely caused by motor state transitions. I am currently identifying the key cells through which UBR-1 regulates motor state transitions. Through investigating the *C. elegans* UBR-1 model, I aim to uncover the molecular pathways through which UBR-1 regulates neuronal and cellular functions.

799C Dissecting a network of the insulin-like peptides underlying olfactory learning. T. Wu1, M. Choi1, A. Caballero2, D. Fernandes de Abreu2, I. Katic3, M. Regenass3, J. Aledo3,4, Q. Ch’ng2, Y. Zhang1 1) Department of Organismic and Evolutionary Biology, Center for Brain Science, Harvard University, Cambridge, MA; 2) Centre for Developmental Neurobiology, King’s College London, London SE1 1UL, UK; 3) Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, CH-4058 Basel, Switzerland; 4) Department of Biological Sciences, Wayne State University, Detroit, MI, USA.

Insulin and insulin-like peptides (ILPs) act through conserved signaling pathways to regulate neural plasticity in both invertebrates and vertebrates. The genomes of many animals, including that of the humans, encode multiple ILPs. Our previous studies characterize two *C. elegans* ILPs that regulate a form of aversive olfactory learning in the nematode. These two ILPs interact with other ILP family members in a transcriptional network (Chen et al., 2013; Fernandes de Abreu et al., 2014). We hypothesize that multiple ILPs act as a network to regulate physiological events, including learning, in response to various contexts. To address this hypothesis, we have examined the learning ability in the deletion mutants of the ILPs in the network. We find that deleting *ins-4*, *ins-8* or *ins-35* significantly disrupt the aversive olfactory learning. We show that these ILPs are expressed in different sensory neurons and that the aversive training alters the neuronal expressions. In addition, other types of experience, such as pheromone exposure and starvation, regulate the expression of these ILPs differently. Together, these results support our hypothesis that ILPs regulate learning in an interactive network that responds to the environment and experience in a context-dependent manner.

800A Gap junction networks within the motor circuit of *C. elegans* facilitate undulatory wave propagation. Tianqi Xu1, Jing Huo1, Shuai Shao1, Min Wu2, Michelle Po2, Mei Zhen2, Quan Wen1 1) Neurobiology and Biophysics, University of Science and Technology of China, Hefei, Hefei, CN; 2) Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada.
Gap junction communication between neurons is prevalent in the motor circuit of invertebrates and vertebrates, yet how electrical couplings might control and coordinate locomotion during normal animal behavior remains largely elusive. The anatomical wiring diagram of C. elegans nervous system suggests that (1) the command interneuron AVB makes en passant electrical synapses with most B-type motor neurons distributed along the worm body; (2) nearby B-type motor neurons are also electrically coupled. What are the functions of gap junction highway and byway in the worm motor circuit? By combining genetic analysis, optogenetic manipulation and computational modeling, we first showed that AVB-B electrical inputs induced a bifurcation in B motor neuron dynamics, and they work synergistically with proprioceptive couplings to enhance sequential activation of motor activities and to equalize the bending amplitude along the worm body. Second, weak electrical couplings between B motor neurons could retrogradely mediate the dynamics of head bending activities. Taken together, gap junction networks within the worm motor circuit facilitate a well-formed and flexible body undulation to propagate from head to tail during C. elegans forward locomotion.

801B  

**trp-1 and trp-2 TRPC channels mediate proprioceptive regulation of C. elegans locomotion.**  

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In animals, locomotion is mediated by highly coordinated sensorimotor feedback system. C. elegans generates sinusoidal locomotion via periodic bending of its head and body. The SMD motor neurons, that innervate to head muscles and project posterior processes to the tail, have been proposed to sense the muscle stretch and regulate head locomotion (White et al., 1986; Hendricks et al., 2012; Shen et al., 2016). However, the molecular mechanisms by which SMD mediates proprioceptive signals to modulate locomotion are still unclear. To identify factors that regulate SMD-mediated head bending, we performed candidate gene search and found that TRPC channels, *trp-1* and *trp-2*, are co-expressed in SMD (Feng et al., 2006). Since we did not observed altered locomotion defects in single mutants of either *trp-1* or *trp-2*, we generated *trp-1 trp-2* double mutants and found that these animals exhibit ventral-directed circles during forward movement; we name this phenotype as ventral circling. Expression of either *TRP-1* or *TRP-2* by using a SMDDD specific promoter rescued ventral circling phenotype of *trp-1 trp-2* mutants, and SMDDD ablated worms also exhibited ventral circling. These results indicate that the ventral circling phenotype of *trp-1 trp-2* mutants is due to the functional defects of SMDDD. Ca²⁺ activity of SMD is correlated with head bending direction in wild-type animals, whereas Ca²⁺ activity SMDDD but not SMDV is not correlated with head bending in *trp-1 trp-2* mutants. These impaired correlation Ca²⁺ dynamic of SMDDD with head bending in *trp-1 trp-2* double mutants was restored by expressing *trp-1* cDNA using the SMDDD specific promoter. Furthermore, ectopic expression of the known stretch receptors, *C. elegans trp-4* or *Drosophila TRP*? in SMDDD were sufficient to rescue ventral circling locomotion of *trp-1 trp-2* double mutants. Currently, we are investigating whether activations of *TRP-1* or *TRP-2* are induced by membrane stretch using electrophysiology in heterologous systems. Taken together, we propose that *trp-1* and *trp-2* act as proprioceptive receptors in the SMD motor neurons to sense the dorsal head movement and correlate SMDDD motor neuronal activity with head bending.

802C  

**Non-associative learning as a behavioral strategy to promote dispersal.**  

A. Yu¹, E. Ardiel¹, C. Rankin¹ ¹²) ¹) DM Centre for Brain Health, University of British Columbia, Vancouver, British Columbia, CA; ²) Department of Psychology, University of British Columbia, Vancouver, British Columbia, CA.

Habituation is a form of non-associative learning defined as decremented likelihood and/or magnitude in response to repeatedly presented stimuli. Habituation is thought to help animal ignore recurring innocuous events and free up its neural resources to attend to other novel or salient stimuli in the environment. *C. elegans* is known to habituate to harmless stimuli such as non-localized tap (Rankin et al., 1990). Recently, we have reported that worms also displayed behavioral characteristics of habituation when a pair of nociceptor neurons, ASH, were repeatedly stimulated with optogenetics (Ardiel et al., 2016). Because ASH detects natural aversive stimuli that are harmful and potentially lethal to worms, it seems puzzling that animals habituate to these noxious stimuli. Our analysis used a non-response-centric approach and found that habituation of ASH-mediated reversal responses are accompanied by the simultaneous sensitization of forward locomotion (Ardiel et al., 2017, unpublished). This behavioral plasticity was found to be mediated by the Pigment Dispersal Factor (PDF) signaling pathway. These coordinated changes in different behavioral components form an optimal behavioral strategy to guide animals to spend less time responding to the recurrent noxious stimuli and more time moving away from them. In this present study, we investigated whether tap habituation was associated with changes in other behavioral components. We observed similar patterns of behavioral plasticity in tap habituation that is also mediated by PDF signaling. Our findings suggest that different forms of non-associative learning participate in a suite of coordinated behavioral changes that together promote a shift in behavioral strategy. Our study also highlights the needs for more detailed behavioral analyses.

803A  

**Stereospecific bioactivity of the eicosanoid 17,18-epoxyeicosatetraenoic acid on the pharyngeal pumping of Caenorhabditis elegans.**  

Yiwen Zhou, Ralph Menzel  Institute of Biology, Ecology, Humboldt-University of Berlin, Germany.  

17,18-epoxyeicosatetraenoic acid (17,18-EEQ), the most abundant cytochrome P450 (CYP)-eicosanoid in *C. elegans*, is a potential signaling molecule in the regulation of pharyngeal pumping activity. Here we tested the hypothesis that stereodiscrimination of this chiral molecule may hint the existence of a receptor in the pharyngeal muscle and/or neuronal cells.
We analyzed the effect of short-term incubation in both well fed and starved condition and found that the stereoisomer 17(R),18(S)-EEQ, clearly rescued impairments of pharyngeal pumping in D-6 fatty acid desaturase mutant strain fat-3(wa22). After treatment with the 17(S),18(R)-EEQ stereoisomer, however, we found no effect on pumping activity. In addition, we will analyze the total endogenous eicosanoid stereoisomer content of *C. elegans* by chiral LC/MS-MS to see whether or not only specific stereoisomers are endogenous resources.

17(R),18(S)-EEQ was more active in *ceeh-1(ok3153)* mutant background in comparison to other stains. We suspect that *ceeh-1(ok3153)*, failing to produce a functional active soluble hydrolase (sEH), is unable to efficiently catalyze the hydrolysis of 17,18-EEQ to the corresponding diol (17,18-DHEQ), which we found correlated with a loss of function of this signaling molecule. 17,18-DHEQ treatment was inactive in a *C. elegans* pumping assay.

**804B Disentangling the CYP-eicosanoids dependent regulation of the pharyngeal pumping in *Caenorhabditis elegans***. Yiwen Zhou, Ralph Menzel  Institute of Biology, Ecology, Humboldt-University of Berlin, Germany.

Cytochrome P450 (CYP)-dependent eicosanoids are epoxygenated and/or hydroxylated metabolites of long-chain polyunsaturated fatty acids (LC-PUFAs). They are important lipid mediators participating in numerous physiological and pathological processes in mammalian systems. The CYP-eicosanoid cascade is initiated by the release of LC-PUFA from cell membrane phospholipids by a phospholipase A2, it follows the CYP-dependent eicosanoids biosynthesis and the interaction of eicosanoids with so far unknown cell-surface receptors that are expected to be part of the G-protein coupled receptor (GPCR) family.

Recent studies revealed that the nematode *Caenorhabditis elegans* also produces a broad spectrum of CYP eicosanoids, in particular, epoxy- and hydroxy-metabolites derived predominantly from eicosapentaenoic acid (EPA). One revealed physiological function in the nematode is involved in the regulation of pharyngeal pumping. 17,18-epoxyeicosatetraenic acid (17,18-EEQ), as the most abundant CYP eicosanoid in *C. elegans*, mimics the stimulating effect of serotonin and rescues LC-PUFA deficient strains from their pumping impairments. Here we tested the hypothesis that 17,18-EEQ serves as second messenger in the underlying signaling pathway and exerts its effect by binding to a specific membrane G-protein-coupled receptor in the pharynx of nematode. We performed a biased screen of selected gene knockout strains responding to 17,18-EEQ treatment. Moreover, manual counting of the pharyngeal pumping frequency in the presence or absence of supplemental 17,18-EEQ was performed. Two of the candidate genes are coding for GPCRs, which indicates that these proteins might be involved in the 17,18-EEQ signaling of *C. elegans*.

**Neurobiology - Neuronal Development**

**805C The Effects of Altered Gravity on Aging in Motor Neurons.** A. Alcantara, S. Kalichamy, J.I. Lee  Division of Biological Science and Technology, Yonsei University, Wonju, Gangwon-do, KR.

As the world’s population increases, humans will begin to populate space. With today’s technological developments, migration of humanity in space might be possible in the near future. Still, human bodies are not conditioned for a long-term altered gravity environment, and the full effects on developmental and physiological process is not known. We wanted to know whether long-term changes in gravity could affect aging and lifespan, which is relatively unexplored. Previously, we showed that hypergravity can affect development of the DD/VD motor neurons in *C. elegans*. Using a similar experimental paradigm, we exposed *C. elegans* to 100G hypergravity and observed the DD/VD motor neurons using a *p(unc-25):GFP* reporter strain. DD/VD motor neurons normally show progressive defects during the aging process including axonal beading and formation of extraneous neurites similar to aging in other neurons. Upon hypergravity exposure, adults showed a 12% increase of DD/VD axon aging phenotypes even after only 4 days. Genetic experiments with aging mutants are ongoing currently. To understand the effects of microgravity on aging and lifespan, we can simulate microgravity in the laboratory using a clinostat. Currently, we are determining a suitable clinostat condition by varying its speed and measuring the expression of *E. coli* and *C. elegans* known microgravity marker genes. After obtaining simulated microgravity conditions, we will conduct experiments to study the microgravity effects on aging in DD/VD motor neurons and even *C. elegans* lifespan with the eventual hope of confirming any results in space aboard the International Space Station.

**806A An activity-dependent insulin-signaling pathway antagonizes p38 MAPK-dependent gene expression in developing chemosensory neurons.** L. Bayer Horowitz, J. Brandt, N. Ringstad  Skirball Institute of Biomolecular Medicine, Molecular Neurobiology Program and Department of Cell Biology, NYU School of Medicine, New York, NY.

The nervous system comprises diverse and highly specialized neuron-types, each expressing a unique set of genes that define its functional properties. What molecular mechanisms generate diverse neuron types remains a central question in neuroscience. Study of *C. elegans* chemosensory BAG neurons showed that a p38 MAP kinase (MAPK), PMK-3, is required for their proper differentiation. How p38 MAPKs function in neurodifferentiation is poorly understood. To identify molecules that function with PMK-3, we performed a reporter-based screen for mutations that restored expression of a BAG fate-marker to pmk-3 mutants. This screen isolated eighteen mutations, including five loss-of-function alleles of *unc-31*. *unc-31* encodes the ortholog of CAPS, which functions to promote Ca²⁺-dependent exocytosis of dense core vesicles (DCVs). This suggests that neural activity and regulated secretion oppose PMK-3 in regulating neuronal gene expression. Indeed we find that genes that promote neural activity and secretion also mutate to suppress pmk-3. Silencing only BAG neurons sufficed to restore gene
expression to pmk-3 mutants, and we found that regulated neurosecretion is required during development to antagonize PMK-3-dependent gene expression. To identify the factors secreted in an unc-31-dependent manner, we tested mutants linked to distinct types of secreted peptide factors for suppression of pmk-3. Knock-down of the insulin receptor DAF-2 in BAG neurons restored gene expression and function to pmk-3 mutant neurons. Strikingly, we found that pmk-3 mutant BAG neurons overexpress insulin-like peptides compared to wild-type BAG neurons. Together, our data suggest that PMK-3 regulates gene expression in developing chemosensory neurons by inhibiting an autocrine insulin-signaling system. Our findings reveal an unexpected role for both neural activity and insulin signaling during neurodevelopment. The mechanisms revealed by our studies will advance our understanding of mechanisms that couple neural activity to neurodifferentiation.

807B Structural Components and Genetic Requirements of Exophers and their Formation. Ryan Guasp1, Meghan Arnold1, Ilija Melentijevic1, Girish Harinath1, Marton Toth1, Ken Nyguen2, Daniel Taub3,4, Christopher Gabel3,4, Jian Xue1, David Hall2, Monica Driscoll1 1) Department of Molecular Biology and Biochemistry, Nelson Biological Laboratories, Rutgers, The State University of New Jersey, Piscataway, New Jersey 08854, USA; 2) Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461, USA; 3) Department of Physiology and Biophysics, Boston University School of Medicine, Boston, Massachusetts 02118, USA; 4) Boston University Photonics Center, Boston, Massachusetts 02215, USA.

Combating late-onset neurodegenerative disease and age-associated cognitive decline are major world health challenges. A striking commonality between neurodegenerative pathology and age-associated decline is the aggregation of proteins. Protein aggregates can move from neurons to surrounding cells, potentially promoting pathology spread, however the mechanism is largely a mystery.

We previously found that some C. elegans neurons can dramatically extrude aggregates within large released vesicles, which we called exophers (Melentijevic 2017). To form an exopher, cytoplasmic materials such as aggregates, damaged mitochondria, and lysosomes become concentrated at the periphery of the soma. The material that will be exported is included as the membrane buds outward, forming a vesicle that moves away from the soma. The large vesicle can remain attached to the soma via a thin filament that can transfer tagged protein and calcium into the exopher compartment. Genetic conditions that compromise proteostasis enhance exopher production and sensitized neurons that produce exophers function better than those that do not. Thus, exophers appear neuroprotective.

Exophers derived from touch neurons must traverse the hypodermal syncytium that surrounds the touch neurons, and EM images support that the hypodermis responds vigorously to exopher content. Electron microscopy images reveal that extruded ALMR exophers have a very heterogeneous, multilamellar, and multicompartmental structure within the hypodermis. It is likely that hypodermal lysosomes degrade the exopher content, however some non-digestible material such as mCherry appears to be later released into the pseudocoelom where it can be taken up by coelomocytes. Exopher formation and expulsion may shed light on how aggregated proteins can be released into neighboring cells, and how those cells react to transcellular cargo.

We will present data on screens intended to define the genetic components needed for exopher formation and the cytoskeletal components needed for exopher extrusion.


808C Transcription factor control of synaptogenesis and neuronal circuit assembly. E.G. Berghoff, O. Hobert Biological Sciences, Columbia University, Howard Hughes Medical Institute, New York, NY.

In nervous system development, neurons must become synaptically connected and assembled into circuits. An important unexplored question is whether there is inherent regulatory logic that is used during this process. Our lab began to ask this question because of our nervous system-wide analysis of the specification of neurotransmitter identity. Through this analysis, we discovered that neurotransmitter identity was specified by transcription factors that are expressed in subsets of neurons of different types and different neurotransmitters. Further, we found that these transcription factors are expressed in subsets of neurons that are often synaptically connected. Thus, we hypothesize that there are “connectivity transcription factors” that control synaptic communication, synaptic wiring, and circuit assembly. To test this hypothesis, I am studying a homeodomain transcription factor, UNC-42, in C. elegans. This transcription factor is expressed in 40 neurons of various type and neurotransmitter identity, and all 40 of the neurons are synaptically connected. I have found that UNC-42 controls the expression of both synaptic communication and synaptic adhesion molecules in UNC-42 expressing neurons only through unc-42(e419) mutant analysis of fluorescent reporter genes. I am currently assessing the impact of UNC-42 on chemical and electrical synapses between UNC-42 expressing neurons through unc-42(e419) mutant analysis of GRASP and innexin fosmid-based reporters, respectively. This study of transcription factor control of synaptogenesis and circuit assembly in an invertebrate organism may reveal organizational strategies that could be used to predict neural circuits across phyla.

809A A direct glia-to-neuron cell fate switch. R. C. Bonnington1, M. Sammut1, B. Kim2, S. Cook2, L. Molina-Garcia1, K. Khambhaita1, D. J. Elliott1, D. H. Hall2, S. W. Emmons2, A. Barrios1, R. J. Poole1 1) Cell and Developmental Biology, University College London, London, London, GB; 2) Albert Einstein College of Medicine, New York, USA.

Whether cell fate is restricted or plastic during development is a key question in developmental biology. In vertebrates, differentiated glia can give rise to neurons but how they retain neurogenic potential remains poorly understood. Our lab recently described how the amphibian scaffold (AMso) glial cells divide during male sexual maturation to self-renew and give rise to a pair of
interneurons, the mystery cells of the male (MCM). Here, we demonstrate that during early L4 in males, one pair of the phasmid socket (PHso1) glial cells undergoes a direct glia-to-neuron cell fate switch, becoming a bilateral pair of cholinergic sensory neurons that we have termed phasmid neuron D (PHD).

In 1980, John Sulston described the postembryonic development of phasmid sensilla. In hermaphrodites, PHso1 is a true socket cell and PHso2 an accessory cell. In males, PHso2 is the main socket and PHso1 projects into the sheath and contains one or two basal bodies but Sulston noted no other neuronal characteristics. Using a lin-48 reporter, we find that during male sexual maturation, the PHso1 cells retract from the socket and simultaneously project anteriorly, synapsing with neurons in the preanal ganglion. Concomitantly, the PHso1 cells downregulate glial markers (mir-228 and grrl-2) and upregulate neuronal markers (rab-3, snb-1, oig-8, unc-17 and osm-6). Expression of osm-6 and unc-17 indicates that PHDs are cholinergic ciliated sensory neurons. Transgenic manipulation reveals the PHso1-to-PHD cell fate switch is cell-intrinsically regulated by the sex-determination pathway. Ultrastructural analysis of PHD confirms the presence of cilia, along with dense-core and synaptic vesicles, and shows several AFD-like villi. Reconstruction of the full connectome indicates the PHDs are heavily connected to male-specific circuits involved in body posture, locomotion, and insemination. Consistent with this, live calcium-imaging in moving animals reveals the PHD neurons are activated during specific mating steps.

We demonstrate that PHso1-to-PHD cell fate switch is a sex-specific direct glia-to-neuron conversion. As the AMso and PHso1 glial cells remain stably differentiated in hermaphrodites, this indicates a key role for glial developmental plasticity during male sexual maturation, remodeling circuits vital for reproductive success. This provides a paradigm to explore mechanisms of plasticity in glia-to-neuron cell fate switches.

810B  A Comprehensive Map of Regulatory Molecules that Determine Terminal Neuron Fate.  Molly Booth¹, Oliver Hobert¹,²  1) Biological Sciences, Columbia University, New York, NY; 2) Howard Hughes Medical Institute.

Cellular differentiation is the foundation of all complex biological life. In the nervous system, we see some of the most diverse cell morphologies to allow processing of vast external stimuli into a measured behavioral response. To create this diversity of neuron subsets, distinct combinations of transcription factors regulate the terminal fate of every neuron - including features like synaptic organization, neurotransmitter machinery, and possibly even circuit assembly. Previous research in C. elegans has demonstrated that these transcription factors directly bind to cis regulatory regions in the DNA to create a unique combinatorial specification code for each neuron. Currently, 28 of these neuronal regulators have been identified and most of them are homeodomain transcription factors belonging to the homeobox gene family. In distinct combinations, the identified regulators are involved in specifying 73 out of 118 neuron classes. However, they are not a complete picture of specification among those classes and the remaining 45 neuron classes have no identified regulators. Without a more complete picture of these regulatory molecules, a fundamental logic of neuron specification may be hard to discern. To discover new regulators and thus uncover this combinatorial logic, I utilize the genetically amendable C. elegans to survey homeobox expression at a cellular resolution and determine if they impact fate specification in every neuron in the nervous system. Presently, I have identified sites of expression for 20 previously uncharacterized homeobox genes through colocalization with 3 well-characterized neurotransmitter reporter strains. And described regulatory functions for 10 of them by mutant analysis. This candidate approach will likely define regulatory molecules involved in specification of every neuron class in the C. elegans nervous system and, in doing so, create the first complete map of regulatory molecules in any organism.

811C  Fasciculation with Axons Patterns Dendritic Arborization in C. elegans.  Chun-Hao Chen, Chun-Liang Pan  Institute of Molecular Medicine, National Taiwan University, Taiwan.

Selective fasciculation of axons and dendrites is a pivotal mechanism that shapes neuronal trajectory and circuit connectivity. While the molecular mechanisms of axon fasciculation have been well studied, little is known about dendrite bundling and its impact on patterning dendritic arborization. Here, we focus on the multidendritic nociceptor PVD neuron in C. elegans, whose primary dendrites extend horizontally in the lateral nerve cord and successively emanate higher order branches to establish elaborate dendrite morphology. Using near super-resolution microscopy and split GFP, we show that the PVD primary dendrites fasciculated with axons of the ALA interneuron in the lateral cord. Consistent with previous studies, we find that many secondary PVD dendrites fasciculated with commissural axons of cholinergic or GABAergic motor neurons. Time-lapse live imaging revealed periodic expansion and shrinkage of the PVD dendritic growth cones that attached to the ALA axon. Extension of the ALA axon is completed during embryogenesis, raising a possibility that the ALA axon guides the PVD dendrites that grow at late second larval stage. Consistent with this, truncating the ALA axon by mutations in the transcription factor ceh-17 or the kinesin vab-8 resulted in misrouted primary PVD dendrites that often traveled in the dorsal or ventral sublateral hypodermis. Our genetic experiments indicated that ceh-17 and vab-8 were required in ALA but not PVD neurons, suggesting that the ALA axon projection patterns the trajectory of primary PVD dendrites. We are now exploring the molecular events that work at the ALA-PVD interface to regulate PVD growth cone dynamics and dendrite extension (supported by the Ministry of Science and Technology, Taiwan, 103-2320-B-002-050-MY3 and MOST 104-2320-B-002-058-MY3)

812A  Activity-dependent dendritic morphology and gene expression in sensory neuron pair URX.  J. Cohn, N. Zarate, J. Pierce  Dept. of Neuroscience, Univ. Texas at Austin, Austin, TX.

Prolonged sensory input and chronic neural activity can lead to several downstream cellular alterations, such as morphological remodeling and changes in gene transcription. The pathways that lead to these changes are often unique in different cell types, and the streamlined nervous system of C. elegans offers a useful system for uncovering genes involved in these processes. To
this end, we are investigating cellular changes induced by chronic activity in the main oxygen-sensing neurons URX. The URX neurons are tonically active in the lab with 21% oxygen. Intracellular cGMP levels controlled by the oxygen-sensing guanylyl cyclases GCY-35 and GCY-36 reflect the absolute level of oxygen to which the worms are exposed. These chronically high levels of cGMP open cyclic-nucleotide-gated ion channels which leads to constant calcium influx into the cell. We have identified two cellular consequences of this chronic activity of the URX neurons. First, we have surprisingly found that a transcriptional reporter for the pro-apoptotic gene egl-1 is expressed in URX in an activity-dependent manner. Animals reared at 1% oxygen have vastly reduced reporter expression when compared to animals raised at 21% oxygen. Similarly, mutations in genes that affect oxygen sensing or signal transduction lead to a loss of egl-1 reporter expression. Secondly, we have found that the dendritic tips of URX that arborize in the nose grow in length and complexity depending on neuronal activity. Animals raised in 21% oxygen grow extensively elaborated URX dendritic tips by Day 4 of adulthood, while the dendritic tips of animals maintained in 1% oxygen fail to elaborate. Many of the same genes found to control activity-dependent gene expression are also involved in this dendritic growth. To identify genes that influence these phenomena, we performed a forward genetic screen and intriguingly recovered a loss-of-function allele of crh-1, the worm homolog of the CREB transcription factor. We are currently exploring several avenues for following up on these findings. An activity-dependent pathway for upregulating the pro-apoptotic egl-1 may shed light on possible activity-dependent cell deaths in C. elegans, and perhaps genes involved in the degeneration of chronically active neurons in other animals. Also, a role for crh-1 in maintaining sensory endings has not yet been reported, so we are currently working to determine whether crh-1 is important for dendritic sensory structure in other neurons as well.

813B Inhibition of a MIG-15/JNK-1 MAP kinase cascade is required for synapse formation. Oliver Crawley, Sudhanva Kashyap, Rayna Birnbaum, Brock Grill. Neuroscience, Scripps Florida, Jupiter, FL.

The Pam/Highwire/RPM-1 (PHR) proteins are conserved intracellular signaling hubs that regulate synapse formation and axon termination. The C. elegans PHR protein, called RPM-1, regulates numerous downstream signaling pathways. One mechanism of RPM-1 function is inhibition of MAP kinase signaling. Previous studies showed RPM-1 ubiquitin ligase activity inhibits the DLK-1 and MLK-1 MAP kinase pathways. We have identified a new MAP kinase pathway that suppresses synapse formation defects in rpm-1 mutants. This pathway includes MIG-15 (MAP4K), NSY-1 (MAP3K), JKK-1 (MAP2K), and JNK-1 (MAPK). Known signaling pathways that mediate RPM-1 function control both synapse formation and axon termination in both the mechanosensory neurons and the motor neurons. In contrast, the MIG-15/JNK-1 pathway specifically suppresses defects in formation of glutamatergic synapses made by the mechanosensory neurons. Consistent with RPM-1 inhibiting the MIG-15/JNK-1 pathway, transgenic overexpression of kinases in this pathway impairs synapse formation. The MIG-15/JNK-1 pathway functions cell autonomously in the mechanosensory neurons, and kinases in the pathway localize to the presynaptic terminal further supporting a role in synaptogenesis. These results indicate that the MIG-15/JNK-1 pathway needs to be restricted for formation of glutamatergic, neuron-neuron synapses, and RPM-1 is a potential mechanism of inhibiting the MIG-15/JNK-1 pathway. Our results also provide an in vivo functional link between the MIG-15/MAP4K and JNK signaling in the nervous system, which has remained elusive.

814C Branched actin promotes synapse disassembly in remodeling GABAergic neurons. A. A. Cuentas Condori1, T. W. Miller-Fleming2, D. M. Miller, III1,2 1) Cell and Developmental Biology, Vanderbilt University, Nashville, TN; 2) Program in Neuroscience, Vanderbilt University, Nashville, TN.

The actin cytoskeleton sculpts postsynaptic structures in the developing brain. Less is understood, however, about the role of actin in presynaptic plasticity. Here we report that branched actin promotes the disassembly of the presynaptic apparatus in a mechanism that resembles activity-dependent bulk endocytosis. We use a developmentally regulated example of circuit remodeling in C. elegans in which Dorsal D (DD) GABAergic motor neurons relocate their presynaptic structures from ventral to dorsal sites during early larval development. We have previously proposed that the DEG/ENaC cation channel, UNC-8, drives disassembly of ventral GABAergic synapses by elevating intracellular calcium and neuronal activity. We have now shown that the branched actin nucleator, the Arp2/3 complex, is also required for synapse elimination in this circuit. This idea is consistent with our finding that two known activators of the Arp2/3 complex, the Wave Regulatory Complex and the F-BAR protein TOCA-1 are necessary and act in a common pathway. We have established that TOCA-1 functions upstream of UNC-8 with the calcium-activated phosphatase, TAX-6/Calcineurin. Interestingly, TAX-6/Calcineurin localizes with Arp 2/3 and TOCA-1 to presynaptic terminals in remodeling DD neurons. Calcineurin is known to function as a key regulator of bulk endocytosis, which is the dominant mechanism for recycling synaptic vesicle components in highly active neurons. Thus, we propose that a similar endocytic pathway involving TOCA-1/Arp2/3-dependent actin branching triggers removal of the presynaptic apparatus in remodeling GABAergic neurons. This idea is important because it provides a molecular description of a key role for branched actin assembly in presynaptic plasticity.

815A A promoter database for embryonic neurodevelopment. Leighton H. Duncan1, Mark Moyle1, Titles Sengupta1, Richard Ikegami1, Abhishek Kumar1, Anthony Santella2, Pavak Shah2, Zhirong Bao3, William Mohler3, Hari Shroff2, Daniel Colón-Ramos1, Ryan Christensen1 1) Department of Cell Biology and Neuroscience, Yale University, School of Medicine, New Haven; 2) Developmental Biology Program, Sloan Kettering Institute, New York, NY; 3) Department of Genetic and Developmental Biology and Center for Cell Analysis and Modeling, University of Connecticut Health Center, Farmington, CT; 4) Section on High Resolution Optical Imaging, National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, MD.

WormGUIDES (Global Understanding in Dynamic Embryonic Systems) is a systems-level resource that facilitates examination of cellular decisions in the developing nervous system of C. elegans. To build this resource we have designed strategies for
labeling neurons during early development. There are 198 neurons that extend processes in the *C. elegans* embryo, and they can be categorized into fifteen neuronal structures. By examining system-level resources such as WormBase, WormAtlas, and EPIC (Expression Patterns in *Caenorhabditis*) databases as a resource for this selection, we have identified a panel of 182 transcriptional promoters that are predicted to sparsely label these 198 neurons. By systematically examining this list, we have identified 28 promoters that label single neuron classes in the embryo, and 74 promoters that label two to five neuron classes each. In addition, we have implemented an imaging and cell identification pipeline that allows us to determine the dynamic expression pattern (via cell-lineaging) during the development of the embryo. We have also built a neuronal promoter database (http://promoters.wormguides.org/) to share our findings with the community. The promoter database enables researchers to submit promoters of interest, which are then imaged throughout embryogenesis and the expression patterns determined via cell-lineaging. We have combined lineaging with imaging for the precise identification of labeled neuronal classes and neurite fascicles during early nervous system development in the embryo. This service will benefit individual investigators, while providing us, and the community, with new tools to examine the nervous system during early embryonic development. In this manner our atlas builds on the *C. elegans* community’s open-sharing of systems-level knowledge and resources and enhances the value of *C. elegans* as a model organism.

**816B  Cellular and molecular mechanisms of left-right asymmetric neurogenesis.**  T.J. Felton1, T.W. Mullan1, J. Tam1,3, J. Yeung1,3, N. Memar2,4, O. Kasem1, A. Aldabergenova1, R. Schnabel2, R.J. Poole1  1) Department of Cell and Developmental Biology, University College London, London, GB; 2) Department of Developmental Genetics, TU Braunschweig, Braunschweig, Germany; 3) Department of Developmental Biology, The Francis Crick Institute, London, GB; 4) Department Biologie II, Biozentrum der LMU München, Martinsried, Germany.

We use the C-lineage as a model to study how neuronal potential is asymmetrically inherited to establish left-right (L/R) asymmetric neurogenesis. The C blastomere undergoes 6 successive rounds of cell division to produce L/R symmetric pairs of body wall muscles and hypodermal cells from both sides of the lineage, and two glutamatergic neurons, DVC and PVR, which arise asymmetrically from the left side of the lineage only. Previously, we showed that asymmetric expression of the bHLH proneural gene *hlh-14/Ascl1* is required for this L/R asymmetric neurogenesis event. To identify genes that regulate *hlh-14* expression and thus affect neuronal specification within the lineage, we have conducted two genetic screens: a forward genetic screen for mutants that fail to express fate makers for DVC and PVR and a 4D-lineage based screen of a collection of temperature-sensitive embryonic lethal mutants. We have isolated eight asymmetric neurogenesis defective (and) mutants. Through mapping-by-sequencing, genetic complementation and rescue experiments, we show that *and-6* is an allele of *hlh-2*, a bHLH transcription factor that heterodimerizes with other bHLH transcription factors, including *hlh-14* and that *and-4* is an allele of *let-19*, a member of the Mediator complex. 4D-lineage analysis of the C-lineage reveals that *hlh-2* and *let-19* mutants exhibit concomitant precocious division of the DVC neuroblast, loss of neuronal cell fate markers, loss of *hlh-14* expression and ectopic expression of a hypodermal marker. All of which are phenotypes shared with *hlh-14* mutants. We find that *let-19* acts during the division of Ca and Caa, and that *hlh-2* expression coincides with, and is dependent on, the activity of *let-19*. These results demonstrate that the Mediator regulates the expression of *hlh-2* and *hlh-14* during asymmetric neurogenesis. We observe three successive unequal (in size) cell divisions leading up to the production of the DVC neuroblast. We find that *let-19* regulates the second of these divisions and in *let-19* mutants this division becomes symmetric with a concomitant loss of neuronal potential. The third unequal division is regulated by *hlh-14* but not *hlh-2* and again there is a concomitant loss of neuronal potential. These results suggest that asymmetric segregation of an unidentified neuronal determinant has a critical role in this lineage and that the Mediator, as well as proneural genes, regulate cell fate and cell size concomitantly. We are currently determining through manipulations of blastomere size if the unequal divisions are an input or output of fate.

**817C  KPC-1 down-regulates DMA-1 receptors to control dendrite self-avoidance and axon pathfinding in *C. elegans*.**  K. Hamada1, H. Chiu2,3, Y. Zou3, W. Zou4, T. Ferreira5, R. W-H. Lin5, K. Shen4, C. Chang1,2  1) Graduate Program in Neuroscience, University of Illinois at Chicago, Chicago, IL; 2) Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL; 3) School of Life Science, ShanghaiTech University, Shanghai, China; 4) Howard Hughes Medical Institute, Department of Biology, Stanford University, 385 Serra Mall, Stanford, CA; 5) Division of Developmental Biology, Cincinnati Children's Hospital Research Foundation, Cincinnati, OH; 6) Division of Biology and Biological Engineering and HHMI, California Institute of Technology, Pasadena, CA.

Mechanical stimuli on the skin of *C. elegans* are detected by the dendritic arbors of the PVD nociceptive neurons, which provide uniform sensory coverage outside the head region across the entire animal. Through genetic screens, we isolated three mutants that display profound dendrite self-avoidance defects in the PVD neurons. Using whole genome sequencing, we identified the responsible mutations in the kpc-1 gene. Compared to wild-type animals, kpc-1 mutants demonstrate a significantly higher number of secondary dendrites, a lower tertiary branch avoidance index, and decreased sensory coverage of skin by PVD dendrite arbors. Although kpc-1 was previously implicated in dendrite self-avoidance, the mechanism by which kpc-1 regulates this process is unknown. Here, we show that the kpc-1 3'UTR is required for kpc-1's function in dendrite self-avoidance. The kpc-1 3'UTR facilitates kpc-1 RNA localization to contact points between sibling dendrites, implicating local translation of kpc-1 in dendrites. We demonstrate that over-expression of kpc-1 leads to greater self-avoidance without limiting initial dendrite outgrowth, supporting a direct role of kpc-1 in self-avoidance. Animals with dma-1 receptor over-expression display a similar tertiary dendrite self-avoidance defect that is suppressed with kpc-1 over-expression, which suggests that DMA-1 is a potential KPC-1 target. Our results support a model where KPC-1 is expressed at the contact points between neighboring dendrites to locally down-regulate DMA-1 receptors to facilitate dendrite self-avoidance. Finally, KPC-1 also down-regulates DMA-1 receptors in PVD axons to prevent axon misguidance.
818A Microtubule nucleation from the basal body organizes the dendritic cytoskeleton for efficient distal transport. Martin Harterink, Bart de Haan, Kah Wai Yau, Lukas Kapitein, Casper Hoogenraad Cell Biology, Utrecht University, Utrecht, NL.

Differences in microtubule polarity organization are thought to be the evolutionary conserved determinant for axon/dendrite polarity, allowing for selective cargo transport to specific compartments. To understand how microtubules are organized in Caenorhabditis elegans neurons we systematically analyzed microtubule dynamics and polarity using the end binding protein EBP-2. As anticipated we found an opposite microtubule polarity between axons and dendrites allowing for selective transport into these neurites using the kinesin or dynein motors respectively (Harterink et al., 2016). Unexpectedly however, in ciliated neurons we observed a marked increase in microtubule growth events from the base of the sensory cilium (basal body) located at the dendrite tip. In addition,?-tubulin, the main microtubule nucleator, localizes to this structure suggesting that the basal body functions as a microtubule organizing center (MTOC). To address if this distal MTOC affects cargo transport we analyzed dendritic RAB-8 transport in ciliated and non-ciliated neurons. We found that only in ciliated neurons RAB-8 is efficiently transported in the distal dendrite and that inhibition of microtubule nucleation resulted in a marked decrease in distal transport. Taken together we suggest that the basal body functions as a MTOC to organize the neuronal microtubule cytoskeleton to ensure proper transport in the distal dendrite towards and from the cilium.

819B MAPK-15 regulates dendrite size in the URX sensory neuron. I. G. McLachlan, M. G. Heiman Genetics, Harvard Medical School, Boston Children’s Hospital, Boston, MA.

The morphology of a neuronal dendrite determines what signals it receives and how those signals are integrated. For example, the positions and shapes of dendritic spines determine synaptic partners and synapse strength. In C. elegans, many sensory neurons extend dendrites that terminate at the nose in a sensory cilium ensheathed by glia, and we previously identified genes required for their morphogenesis. In contrast, the oxygen-sensing neuron URX extends a dendrite to the nose that lacks a cilium, is not ensheathed by glia, and does not require any of the genes we previously identified. Therefore, to identify novel regulators of dendrite morphogenesis, we performed an unbiased visual genetic screen for mutants with defects in URX dendrite morphogenesis. We isolated 17 recessive mutants exhibiting four classes of phenotype: (I) short dendrites; (II) ectopic branching; (III) disorganized dendrites, and (IV) dendrite overgrowth, in which the URX dendrite extends up to ~150% of its normal length. We identified two genes that cause this overgrowth phenotype: C05D10.2/mapk-15, a previously uncharacterized gene encoding a kinase with homology to mammalian MAPK15/ERK8, and sma-1, a homolog of beta-H spectrin. Both mapk-15 and sma-1 phenotypes appear during L4 stage and become increasingly pronounced as the animal ages. We determined via mosaic and rescue experiments that MAPK-15 acts cell-autonomously in URX neurons and requires its kinase activity to function. The URX dendrite ending is enriched in signaling molecules including the receptor-type guanylyl cyclase GCY-35, and we found that MAPK-15 also localizes to the dendrite ending in wild-type animals. Further, in mapk-15 mutants, GCY-35 becomes localized throughout the region of dendrite overgrowth, suggesting the additional dendrite length reflects expansion of this sensory compartment. Surprisingly, overexpression of wild-type, but not inactive, GCY-35 suppresses the mapk-15 phenotype, suggesting that the size of this sensory compartment may be regulated by sensory signaling in a manner epistatic to mapk-15. Together, these results suggest that mapk-15 may mediate activity-dependent size regulation of a dendritic sensory compartment, possibly analogous to regulation of dendritic spine size and shape.

820C The Eph receptor/ephrin pathway is required for AYI interneuron development and food-seeking behavior. T. Hill, A. Johnston, E. Santorella, M. Bentley, C. Benton, M. Hudson Molecular and Cellular Biology, Kennesaw State University, Kennesaw, GA.

In order to survive, an organism must be able to receive, integrate, and respond to sensory stimuli. However, the cellular basis of sensory perception and response is difficult to study in complex animals such as humans, and is therefore poorly understood. The nematode Caenorhabditis elegans is a relatively simple organism yet displays many distinct behaviors, making it an ideal system to understand the relationship between gene function, cell shape, cell physiology, and behavioral output. Much of the thermosensory and chemosensory information that the nematode receives from its sensory neurons is processed via a pair of interneurons called AYIL and AYIR. In wildtype animals, the AYI cell bodies lie just posterior to the pharynx, and extend an anterior process that contacts its contralateral partner at the base of the nerve ring. The AYIL and R processes then diverge and extend around the nerve ring, ultimately making contact again on the dorsal side of the animal via a gap junction. We previously showed that the Eph receptor tyrosine kinase vab-1 is required for AYI cell body placement and ventral AIY/R contact. Conversely, the ephrin efn-4 is required for dorsal AYIL/R connectivity. We have extended these studies and show that the AYIL/R ventral contact is mediated via the ephrin gene efn-1. In addition, we show that this connectivity requires both vab-1 kinase activity and also a non-kinase dependent vab-1 function. We are currently performing tissue specific rescue experiments to understand whether efn-1 functions cell autonomously in AIY development.

To integrate AYIL/R morphology and function with behavior, we are using WormLab software to image and analyze EphR/ephrin mutants both on and off food. Wildtype animals search for food using long “runs” interspersed with reversals and ~170-degree “omega” turns. We find that vab-1 mutants lack the ability to perform straight runs, although they can perform omega turns normally. Instead, they display a strong circling locomotion, both on and off food. When EphR/ephrin pathway mutants are conditioned in a 150mM NaCl environment with food, then assayed on a food-free NaCl gradient, vab-1, efn-1 and efn-4 mutants all display normal chemotaxis towards a point NaCl source, suggesting no overt defects with NaCl conditioning and chemotaxis. We are currently investigating neuromuscular junction morphology in EphR and ephrin mutants to see if this correlates with dorsal versus ventral locomotion bias.
The CPG underlies several diverse and essential functions, functionally streamlined and localized for their non-divorceable utility. Until recently, however, the CPG has conventionally been understood unjustly in the confines of rhythmicity and motorized behavioral processes. Beginning and framing the C. elegans as a fundamental model for the incredible versatility of the basic CPG, we delve into the sensorimotor integration, maintenance of the locality and causality with a temporal-spatial model, and the conserved processes it demarcates in more complex behaviors, particularly human saccadic eye movement. Afterwards, we discuss the global trends and emergent properties across several organisms that characterize the manifestation and institution of a meaningful rhythmic neural pattern, premised initially by the highly elucidative and revealing neural system of the C. elegans. In finality, we demarcate the evolutionary morphology of the information code and neuromodulatory vehicles that biological pressures have enforced on the increasingly complex design of organisms, starring the C. elegans, once again, as the basis and transitional point for the adaption of an information code from an amplitude based phenomena (graded potential code) to a frequency based phenomena (action potential code). Ultimately, the primary motif of this article demonstrates the immense utility of understanding the 302 neuron system of the C. elegans towards unveiling unobserved homologies and information trends in the evolutionary ladder, across pattern generated behaviors. Our analyses incepts and extends from a compilation of personal lab discoveries and external literature interpretations of the C. elegans nervous system, and has been protracted to several homologies across various animalia and processes, including the aforementioned human saccades.

822B Effect of gravity on the development of motor neurons of C. elegans.  
S. Kalichamy, TY Lee, JI Lee  Biological Science and Technology, Yonsei University, Wonju.

Recently, space agencies are developing capability to inhabit space. However, the effects and dangers of long-term space travel or habitation to the human body are not fully known. During space exploration, astronauts have encountered health issues due to low gravity including muscle atrophy. Most of the studies on gravity's effect on muscle have focused on muscle development itself. However the effect that gravity has on motor neuron development and axonal outgrowth and pathfinding has not been explored. Using C. elegans as a model for motor neuron development, we decided to look at the effect that hypergravity may have on axonal outgrowth and pathfinding in the D-type GABAergic motor neurons. We first designed a hypergravity cultivation system in which C. elegans eggs can be exposed to 100G force gravity and then analyzed the motor neurons. We found a 3.4-fold axons were defected in 100G exposed worms, and identified a critical period before the L2 larval stage that is required for hypergravity to induce defects in worms. Using candidate mutant analysis, we found hypergravity-induced axon defects can be suppressed in mutant of unc-70 which encodes β-spectrin protein. In addition, we are also trying to identify effects that microgravity may have on motor neuron development using a 2D-clinostat that simulates microgravity condition in the laboratory. Currently, we are trying to attain simulated microgravity conditions by measuring the expression of known microgravity marker genes in both E. coli and C. elegans. This study may have a huge impact in the field of space biology and helps to understand the motor neuronal development in altered gravity conditions. It may also contribute to resolve the problems of muscle atrophy observed in astronauts during space flight and habitation.

823C Homeotic mutation and undergraduate collaboration: exploration of Hox-mediated specification of neurons in males.  

In metazoans, Hox transcription factors provide positional cues that define neuronal identities on the anterior-posterior (AP) body axis; disruptions to Hox genes can cause transformation of neuronal identities. In C. elegans males, regional identities of ventral cord CP neurons are defined by overlapping zones of Hox activity: Zone 1 is characterized by lin-39-dependent expression of tph-1::mCherry and flp-22::gfp in CPs 1-4; Zone 2 is characterized by lin-39-dependent expression of tph-1::mCherry in CPs 5-6; Zone 3 is characterized by mab-5-dependent expression of flp-21::gfp in CPs 7-9. In Zone 2, flp-22::gfp and flp-21::gfp are each repressed in a manner that depends on mab-5 and lin-39 respectively, indicating that MAB-5 and LIN-39 act reciprocally to define aspects of CP fate.

The homeotic mutation lin-39(ccc16) transforms fates of CPs in Zone 2 into CPs with Zone 3 identities. In lin-39(ccc16) mutants, tph-1::mCherry is expressed in Zone 1 CPs, but is absent in Zone 2 CPs. lin-39(ccc16) males express flp-22::gfp normally in Zone 1, but fail to repress flp-21::gfp in Zone 2. Because lin-39(ccc16) preferentially affects only CPs in which both MAB-5 and LIN-39 are active, we hypothesize that the protein encoded by lin-39(ccc16) is able to specify CP fates in the absence of MAB-5 (Zone 1) but cannot achieve the MAB-5 interactions necessary to specify CP fates in Zone 2. If this is the case, mab-5 mutations should restore normal tph-1::mCherry expression to Zone 2 in a lin-39(ccc16) mab-5(0) double mutant. Our preliminary genetic analysis supports this idea: mab-5(e1239) restores tph-1::mCherry expression in CPs 5-6 in lin-39(ccc16) males.

To further explore regulation of neurotransmitter gene expression in the male ventral cord, we have initiated a course-based undergraduate research collaboration between students at St. Catherine University (SCU) and at Carleton College (CC). During Fall 2016, SCU genetics students constructed tph-1::pes-10::gfp reporters containing fragments of the tph-1 regulatory region. These were analyzed by CC genetics students during Winter 2017, revealing preliminarily that a 485 bp tph-1 regulatory region drives robust GFP expression in CPs 5-6. In addition to forwarding research goals, this collaboration requires students to connect concepts of cis-regulatory elements with gene expression. Our tph-1 promoter analysis, along with the Zone-2-specificity of the lin-39(ccc16) phenotype, lends support to the idea of a Zone-2-distinct program of neuronal fate specification that has the potential to shed light on how activity of neighboring Hox genes is coordinated.
824A  FAX-1 and UNC-42 transcription factors mediate insulin-dependent quiescence and arrest.  F. Koitz1, E. Bayer1,2, S. Clever1, B. Wightman1  1) Biology Department, Muhlenberg College, Allentown, PA; 2) Columbia University, New York, NY.

The fax-1 nuclear hormone receptor and unc-42 homeobox gene control interneuron identities in C. elegans. fax-1 is the ortholog of unfilled in Drosophila and PNR/NR3E3 in vertebrates, where it functions in the development and function of mushroom bodies and photoreceptors, respectively. The fax-1 and unc-42 transcription factors function in specifying the identities of an overlapping subset of nematode interneurons, including the command interneurons AVA and AVE, which function in coordinated movements. Both genes are required for the expression of neuron-specific genes, including glutamate receptors subunits, and axon pathfinding.

Mutations in both fax-1 and unc-42 cause an incompletely-penetrant slow-growth phenotype that arises from temporary arrest after hatching at the L1 stage. L1 arrest has been shown to be controlled by the insulin-like signaling pathway that also controls dauer formation and longevity. Thedaf-2 insulin receptor is a primary mediator of insulin signaling in C. elegans. Strongdaf-2 mutations cause L1 arrest, while weakdaf-2 mutations cause dauer-arrest. Both fax-1 and unc-42 mutations cause a fully-penetrant late embryonic arrest in combination with a weakdaf-2 mutation. The arrest can be reversed by a mutation in thedaf-16 forkhead transcription factor, which functions downstream ofdaf-2, but not by mutations in the parallel TGFβ pathway. Arrestedorfax-1;daf-2 and unc-42;daf-2 embryos typically displayed normal L1 morphology, but remained coiled in a broken eggshell in a state of extreme quiescence. Arrested embryos exhibited weak or no pharyngeal pumping. Nonetheless, arrested embryos could be prompted to vigorous movement by stimulation with green light. The arrest could also be partially reversed by a mutation inegl-4, which is required for sleep-like quiescence. We also examined the strongdaf-2(e979) mutation and found that it displays a complex phenotype, including morphological collapse at elongation in mid-embryogenesis, late embryo quiescence (like the double mutants), and L1 arrested animals that display fairly normal pumping and activity. These observations indicate that the fax-1 and unc-42 transcription factors function in an insulin pathway that controls arousal and developmental progression out of embryogenesis. Given that bothfax-1 and unc-42 are required for the development of a limited set of interneurons, these experiments suggest a previously unappreciated role for interneuron function and insulin signaling in regulating developmental arrest and arousal.

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825B  An unexpected role for the conserved PBAF chromatin remodeling complex in neuronal diversity.  A. Osuma, J. Aburas, P. Kratsios  Department of Neurobiology, University of Chicago, Chicago, IL, USA.

A key question in developmental neurobiology is to reveal the gene regulatory mechanisms that control neuronal diversity. Although remarkable progress has been made in understanding how transcription factors (TFs) generate diversity, the role of epigenetic regulators in this process remains largely unknown. The motor circuit of Caenorhabditis elegans provides an ideal model system to probe the genetic and epigenetic mechanisms of neuronal diversity. We have previously shown that the evolutionarily conserved COE (Collier, Olf, Ebf) type TF UNC-3 acts as a terminal selector in the majority of ventral nerve cord (VNC) cholinergic motor neuron (MN) classes. Apart from controlling shared features among all MN classes (e.g. the expression of enzymes and transporters in the acetylcholine biosynthetic pathway), UNC-3 is also required for MN diversity by directly activating the expression of terminal identity genes (e.g. ion channels, neurotransmitter receptors) that are selectively expressed in specific MN classes (SAB, DA, DB, VA, VB, AS). However, unc-3 is expressed in all these MN classes, leading us to hypothesize the existence of repressor proteins that restrict the ability of UNC-3 to activate these terminal identity genes in a broad, not class-specific manner. To test this hypothesis, we performed a forward genetic screen using a fluorescent reporter for the terminal identity gene glr-4, which encodes a glutamate receptor subunit selectively expressed in the SAB class and DA9 MNs. We discovered that pbrm-1, the sole C. elegans ortholog of the evolutionarily conserved chromatin regulator BAF180, acts as a repressor of the unc-3-dependent gene glr-4. Despite its ubiquitous and continuous expression, pbrm-1 selectively controls AS terminal identity features by repressing glr-4 only in the AS class of MNs. We further found that all other VNC MN classes are normally generated inpbrm-1 mutants and the expression of several known MN class-specific, unc-3-dependent genes is unaffected by PBRM-1. Since PBRM-1/BAF180 is a subunit of the PBAF, a chromatin remodeling complex of the SWI/SNF family, we reasoned that animals lacking gene activity for other PBAF subunits may display a similar AS phenotype. We indeed found that SWSN-9, the sole C. elegans ortholog of human BRD7 and BRD9, is also required for glr-4 repression in AS MNs. Neuron-specific rescue further demonstrated that PBRM-1 and SWSN-9 act cell-autonomously. Altogether, we provide novel insights on the epigenetic mechanisms that generate neuronal diversity by uncovering a previously unrecognized, neuron-specific role for the PBAF chromatin remodeling complex in the selective repression of terminal selector target genes.

826C  Morphogenesis of a neuron-glia attachment that shapes dendrite extension.  E.R. Lamkin1,2,3, I.G. McLachlan1,2,3, M.G. Heiman1,2  1) Genetics, Harvard University, Boston, MA; 2) Division of Genetics and Genomics, Boston Children’s Hospital, Boston, MA; 3) Program in Neuroscience, Harvard University, Boston, MA.

The function of the nervous system depends on precise connections among neighboring cells, including specialized contacts between neurons and glia. In vivo studies of neuron-glia contacts have been hindered by the challenge of visualizing individual contacts amidst the vast number of cells in the vertebrate nervous system. To study the development of neuron-glia contacts at the single-cell level, we have turned to C. elegans. Two classes of neuron-glia interactions have been studied in C. elegans: (1) gial wrapping of axons in the nerve ring and (2) epithelial-like interactions between sensory dendrite endings and their ensheathing glia. We have focused on a third class of neuron-glia interactions reminiscent of contacts between Bergmann glia and Purkinje cell dendrites in the mammalian cerebellum. The most striking example within this class is the interaction between
the BAG sensory neuron and its neighboring glial cell, in which the BAG dendrite ending precisely wraps a protrusion from the glial cell. This interaction was first seen by electron microscopy over four decades ago but has not been further studied. We used super-resolution optical microscopy to visualize the BAG-glia contact in living animals. We also performed forward genetic screens to identify factors required for BAG dendrite development. We showed that the nascent dendrite ending attaches to the glial cell and is pulled to its final length during embryo elongation. Our genetic screens revealed that this attachment requires the cytoskeletal adaptor protein GRDN-1/Girdin, which acts non-cell autonomously in the glial cell to promote dendrite extension in the neuron, and the adhesion molecule SAX-7/L1CAM, which is localized via GRDN-1 to the glial ending where the attachment forms. Based on the partial penetrance of these mutants, we reasoned that additional factors may contribute to the specification and morphogenesis of the BAG-glia contact. Using unbiased and candidate screens for modifiers of the sax-7 dendrite defect we identified roles for the adaptor protein AFD-1/afadin, which is known to localize to cell junctions, the membrane-associated guanylate kinase MAGI-1, and the fibroblast growth factor receptor EGL-15. Our working model is that these molecules form an adhesion complex that specifies the site of the neuron-glia contact. All the adhesion factors we have identified are conserved in mammals, suggesting that mechanisms of neuron-glia contact formation may be shared across systems. We anticipate that, in addition to adhesion factors, other molecules facilitate communication between the neuron and the glial cell to coordinate cell shape changes and generate the precisely interlocking morphologies of the mature contact. The BAG-glia contact thus provides an innovative model for studying the development of a special class of neuron-glia contacts at single-cell resolution.

827A  Feedback regulation of tetrahydrobiopterin (BH4) synthesis in C. elegans.  C.M. Loer, D. Sykora  Biology, Univ San Diego, San Diego, CA.

Synthesis of the neurotransmitters serotonin and dopamine, conversion of Phe to Tyr, and the breakdown of ether lipids all involve enzymes that require the cofactor 5,6,7,8-tetrahydrobiopterin (BH4) to function. Worms that can’t synthesize BH4 are serotonin- and dopamine-deficient, and have leaky, fragile cuticles caused by aberrant lipid metabolism in the hypodermis. The defective cuticle in BH4-deficient worms also alters their susceptibility to bacterial pathogens (Loer et al., 2015, Genetics 200: 237). In humans, regulation of BH4 levels is important for several physiological functions. BH4 is synthesized in four steps starting with GTP; the first step is performed by GTP Cyclohydrolase I (GTPCH1, C. elegans gene cat-4). Like in many biochemical synthesis pathways, the first enzyme in the pathway is regulated by ‘end product feedback inhibition.’ This typically occurs by the end product itself binding to another site on the enzyme – allosteric regulation. For BH4, it is more complicated – in mammals, GTPCH1 inhibition by BH4 requires an additional small protein called feedback regulatory protein (GFRP). Crystal structures show that the analog BH2 binds at the interface between pentamers of GTPCH1 & GFRP to inhibit GTPCH1 function, mainly interacting with GTPCH1 (Maita et al., 2004, J Biol Chem 279: 51534). Phe, which stimulates GTPCH1, also binds at the interface between the two proteins, mainly interacting with GFRP. C. elegans encodes a homolog of GFRP (gene gfrp-1) that at least partly overlaps in expression with the cat-4 gene (Loer et al., 2015). We are seeking to demonstrate the function of the putative worm GFRP in regulating BH4 synthesis. Sequence analysis and structural predictions of the putative worm GFRP suggest that it will function like the mammalian protein, binding both BH4 and Phe. In mammals, GTPCH1 and GFRP can also bind the selective inhibitor 2,4-Diamo-6-hydroxypyrimidine (DAHP), which is structurally similar to both GTP (substrate) and BH4 (pathway end product). At low concentrations, DAHP apparently inhibits GTPCH1 like BH4 by binding with GFRP; this inhibition is GFRP-dependent. At higher concentrations, DAHP acts as a competitive inhibitor, binding at the GTPCH1 active site like GTP; this inhibition is GFRP-independent (Xie et al., 1998, J Biol Chem 273: 21091). Although DAHP treatment has no obvious effect on wildtype C. elegans, serotonin levels (a proxy for BH4 levels) are reduced by DAHP in worms with a reduction-of-function mutation [cat-4(e3015)] that already have less BH4. We plan to test whether this presumed inhibition of worm GTPCH1 is GFRP-dependent by knocking down gfrp-1 gene function in DAHP-treated cat-4(e3015) worms.

828B  Neuron-specific profiling to identify dendrite branching genes.  R. McWhirter, B. O’Brien, D. Miller, III  Vanderbilt University, Nashville, TN.

The C. elegans genome is completely sequenced and its developmental anatomy is defined at single cell resolution. In an effort to link gene expression to cellular identity, we are generating expression profiles of specific cell types at defined developmental stages. To accomplish this goal, we developed the SeqCel (RNA Seq of specific cells) technique that uses FACS to isolate specific GFP-labeled for RNA-Seq analysis. To date, we have generated profiles of over 15 different cell types from larvae and adults including specific neurons and muscle cells. This approach has identified transcripts that are highly expressed in specific cells and also revealed genes with roles in important developmental processes. Here, we report the use of SeqCel to identify transcripts that drive dendritic branching in the PVD nociceptive neuron. Each PVD neuron, one on each side of the body, is defined by a single longitudinal process that gives rise to regularly-spaced menorah-like structures that envelop the animal. This elaborate structure is dramatically simplified by mutations that disable the MEC-3 LIM homeodomain transcription factor. In mec-3 mutants, PVD lateral branching fails and menorahs are rarely generated. To identify mec-3-regulated targets, we have used SeqCel to profile both wild-type (WT) and mec-3 mutant PVD neurons during the L3 larval stage in which lateral branching normally occurs. Differentially regulated transcripts detected in a comparison of WT and mec-3 mutant PVD SeqCel profiles will be screened by RNAi and with available genetic mutants to identify downstream determinants of dendritic branching.

829C  The claudin-like adhesion protein MOK-2 has a cell autonomous role in asymmetric differentiation of olfactory neurons.  Z. D. Morrissey¹, X. Wang², C.-F. Chuang¹,³  1) Graduate Program in Neuroscience, University of Illinois at Chicago, Chicago, IL, USA; 2) Division of Developmental Biology, Cincinnati Children’s Hospital Research Foundation, Cincinnati, OH, USA; 3) Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL, USA.

Nervous systems within bilateral organisms display a remarkable degree of left-right symmetry. However, notable instances of
left-right neuronal asymmetry have been evolutionarily conserved and are established by mechanisms that are not fully understood. The *C. elegans* AWC olfactory neuron pair undergoes a stochastic decision during late embryogenesis to differentiate asymmetrically into two subtypes, default *AWC\(^\text{OFF}\)* and induced *AWC\(^\text{ON}\)*. Intercellular calcium signaling between the two AWC and non-AWC cells within a NSY-5 gap junction network coordinates AWC asymmetry. The innexin gap junction protein NSY-5 and the claudin adhesion protein NSY-4 act in parallel to suppress UNC-2/UNC-36 and EGL-19/UNC-36 calcium channel-mediated signaling by activating both SLO-1 and SLO-2 BK potassium channels to promote *AWC\(^\text{ON}\)*. However, it is unknown how *slo-1* and *slo-2* function to specify *AWC\(^\text{ON}\)*.

To identify the molecular mechanisms by which *slo-1* and *slo-2* promote *AWC\(^\text{ON}\)*, we performed a forward genetic suppressor screen with *slo-1* gain-of-function (2AWC\(^\text{ON}\)) mutants and identified several downstream genes of *slo-1* called *mok* genes (modifier of *K*) channels. Among these, the *mok-2*(vy149) missense mutation exhibits a 2AWC\(^\text{OFF}\) phenotype, indicating that it has a role in inducing the AWC\(^\text{ON}\) subtype. Whole genome sequencing revealed that *mok-2* encodes a claudin-like protein with four transmembrane domains that is typically associated with tight junction complexes. We generated a *mok-2* null allele using CRISPR/Cas9, and showed that it also displays a 2AWC\(^\text{OFF}\) phenotype, further supporting the essential role of *mok-2* in promoting *AWC\(^\text{ON}\)*. In addition, our genetic studies suggest that *mok-2* functions in a cell autonomous manner downstream of *slo-1* to inhibit the calcium channel-mediated signaling to promote the AWC\(^\text{ON}\) subtype. Furthermore, translational GFP reporters under the control of an AWC-specific promoter revealed that while MOK-2 is predominantly localized to the AWC dendritic tip region, the MOK-2(vy149) mutant fails to be trafficked towards the dendritic tip and accumulates in the cell body, resulting in a heterogeneous distribution pattern. Based on its putative subcellular localization and claudin-like structure, these data suggest that *mok-2* may communicate with other cells in the ciliary region to have non-cell autonomous roles in the AWC\(^\text{ON}\)/AWC\(^\text{OFF}\) asymmetric subtype decision. Our preliminary data using translational mNeonGreen reporter strains generated with CRISPR/Cas9 show that *mok-2* may be expressed in supporting cells, such as glial cells in the head. Our study will provide novel insight into how tight junction proteins mediate interactions between sensory neurons and supporting cells to generate sensory neuron diversity.

**830A  The Dynamics of Synaptic Remodeling: insights from the *C. elegans* Motor Circuit.**  *Ben Mullan*¹, James Mitchell², Daniel Witvliet¹, WanXian Koh¹, Maggie Chang¹, Peter Berman², Yixin Qu¹, Douglas Holmyard¹, Rick Fetter², Ryan Christensen³, Hari Schroff², Richard Schalek³, Jeff Lichtman², Aravi Samuel², Andrew Chisholm⁴, Mei Zhen¹ ¹) Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, CA; 2) The University of Texas Southwestern Medical Center, Dallas, Texas, US; 3) The Rockefeller University, New York, NY, US; 4) Janelia Research Campus, Howard Hughes Medical Institute, Washington, US; 5) Division of Biological Sciences, UCSD, La Jolla, US; 6) these authors contributed equally to this work.

During development, neural circuits undergo constant changes: they incorporate new neurons, and maintain, prune or remodel existing synapses. The small and rapidly developing nervous system of *C. elegans* allows us to address the developmental dynamics of synapses and neurons in the context of an intact neural ensemble. The vast majority of *C. elegans* postembryonic neurons are motor neurons. Previous studies suggest that at least one group of motor neurons, DD, reverse their neurite polarity entirely: they innervate ventral body wall muscles in the first larval stage, whereas they innervate dorsal muscles in adults. Their role of innervating ventral muscle is taken over by a group of post-embryonically born motor neurons, VD. Precisely how and when such processes occur, without disrupting motility as the motor circuit transitions into the adult form, is poorly described.

We used serial section electron microscopy to reconstruct DD and VD motor neurons across development to define the beginning and ending of remodeling. We identified a series of elegantly coordinated synaptogenesis and remodeling events where synaptogenesis events follow different sequential orders for embryonic (DD) and post-embryonic (VD) neurons. Developing VD neurons have some interaction with DD neurons that may help to guide neurite extension and synaptogenesis. These events implicate an elegant mechanism that allows a gradual and seamless transition of the motor circuit to take place without disrupting its functional output.

**831B  4D lineage based screen of temperature sensitive embryonic lethal mutants to identify regulators of left-right asymmetric neurogenesis.**  *T.W. Mullan*¹, R.F. Wademan¹, T. Felton¹, O. Kasem¹, R. Schnabel², R.J. Poole¹ ¹) Department of Cell and Developmental Biology, University College London, London, UK, WC1E 6BT; 2) Institut für Genetik, Technische Universität Braunschweig, Braunschweig, Germany.

We are interested in uncovering cellular and molecular regulators of proneural gene expression in the context of left-right asymmetric neurogenesis. To achieve this, we focus on the C lineage. Asymmetric expression of the proneural bHLH transcription factor *hlh-14/Ascl1* on the left of the lineage is required to produce two glutamatergic tail neurons, DVC and PVR, on the left only.

Taking advantage of the invariant cell lineage and single-cell resolution of *C. elegans* we are performing a 4D-lineage based screen of a collection of over 2000 temperature sensitive embryonic lethal mutants from our collaborator Ralf Schnabel. In wildtype, the cell cycle of the DVC neuroblast (Caapa) is twice the duration of those of its hypoblast cousins. In *hlh-14* mutants, neurogenesis is lost with the transformed DVC neuroblast dividing precociously in line with the hypoblasts, its daughters adopting a hypodermal fate.

Through preliminary screening for this precocious division in 150 4D-recordings, comprising 75 mutant strains and secondary
screening of our own we have identified three strains of interest. These are \textit{and-7(t3224)}, \textit{and-8(t3294)} and, \textit{and-4(t3200)}; which has been mapped and cloned and is an allele of \textit{let-19}, a Mediator complex component. \textit{and-4} also displays a symmetrisation of an asymmetric division in the C lineage correlating with loss of neurogenesis (see poster by Terry Felton).

Closer study of \textit{and-7(t3224)} reveals additional ectopic DVC neurons rather than loss. 4D-lineage characterization assessing number and timing of divisions has shown the strain displays highly variable lineage transformations, with the P4 blastomere displaying the greatest and most obvious aberrations. Whole genome sequencing has identified two promising candidates: an endosomal budding gene, \textit{pad-1/Dop1p} and zinc finger TF \textit{sma-9/Schnurri}. Loss of \textit{pad-1} in embryos is lethal and causes misplacement of cells by an unknown mechanism. This could be due to aberrant lineal origins. In the postembryonic M lineage \textit{sma-9} has been shown to antagonize anterior-posterior lineage identity though modulation of TGFß signaling, yet to our knowledge, a role for TGFß in the embryo has not been described.

Combining modern and classical methods the screen is proving successful in uncovering mutants of the class it was designed for in addition to others. Furthermore, through the identification of \textit{let-19} the screen has uncovered another avenue of inquiry in asymmetric control of daughter size in the lineage, a potential cellular regulator of asymmetric neurogenesis in the lineage.

832C  **BLOC-1 related complex (BORC) regulates the axonal transport of synaptic vesicle precursors by activating ARL-8.**  Shinsuke Niwa\textsuperscript{1}, Li Tao\textsuperscript{2}, Sharon Lu\textsuperscript{2}, Gerald Liew\textsuperscript{2}, Maxence Nachury\textsuperscript{3}, Kang Shen\textsuperscript{2,4}  \textsuperscript{1}Frontier Research Institute for Interdisciplinary Sciences, Tohoku University, Sendai, Miyagi, Japan; 2) Department of Biology, Stanford University, Stanford, CA; 3) Department of Molecular and Cellular Physiology, Stanford University, Stanford, CA; 4) National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China.

Axonal transport of synaptic vesicle precursors (SVPs) is essential for synapse development and function. Previous studies have shown that the conserved ARF-like GTPase ARL-8 is localized to SVPs and directly activates UNC-104/KIF1A, the SVP kinesin in \textit{C. elegans}. Here we show that BLOC-1 related complex (BORC) is required to recruit and activate ARL-8 on SVPs. In order to find ARL-8 regulators, we searched for mutants that disrupt the attachment of ARL-8 onto intracellular vesicles by EMS mutagenesis and candidate screenings. As a result, we found ARL-8 is dissociated from intracellular vesicles in \textit{blos-9(jpn2)} and \textit{sam-4(tm3828)}.

Both of these genes encode subunit of BORC which regulates lysosomal transport in mammalian cells. Next, we obtained all other BORC subunit mutants (i.e. blos-1, blos-2, snpn-1, kxd-1, blos-7 and blos-8) from mutant libraries as well as by CRISPR/Cas9 knockout and analyzed the axonal transport of SVPs. In BORC mutants except for kxd-1, axonal transport of SVPs is compromised, leading to ectopic accumulation of synaptic vesicles in the proximal axon. This phenotype is genetically suppressed by constitutively active ARL-8 or \textit{UNC-104}, indicating that BORC is upstream of ARL-8 and \textit{ARL-8} regulates the axonal transport of synaptic materials and synapse formation by controlling the nucleotide state of ARL-8.

The involvement of BORC and ARL-8 in regulating lysosome trafficking suggests a broad role of this signaling pathway in regulating cellular organelles.

833A  **A role for dip-2 (Disco-Interacting Protein-2 homolog) in neuronal migration and the maintenance of neuronal morphology.**  N. Noblett\textsuperscript{1,3}, Z. Ding\textsuperscript{1,3}, T. Roenspies\textsuperscript{1,3}, S. Flibotte\textsuperscript{2}, A. Colavita\textsuperscript{1,3}  \textsuperscript{1) Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON; 2) Department of Zoology, University of British Columbia, Vancouver, BC; 3) Neuroscience Program, Ottawa Hospital Research Institute, Ottawa, ON.

Mechanisms that inhibit inappropriate neurite outgrowth in the mature nervous system are likely to play important roles in maintaining normal neuronal function. A genetic screen for ectopic neurites in the VC motor neurons identified mutations in \textit{nde-5 (neurite outgrowth defective-5)} (1). We now show that \textit{nde-5} encodes the worm orthologue of Disco Interacting Protein-2 (Dip2) and has thus been renamed \textit{dip-2}. \textit{DIP2} proteins belong to a highly conserved protein family that contain an N-terminal DNA methyltransferase-associated protein 1 (DMAP1) binding domain and two adenylate-forming enzyme family domains. In addition to \textit{VC} defects, we found that \textit{dip-2} mutants show morphology defects in several other neurons. Most prominently in the touch neurons where morphology defects show an age-dependent increase suggestive of a role in neuronal maintenance. \textit{dip-2} mutants also show HSN neuronal migration defects. A CRISPR/Cas9-mediated functional GFP insertion into the native \textit{dip-2} locus revealed expression in many neurons including the touch neurons and HSN as well as epidermal cells. \textit{DIP2} localization in neurons was found to be predominantly cytoplasmic. In contrast, \textit{DIP2} in epidermal cells was predominantly plasma membrane localized. Cell specific rescue experiments are consistent with a cell autonomous role in neurons to promote proper morphology and migration. These findings indicate that \textit{DIP2} proteins are important for the development and maintenance of neuronal connections. (1) D. Carr et al., 2016 Plos One.

834B  **The CELF-family putative RNA binding protein ETR-1 acts with Wnt signaling to regulate Q neuroblast descendant migrations.**  M. Ochs, Erik Lundquist  Molecular Biosciences, University of Kansas, Lawrence, KS.

Migration of neurons is a critical process to ensure the proper development of the nervous system. The Q neuroblasts are bilaterally symmetrical, undergoing similar divisions and migrations. QR is born on the right side of the worm and its descendants migrate anteriorly. QL is born on the left side of the worm and its descendants migrate posteriorly. Here we show mutations in the gene \textit{etr-1} result in improper migrations of Q neuroblast descendants AQR and PQR. \textit{etr-1} encodes for an CELF-type RNA binding protein, and is an essential gene required for muscle development (Milne and Hodgkin, 1999)). Our alleles cause premature stop codons in an alternatively-spliced exon and are viable and fertile, because they affect only a subset of \textit{etr-1} isoforms required for AQR and PQR migration. We show that \textit{etr-1} acts not in the Q cells themselves but rather
non-autonomously in muscle to regulate AQR and PQR migration, ETR-1 is possibly involved in the production of a muscle-derived signal for AQR and PQR migration. The five Wnt ligands have roles in guiding the long-range migration of the Q descendants (Josephson et al., 2016; Zinovyeva et al., 2008), and at least three are expressed in muscles (cwn-1, cwn-2, and egl-20). eetr-1 AQR/PQR migration defects resemble those of Wnt double and triple mutants, and etr-1 and egl-20 interact genetically. Possibly, ETR-1 affects production of a muscle-derived Wnt signal or the production of a signal that acts in parallel to the Wnts in guiding AQR and PQR migration. CELF1, the mammalian molecule most similar to ETR-1, regulates alternative splicing and transcript stability. Future studies will be directed at identifying the targets of ETR-1 expressed in muscles that regulate AQR and PQR migration.

835C A conserved ser/thr kinase UNC-51 and its binding partner UNC-14 are required for axon-dendrite polarity in *Caenorhabditis elegans*. Ken-ichi Ogura Department of Molecular Pharmacology and Neurobiology, Yokohama City University Graduate School of Medicine, Yokohama, JP.

A neural cell is a highly polarized cell, which contains one cell body, one axon (for output) and dendrites (for input). The axon-dendrite polarity mechanism of the neural cell is extensively studied by using a cultured hippocampus neuron (in vitro), and many molecules are identified as the regulators (e.g. CRMP2). However, in vivo mechanism on the axon-dendrite polarity is poorly understood.

I found that, in unc-51 and unc-14 mutants, the synaptic vesicle precursors (SVs, Venus::RAB-3) and an axon marker Venus::TBA-1(alpha-tuburin) were abnormally localized at the dendrites of PVD neurons. UNC-51 is an evolutionally conserved ser/thr kinase. A RUN domain protein UNC-14 is an UNC-51 binding partner. It is known that the SVs are transported to the axons by a kinesis motor molecule UNC-104/KIF1A. I found that the unc-104 mutation suppresses the abnormal SVs localization of unc-51 and unc-14 mutants, suggesting that, UNC-104/KIF1A transport the SVs to the dendrites in unc-51 and unc-14 mutants. In addition, I found that, in unc-51 and unc-14 mutants, UNC-33/CRMP2 that is required for the PVD polarity were abnormally localized at the dendrites of the PVD neuron. These results suggest that UNC-51 and UNC-14 regulate localization of UNC-33 for axon-dendrite polarity in the PVD neuron.

I will discuss possible functions of UNC-51, UNC-14 and UNC-33 on the axon-dendrite polarity of the PVD neuron.

836A Parallel acting signaling pathways regulate gap junction specificity in the *C. elegans* motor circuit. S.D. Palumbos1, R. Skelton1, S.E. Von Stetina2, R. McWhirter2, D.M. Miller1,2 1) Neuroscience, Vanderbilt University, Nashville, TN; 2) Cell and Developmental Biology, Vanderbilt University, Nashville, TN.

Gap junctions provide the only known form of electrical communication between neurons. Although much has been learned about gap junction assembly, the mechanisms that direct the formation of these “electrical synapses” between specific neurons are largely unknown. Here we address this question in a study to identify transcriptionally-regulated components that control the neuron specificity of gap junctions in the *C. elegans* motor circuit. VA and VB class motor neurons normally establish electrical synapses with different sets of presynaptic interneurons to drive either backward (VA) or forward (VB) locomotion. The UNC-4 transcription factor is expressed in VA neurons to prevent the adoption of VB-type inputs. *unc-4* mutants are unable to crawl backward because VA neurons are miswired with gap junctions from interneurons (AVB) normally reserved for VBs. Work in the Miller lab has shown that UNC-4 preserves functional VA inputs by antagonizing two independent signaling pathways. UNC-4 normally blocks EGL-20/Wnt signaling as well as a parallel acting G-protein-dependent pathway involving GOA-1/GaO to prevent the formation of VB-type connections. In both cases, however, the UNC-4 targets that effectively regulate these downstream pathways are not known. To address this question, we are using a new cell-specific profiling strategy, SeqCel (RNA-Seq of *C. elegans* cells), to identify *unc-4*-regulated transcripts in VA neurons. We hypothesize that UNC-4 acts as a trancriptional repressor, and thus predict that *unc-4* regulated targets should be over-expressed in *unc-4* mutant vs WT VA neurons. Candidate *unc-4* targets will be screened by RNAi for suppression of the Unc-4 phenotype (e.g., ectopic gap junctions with AVB). This strategy will likely reveal new regulators of gap junction specificity. Thus, this project applies a quantitative RNASeq profiling strategy to elucidate a fundamental question in developmental neuroscience.


In many animal species, sexual maturity is accompanied by gender-specific developmental changes in the nervous system. Specifically, a number of neurons within the nervous system adopt sex-specific structural and functional identities during sexual maturation. These sexually dimorphic neurons are part of sexually dimorphic neuronal circuits that, once active, regulate sex-specific behaviors. Besides their relevance in healthy nervous system function, temporally regulated sexually dimorphic neuronal circuits can be differentially susceptible to pathological processes that result in mental health disorders. While nervous system development during embryogenesis has been extensively studied, the genetic programs involved in temporally regulating neuronal differentiation and circuit formation during sexual maturation are poorly understood. After making a comprehensive chemical map of the cholinergic nervous system of the nematode, I found that sex-specific motor and sensory cholinergic neurons are born early in development but do not express acetylcholine (ACh) pathway genes until sexual maturity. In addition, the AIM interneuron present in both sexes undergoes a neurotransmitter switch only during male sexual maturation. By following a candidate gene approach I found that the evolutionary conserved heterochronic factors are required for the correct timing of neuronal differentiation during sexual maturation. When mutants for the zinc-finger transcription factor *lin-29* were analyzed, AIM neurons failed to switch from a glutamatergic to a cholinergic identity in males during sexual maturation. *let-7* mutants phenocopied *lin-29* mutants suggesting that the *let-7* miRNA acts upstream of *lin-29* in AIM neurons. In contrast, the analysis of *lin-28* loss of function mutants showed that the AIM neurotransmitter switch happened earlier in development.
we utilized the somatosensory neuron, PVD, with its highly stereotyped 'menorah'-like dendrites. These dendrites are formed by molecular signals that may be required during different stages of development. In order to better understand dendrite development, we mapped one of the suppressors to a gene, *muscleblind-1/mbl-1*. We found that mbl-1 is necessary and sufficient for touch neuron axon extension in a cell-autonomous manner. Gentle touch response in posterior and anterior side is compromised in *mbl-1* mutant. Mbl-1 function is mostly known in muscles as a splicing regulator. Recent study in worm suggested that MBL-1 is required for synapse stabilization in DA9 motor neuron (2). We hypothesize that MBL-1 is regulating splicing of genes required for microtubule stability.

References:

### 838C Regulation of neuronal microtubule cytoskeleton.

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In the nervous system, directional flow of information depends on the polarized architecture of neuron. Neuron has a unique structure — many dendrites and one axon. Microtubule polymers are the building blocks of the polarized architecture of neuronal cell. Although MT protofilaments give stability in axon, they themselves are dynamic - undergoing polymerization and depolymerization. The dynamics of the microtubule network needs to be regulated in a context-dependent manner during polarization, maintenance or other remodeling processes.

Loss of kinesin-13 family microtubule depolymerizing enzyme *klp-7* (kinesin-like protein) leads to excess stabilization of microtubules, leading to a multifaceted neuronal phenotype in *C. elegans* mechanosensory (1) and other neurons. We found that this phenotype in *klp-7(lf)* can be reversed by a microtubule-stabilizing drug Colchicine or in backgrounds lacking either alpha or beta tubulin. We hypothesized that a genetic screen for the suppressors of *klp-7(lf)* will help us identify regulators of microtubule cytoskeleton. We conducted a genetic screen saturating the mutagenized genome. By combining meiotic recombination mapping and whole genome sequencing, we have identified candidates such as tubulin genes, post-translation modification factors and other novel factors.

We mapped one of the suppressors to a gene, *muscleblind-1/mbl-1*. We found that mbl-1 is necessary and sufficient for touch neuron axon extension in a cell-autonomous manner. Gentle touch response in posterior and anterior side is compromised in *mbl-1* mutant. Mbl-1 function is mostly known in muscles as a splicing regulator. Recent study in worm suggested that MBL-1 is required for synapse stabilization in DA9 motor neuron (2). We hypothesize that MBL-1 is regulating splicing of genes required for microtubule stability.

### 839A Genetic and biochemical analyses between the Heparan sulfate proteoglycans and Wnt signaling proteins in *Caenorhabditis elegans*.

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Heparan sulfates (HS) are unbranched glycans containing substantial modification patterns in the way of acetylation, sulfation, and epimerization. These extracellular polysaccharides are bound through conserved serine residues to core proteins to form Heparan sulfate proteoglycans (HSPGs). HSPGs are known to regulate cell adhesion, motility, and signaling by modulating protein-protein interactions. Moreover, they have been implicated as co-factors of many morphogens and extracellular proteins including FGF, Wnt, Slit/Robo, among others. Extensive research in *Caenorhabditis elegans* has established a role of the Wnt family genes in anterior-posterior cell migrations and axon pathfinding. However, the mechanisms regulating Wnts and the frizzled receptors in these processes are largely unknown. To investigate the role of HSPGs with Wnt signaling during cell migration, we performed a genetic analysis between these gene networks in *C. elegans*. Our findings show that syndecans and glypicans, both membrane-bound proteoglycans, function to promote migrations and determine final cellular positions in *Caenorhabditis elegans*. Double mutant analyses between HSPG genes demonstrate distinct redundancies among different anterior-posterior neuronal migration events. Moreover, HSPGs genetically interact with Wnt-ligands and frizzled receptors in a context-dependent manner to mediate cell migration and axon guidance. Based on our genetic analyses, we hypothesize that the transmembrane SDN-1/Syndecan forms a biochemical complex with MIG-1/Frizzled and EGL-20/Wnt. Co-immunoprecipitation experiments suggest that the Frizzled receptor MIG-1/Fz physically interacts with the EGL-20/Wnt-ligand and SDN-1/Syndecan in an *in vitro* heterologous system. Future work will focus on the structure-function analysis of SDN-1, including the involvement of its conserved heparan sulfate attachment sites, and their significance for biochemical interactions with Wnt signaling proteins. We propose that HSPGs act redundantly as co-receptors of Wnt-signaling to regulate defined neuro-developmental cell and axonal migrations.

### 840B Characterizing the function of a novel allele (dz205) in the development of PVD.

C. J. Salazar, C. A. Diaz-Blazac, H. E. Bülöw Genetics, Albert Einstein College of Medicine, Bronx, NY.

Neuronal development depends on numerous extracellular and intracellular cues to ensure proper dendritic structure and function. However, the mechanisms, which control and ensure proper dendritic development, are incompletely understood and are difficult to study *in vivo* within vertebrates. *C. elegans* is an excellent model to determine when and where genetic and molecular signals may be required during different stages of development. In order to better understand dendrite development, we utilized the somatosensory neuron, PVD, with its highly stereotyped 'menorah'-like dendrites. These dendrites are formed...
through consecutive branching formation of primary, secondary, tertiary, and quaternary dendritic branches. During a genetic screen, we have isolated a mutant allele, dz205, which elicits a characteristic "hyper-branching" phenotype in which the number of dendritic branches of the PVD somatosensory neuron increases. In order to quantify this phenotype, we utilized morphometric analysis to characterize the morphology of PVD at the L4 larval stage. We found the number of ectopic secondary and tertiary increased in mutant animals compared to the wildtype. This finding suggests that the normal function of dz205 is to limit the formation of ectopic branches of PVD dendrites. Currently, our research aims to identify the gene mutant in dz205 and to determine in which tissue the corresponding gene may act during the development of PVD dendrites.

841C WormGUIDES: Assembling and Accessing an Integrated Record of Neural Development. Anthony Santella1, Mark Moyle2, Ryan Christensen3, Kris Barnes1, Gabriela Bosque2, Leighton Duncan2, William Duncan2, Li Fan1, Brandon Harvey3, Richard Ikegami2, Braden Katzman1, Abhishek Kumar3, Nhan Nguyen1, Titas Sengupta2, Pavak Shah1, Doris Tang1, Daniel Colón-Ramos2, Hari Shroff2, William Mohler2, Zhirong Bao1 1) Developmental Biology, Sloan Kettering Institute, New York, NY; 2) Cell Biology and Neuroscience, Yale University School of Medicine, New Haven, CT; 3) Section on High Resolution Optical Imaging, NIBIB NIH, Bethesda, MD; 4) Genetics & Dev Biology & Ctr. For Cell Analysis and Modeling, UConn Health Ctr., Farmington, CT.

WormGUIDES is an interactive 4D atlas of C. elegans embryogenesis. Its goals are to (1) provide a model of neural development based on detailed time lapse measurements of nuclear positions and neurite outgrowth; (2) cross reference worm community data with the 4D model and (3) provide an easy to use visualization platform for exploring, understanding and annotating the model and sharing insights.

The major tracts of the adult nervous system are laid out early and added to over time. By the 1.5 fold stage many major structures are established. The nerve ring (NR) forms a complete loop that includes dorsal and ventral cells. Sensory nerves have extended to the dent, and the amphid commissure is established. Motor neurons in the VNC have intercalated and others have extended into the VNC and toward the NR.

The early emergence of tracts motivates a hierarchical approach to modeling and measuring neural development. Our model contains three levels of structure: (1) Tracts, major nerve tracts; (2) Multi-cellular structures, small groups of co-labeled neurons; and (3) Individual cells. The latter two include cell bodies as well as fascicles or individual neurites. Neurites are threaded through the tracts based on measured lengths and tip positions to minimize noise in alignment and maximize legibility. The current model contains 9 tracts representing the amphid sensory nerves, amphid commissures, NR, VNC and connections, 21 neurons (5 single cell) and nuclear positions up to twitching. We have mapped 6 groups of neurites in the NR with stereotypical positions involving 30 neurons, and sorted the 38 ventral-going amphid commissure axons into 4 temporal groups. 182 markers slated for analysis cover almost all neurons. Stochastic labeling by heat shock, mosaicism of reporter arrays and single cell photo conversion are being pursued to distinguish intertwined neurites. Additional imaging and analysis tools are being developed to push our model to hatching.

The wormguides.org website provides comprehensive information on the project, access to our reagents and image data, and a download of the latest atlas. We accept nomination of markers and cells as priorities, and promote sharing of user-driven annotations of developmental processes.

842A Neurite position and synaptogenesis in the developing AIB neuron. T. Sengupta1, M. Moyle1, A. Kumar2, H. Shroff2, D.A. Colón-Ramos1 1) Departments of Cell Biology and Neuroscience, Yale University School of Medicine, New Haven, CT; 2) Section on High Resolution Optical Imaging, National Institute of Biomedical Imaging and Bioengineering, NIH, Bethesda, MD.

The position of a neuronal process (neurite) within a bundle or fascicle limits contact with other neurons to restrict potential synaptic partners. The AIB neuron of C. elegans travels through two distinct fascicles, forming presynaptic specializations in the posterior fascicle and postsynaptic sites in the anterior fascicle. The mechanism by which AIB positions its neurite in these two fascicles and restricts pre- and post-synaptic contacts to the appropriate neurons is not understood. We developed fluorescent markers to visually separate the two fascicles and label pre- and postsynaptic sites within the AIB neurite. Using dSPIM (dual-view inverted selective plane illumination microscopy), we visualized the process of outgrowth and synaptic polarity establishment in AIB during embryogenesis. By performing a forward genetic screen, we isolated candidate mutations that likely disrupt fascicle position and others that affect presynaptic polarity of the AIB neurite. Uncovering the causative genetic lesions for these mutants will provide insights into mechanisms that position neurites precisely within their fascicles to enable appropriate synaptic contacts.

843B The ROR/CAM-1 determines synaptic spatial specificity through opposite functions in synaptogenesis. Yanjun Shi, Kai Wang, Qian Li. Zhiyong Shao Institute of Brain Science, State Key Laboratory of Medical Neurobiology and Collaborative Innovation Center for Brain Science, Fudan University, Shanghai 200032.

Neurons form synapses with remarkable specificity both at the cellular and subcellular level. However, the molecular mechanisms underlying the synaptic specificity is not well understood. Here we use C. elegans A1Y interneuron as a model to address this question. A1Y forms presynapses with distinct and highly reproducible pattern: the ventral region proximal to soma without synapse (Zone 1), the turn region from the ventral to the dorsal fragment with enriched synapses (Zone 2) and the distal region with a few scattered synapses (Zone 3). We found that the receptor tyrosine kinase ROR/CAM-1 is required for A1Y synaptic specificity. Cam-1 regulates A1Y presynaptic spatial specificity through the opposite roles in synaptogenesis: it promotes synapse formation at the synaptic enriched region (Zone 2) and suppresses synaptogenesis at the asynaptic region (Zone 1). To determine the temporal role of cam-1, we monitored the dynamic change of the synaptic pattern during different...
development stages. We found that cam-1 act during both embryonic and larval stages. Further study indicated that the prosynaptogenesis role is mediated through wnt pathway: cwm-2/cfz-2/dsh-2/sys-1. While the antisympaptogenesis role of CAM-1 is independent of any known wnt receptors, Disheveled or ß-catenin. We conclude that ROR/CAM-1 regulates synaptic spacial specificity through opposite functions in synaptogenesis mediated by distinct signaling pathways.

844C Investigating the role of the C-terminal binding protein-1 (CTBP-1) in neuronal development and maintenance in *Caenorhabditis elegans*. T. Sherry¹, A. Reid¹², A. Chen¹, H. Nicholas¹ ¹) School of Life and Environmental Science, University of Sydney, Camperdown, NSW, AU; 2) Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, 13125, Germany.

C-terminal binding proteins (CTBPs) are transcriptional co-repressors which are conserved across many species, including *Caenorhabditis elegans*. *C. elegans* CTBP-1 is expressed in both the nervous system and hypodermis, and regulates several processes, including lifespan. We previously identified defective exploration behaviour and abnormal axonal morphology of dorsal SMD (SMDD) neurons in developing and adult *ctbp-1* mutant animals, highlighting a role for CTBP-1 in the development and maintenance of the nervous system. Further characterisation of the morphology of SMDD axons revealed that *ctbp-1* mutant animals display longer axons than wild-type animals, suggesting that axon guidance and/or termination cues are disrupted.

From the single *C. elegans* *ctbp-1* locus, two isoforms are transcribed: *ctbp-1a* and *ctbp-1b*. These transcripts encode distinct proteins: CTBP-1a, which contains an additional Thanatos-associated protein (THAP) domain, and CTBP-1b. We set out to further understand the function of the two CTBP-1 isoforms in the nervous system. The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)- CRISPR Associated Protein 9 (Cas9) system was used to generate a null mutant that removes the function of both CTBP-1a and CTBP-1b, and a mutant that only removes the function of CTBP-1b. The abnormal SMDD morphology and defective exploration behaviour of CTBP-1a mutant animals is not observed in CTBP-1b mutant animals. Furthermore, null mutant animals display the same defective axonal morphology and defective exploration behaviour as the CTBP-1a mutant animals, suggesting that CTBP-1b is not involved in these processes.

Overall, these results demonstrate differential roles for CTBP-1a and CTBP-1b in the regulation of SMDD axon morphology and exploration behaviour in *C. elegans*. Further roles for CTBP-1 in the nervous system are being investigated.

845A Gene expression regulating terminal maturation of HSN neurons is affected by preformed chromatin alterations during early development. Y. Shinkai, M. Doi  Biomedical Research Institute, AIST, Tsukuba, Ibaraki, JP.

Post-mitotic neurons mature through distinct and sequential cellular steps including migration, neurite elongation, synapse formation, and membrane excitability of the cell. Each step of the neuronal maturation requires the expression of specific genes in concert with epigenetic changes at an appropriate period. Representatively, activity-dependent DNA methylations could take place to induce timely gene expression programs during the maturation of post-mitotic neurons, suggesting direct coupling of epigenetic modifications to gene expression changes during neuronal development. In contrast to this generally accepted view, the majority of changes in chromatin accessibility was reported to occur in immature newborn neurons. This raises the possibility that some chromatic changes mediated by chromatin remodeling enzymes are temporally dissociated from gene expression. However, whether these predefined epigenetic changes in premature neurons directly affect gene expression programs in later terminal maturation step still remains unclear.

To uncover the roles of predefined epigenetic alterations in neuronal terminal maturation, we employed a pair of motor neurons called HSN in *Caenorhabditis elegans*. In the hermaphrodite worms, HSN neurons are born from neuroblast cells at the tail during embryogenesis and migrate to mid-body region by the start of L1 larval stage. Next, axon outgrowth initiates during L2 and L3 larval stage. Finally, HSN neurons express genes required for the functional maturation from L4 larval stage onward. Its transparent body allows easy access to gene expression activity and chromatin state by using extrachromosomal reporter arrays. Therefore, these features provide a powerful means to analyze the interplay between transcriptional activities and chromatin modifications during neuronal maturation in a single neuron level.

Here, we demonstrate that a zinc finger transcription factor EOR-1 positively regulates the expression of genes that mediate the terminal maturation of HSN neurons. ChIP-seq and mutation analysis reveal the presence of EOR-1 binding sites in the promoter region of the terminal maturation genes. A component of SWI/SNF chromatin remodeling complex SWSN-1 and cohesin loader MAU-2 also regulate the expression of genes for HSN terminal maturation. By visualizing chromatin dynamics in living worms, we show that chromatin composition at the promoter regions of terminal maturation genes has been already modulated at an early developmental stage dependently on the function of EOR-1 and MAU-2. Together, we propose that predefined epigenetic modifications mediated by EOR-1, MAU-2, and SWI/SNF complex are critical to prepare for future gene expression program in neuronal terminal maturation.

846B The development and functions of GLR glia. N. Stefanakis, S. Shaham  Laboratory of Developmental Genetics, The Rockefeller University, New York, NY.

*C. elegans* glia can broadly be divided into three classes: 46 sensory-neuron associated glia (sheath and socket cells), four nerve-ring wrapping glia (CEPsh cells), and six GLR (Glia Like cells of the nerve Ring) glia. While roles of *C. elegans* sensory organ glia and nerve-ring wrapping glia have been characterized, virtually nothing is known about the development and functions of GLR glia. The GLR cell bodies lie in a six-fold symmetry just posterior to the nerve ring. They extend anterior leaf-like processes that form a seal around the pharynx, isolating the nerve ring from the body cavity. These processes lie in close proximity to the muscle arm plate in the nerve ring, where neuromuscular junctions (NMJ) form with head motor neurons. GLR cells have unique characteristics distinguishing them from other *C. elegans* glia: (a) they derive from a mesodermal lineage that gives rise to body wall muscle, (b) they express the *C. elegans* ortholog of MyoD, (c) they are electrically coupled by gap
junctons to the RME motor neurons and head muscles, and (d) they have GABA immunoreactivity. Based on their morphological characteristics, position, and gene and neurotransmitter expression patterns, roles in neuromuscular junction development, maintenance and function are likely, suggesting possible similarities to persynaptic Schwann cells in vertebrates. To shed light on the development and functions of GLR cells, we plan to perform forward genetics screens to uncover genes controlling muscle vs. glial fate specification, to identify terminal identity selectors, and to uncover regulators of GLR shape. GLR glia ablated animals and mutants obtained from the screens will be used to study potential GLR glia roles in NMJ and sensory dendrite development and maintenance, and in head motor behavior. We are currently generating transgenic reporters and tools for conditional genetic manipulation of GLR cells. We are also performing transcriptome analysis of the GLR glia which will not only be used to identify genes mediating GLR glia functions, but also to inform us on molecular similarities with vertebrate glia subtypes.

847C Heterochronic regulation of the sex-specific maturation of the C. elegans nervous system. Hannah Steinert1, Douglas S. Portman1,2,3 1) Department of Biology, University of Rochester, Rochester, NY; 2) Department of Biomedical Genetics, University of Rochester, Rochester, NY; 3) Center for Neural Development and Disease, University of Rochester, Rochester, NY.

Structural and functional remodeling of the nervous system is a pervasive feature of adolescence in many animals. One critical purpose of this remodeling is to enable adult-specific sex differences in behaviors, such as mate-searching and parental care. While some of these changes involve neurogenesis and changes in connectivity, others occur through gene expression changes in existing neural circuits. In C. elegans, several pre-existing neural circuits undergo gene expression changes during the juvenile-to-adult transition, particularly in males. In one important example, juvenile males and hermaphrodites have similar levels of the food-associated chemoreceptor odr-10 in the sensory neuron AWA; however, upon maturation males specifically downregulate expression of this chemoreceptor, reducing food sensitivity and facilitating mate-searching behavior. tra-1, the master regulator of C. elegans sex-determination, acts cell-autonomously to repress male development, allowing us to alter the sexual identity of single cells by manipulating its expression. Feminizing AWA by inducing tra-1 is sufficient to generate high, hermaphrodite-like odr-10 expression in adult males, demonstrating odr-10 expression is dependent on genetic sex. This raises the question of how genetic sex and developmental timing intersect to regulate neural maturation. The heterochronic pathway is known to control developmental timing by regulating stage-specific events in the hypodermis; however, its functions in the nervous system are less well understood. Using developmentally regulated genes, such as odr-10, as markers for nervous system maturity, we investigated whether the heterochronic pathway mediates nervous system maturation. Consistent with this hypothesis the “precocious” lin-28 and “retarded” let-7 mutants have early and delayed maturation, respectively, while the retarded lep-5 and lep-2 mutants retain larval gene expression patterns as adults. For example, lep-5 and lep-2 mutants exhibit high levels of odr-10 expression in AWA. Like larvae, they also fail to exhibit food-seeking behavior. Surprisingly, our results suggest that other important heterochronic genes, such as daf-12 and lin-41, may not be important for this process. Currently we are determining where the heterochronic pathway acts to time neural maturation by performing cell-specific rescue. One interesting possibility that we are currently considering is that the male nervous system adopts a masculinized state at the juvenile-to-adult transition by heterochronic regulation of the sex-determination pathway.

848A C. elegans immunoglobulin superfamly members, syg-2 and syg-1, control anteroposterior neurite growth of GABAergic motor neurons. D. Tucker, B Ackley Molecular Biosciences, University of Kansas, Lawrence, KS.

Proper development of the nervous system requires directed axonal outgrowth. During development in C. elegans, the DD and VD D-type GABAergic motor neuron axons are directed anteriorly along the ventral nerve cord. The 6 DD neurons form during embryogenesis and the 13 VD neurons form later at the end of the L1 stage. Previously, we found that fmi-1/Flamingo, the single C. elegans member of the Celsr-like cadherin superfamily interacts synergistically with Wnt pathway components to regulate anteroposterior (A/P) axonal outgrowth of the DD and VD neurons. In mutant animals, some DD and VD neurons exhibit posteriorly-directed neurite (Pdn) outgrowth instead of the stereotypical anterior neurite (AN). We conducted a forward enhancer genetic screen in mig-5/Dishaved mutants for new mutations that synergistically increased the occurrence of Pdns. One of the alleles recovered was a nonsense mutation in the cell-adhesion molecule syg-2. To simplify our genetic analyses, we focused entirely on Pdns that occurred in the tail of the animal, where there are normally no GABAergic axons present. We found that, individually, loss of function in either mig-5 or syg-2 resulted in a Pdn in approximately 2% of animals, while 28% of the double mutants had a Pdn. Mutations in syg-1 also demonstrated synergy with mig-5, resulting in a similar penetrance to the syg-2;mig-5 double mutants. To test a second component of the Wnt signaling pathway, we used lin-17/frizzled. Loss of lin-17 function resulted in a Pdn in roughly ~20% of animals, while 46% and 55% of syg-2;lin-17 and syg-1;lin17 animals exhibited Pdns, respectively. The syg-1;syg-2;lin-17 triple mutant exhibited a 55% penetrance of the Pdn phenotype, indicating that syg-1 and syg-2 are in a common genetic pathway. Finally, we sought to determine whether the DDs or VDs were contributing to the Pdns observed. To do so, we compared the penetrance of Pdns in L1 stage animals to adults. Our previous work showed that the DD neurons were not affected by loss of function in fmi-1, however both DD and VD neurons are affected in mig-5;fmi-1 double mutants. Recent results indicate that that mig-5 affects all D-type neurons, while syg-1 and syg-2 more specifically affect DDs in both a mig-5 and lin-17 background, while fmi-1 is VD selective. Currently, we are examining where syg-1 and syg-2 are functioning during GABAergic axon development. Our results provide novel insights as to how neurons with the same morphology may use different patterning cues during nervous system development.

849B An atlas of C. elegans G protein-coupled receptor expression. B Vidal Iglesias1,2, U Aghayeva1, H Sun1, L Glenwinkel1, C Wang1,2, E Bayer1, O Hobert1,2 1) Dept. of Biological Sciences, Columbia University, New York, NY; 2) Howard...
Hughes Medical Institute.

One goal of modern day neuroscience is the establishment of molecular maps that assign unique molecular, and hence, functional features to individual neuron types. Such maps provide important starting points for a number of downstream applications. We describe here nervous system-wide maps of the expression of 250 members of the largest gene family in the C. elegans genome, rhodopsin-like (class A) GPCR receptors, composed of more than 1300 genes. We synthesize our expression patterns with those previously described and we have now expression information for about 25% of the gene family. As previously anticipated, 90% of the genes are expressed in sensory neurons, although not always exclusively. GPCR expression is significantly enriched in amphid and phasmid neurons, which in some cases express over 50 different receptors. Remarkably, the neurons with most GPCRs expressed are all involved in nociceptive behaviors. Independent of its “classic” sensory modality, we find that every sensory neuron, except URY, expresses at least one sensory-type GPCR. Moreover, around 20% are expressed in interneurons and motorneurons, implying that they have other functions beyond being sensory receptors. Including sensory, inter- and motorneurons we have so far revealed 25 new neuron types showing sensory-type GPCR expression.

We have identified 6 new GPCRs asymmetrically expressed in the AWC olfactory neuron, which we found to be regulated by known mechanisms establishing AWC asymmetry. The fact that we did not find asymmetric GPCR expression in any new neuron type suggests that C. elegans might not use functional lateralization as a widespread mechanism to increase sensory discrimination. After examining a subset of our reporters at different larval stages including dauer, we found two GPCRs that show different expression in L1 versus adult and 16 GPCRs that change their expression pattern during dauer diapause. Thus, sensory-type GPCRs seem to be very dynamically regulated in dauer, when the animal is exposed to a dramatically different sensory environment.

Our analysis constitutes a resource that provides multitude of starting points for multi-level downstream analysis, including the functional analysis of GPCRs in specific neuron types or the bioinformatic and experimental analysis of cis-regulatory control mechanisms and their evolution. Reporter transgenes are also invaluable neuronal identity markers that will help shed light on neuronal differentiation processes.

850C  **Homeodomain proteins MLS-2 and UNC-62 function to specify general identity and asymmetrical differentiation of olfactory neurons.**  Y.-W. Hsieh1,2, R. Xiong1,2, C.-F. Chuang1  1) Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL, USA; 2) Equal contribution.

The C. elegans AWC olfactory neuron pair asymmetrically differentiates into two subtypes, default AWC OFF and induced AWC ON, with distinct functions after general identity is specified. The Otx homeodomain transcription factor CEH-36 acts as a terminal selector of general AWC identity by directly regulating expression of AWC identity features. In addition, the HMX/NLX homeodomain protein MLS-2 is a transiently expressed inducer of ceh-36 expression. However, AWC identity is only partially abolished in mls-2 loss-of-function mutants, suggesting additional factors may have a partial redundant role to induce ceh-36 expression in the specification of AWC identity. We identified unc-62, encoding a homothorax homeodomain protein, from a small candidate gene approach screen for mutants of transcription factors with defective AWC identity. Like mls-2 loss-of-function mutants, unc-62 reduction-of-function mutants show a partial loss of general AWC identity. In addition, unc-62 and mls-2 double mutants show a nearly complete loss of AWC identity, suggesting that unc-62 and mls-2 have a partial redundant role in the specification of general AWC identity.

In addition to a defect in general AWC identity, our results show that mls-2 and unc-62 mutants also display defective AWC asymmetry. mls-2 and unc-62 single mutants have a high penetrance of 2AWC OFF phenotype, in which both AWC neurons become the default AWC OFF subtype, indicating an essential role of mls-2 and unc-62 in the specification of the induced AWC ON subtype. In addition, our genetic studies suggest that mls-2 and unc-62, with mls-2 acting upstream of unc-62, acts cell autonomously downstream of nsy-4 (claudin-like protein) to suppress the unc-43(CaMKII)-mediated calcium signaling in promoting the AWC OFF subtype. Consistent with the genetic data, we show that the number of cells expressing mls-2 is significantly reduced in nsy-4 mutants. Furthermore, our results suggest that mls-2 and unc-62 have separable functions in the specification of general AWC identity and AWC asymmetry. Thus, our study provides novel findings that mls-2 and unc-62 act redundantly to specify general AWC identity in early embryogenesis and are subsequently repurposed in the specification of asymmetric AWC subtypes in late embryogenesis.

851A  **The spatial and temporal regulation of gliogenesis in C. elegans by lin-32.**  A. Zhang1, D. Yan1,2,3  1) Department of Molecular Genetics and Microbiology, Duke University, Durham, NC; 2) Department of Neurobiology, Duke University Medical Center, Durham, NC; 3) Duke Institute for Brain Sciences, Duke Medical Center, Durham, NC.

Glia play an important role in most aspects of neural biology, and regulating the number of these cells is important for the proper functioning of the nervous system. During development, gliogenesis is a controlled process that involves many different genes functioning in different cells and at different time points, many of which are not yet known. The purpose of our study is to the molecular mechanisms behind gliogenesis, and we have found that a homolog of the human proneural gene Atoh1, lin-32, is involved in regulating the number of glia in C. elegans. Through genetic analyses, time lapse imaging, and screens, we have found that the transcription factor has a gliogenesis suppressing role that is independent of its proneural function during early embryogenesis. Furthermore, we have identified other proneural transcription factors including cnd-1 and ngn-1 that also appear to negatively regulate glial number. This finding is in line with previous studies showing the mouse ngn-1 homolog promotes a neural fate while independently represses the pathway controlling astrogliogenesis in the developing mouse cortex. The
processes regulating gliogenesis and neurogenesis are thus likely to be conserved from invertebrates to vertebrates, and this study suggests that in-32/Atoh1 may have a conserved and previously unknown function in regulating gliogenesis as well.

**Neurobiology - Novel Neuronal Methods**


Calcium dynamic imaging and free motion tracking coupled with external stimulations allow for in depth analysis of C. elegans behavior. We have developed an integrated platform for monitoring and controlling C. elegans under a variety of external stimulations, including thermal, electrical, and photo stimuli. This innovative platform combines rapid volumetric (20 volume/s) diffraction limited dual line-confocal microscopy (0.5 um x 1 um x 5 um voxel) to determine the neural pathways different external stimuli induce, while tracking worm's two dimensional motion. Never before has dynamic signal propagation, from neuron to neuron, been observed for C. elegans in free motion at such high volume scanning rate. External stimuli are computer controlled with <10 ms resolution for precise spatio-temporal synchronization with free motion behavior and whole-brain calcium dynamics. Physical linear and circular thermal gradients were implemented using customized temperature plates with thermal fluctuations of less than 0.05 °C. In addition, thermal stimulation was applied via a 1490 nm infrared laser to create virtual temperature conditions, synchronized with head motion. Infrared laser stimulation allows C. elegans' thermoreceptor (AFD neuron) to perceive temperature fluctuations exclusively in the time domain, thereby allowing for the complete virtual manipulation of the nematode's thermal environment. Electrical responses were induced using a technique that involves applying a linear or spatially alternating electrical field through a gelatin sample with fields ranging from 4 to 14 V/cm. Photon stimulation was implemented using a 405 nm laser with intensities ranging from 0 to 10 mW/mm². Volumetric Calcium imaging of QW1217 has also allowed for the complete mapping of the neurons responsible for each of the aforementioned stimuli. The microscope and software accommodate multiple simultaneous stimuli applications, such as electrical and photon simulations. Also tested were the neural pathway differences between infrared and photo avoidance behavior due to their very similar behavioral responses.

**853C A computational model of the calcium dynamics in Caenorhabditis elegans ASH sensory neuron.** Ehsan Mirzakhalili1, Bogdan Epureanu1, Eleni Gourgou1,2 1) Mechanical Engineering, University of Michigan, Ann Arbor, Mi; 2) Internal Medicine—Geriatrics, Medical School, University of Michigan, Ann Arbor, Mi.

*C. elegans* is widely used as a model system for monitoring stimulus-evoked 

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Calcium transients in neurons. The ASH sensory neuron is the subject of several such studies, primarily due to its key importance as a polymodal nociceptor. However, despite the pivotal role of ASH in *C. elegans*, the overall biology and the characteristics of its Ca²⁺ transients (e.g., the "off" response), no mathematical model has been developed to describe the full mechanism of ASH Ca²⁺ dynamics. We propose a phenomenological computational model which captures the Ca²⁺ transients in the *C. elegans* ASH neuron upon its activation. The model is built on biophysical cascades that unfold as part of the neuron's Ca²⁺ signaling events and homeostatic mechanism (e.g., TRPV channels and voltage-gated channels activation, Ca²⁺ release from intracellular stores, IP3 dynamics, PMCA and SERCA pumps function). The state of the ion channels is described based on Hodgkin-Huxley equations and the remaining molecular states are based on kinetic equations with phenomenological adjustments. We fit the model using experimental data of osmotic stimulus-evoked ASH Ca²⁺ transients, detected with a FRET sensor (TN-XL), in young and aged worms, both untreated and exposed to oxidative stress. We use a multi-objective genetic algorithm to find the parameters for young untreated worms' data set. Parameters are estimated using a hybrid method that consists of a genetic algorithm and nonlinear least-squares. We use the same approach to fit the model for the other groups of experimental data. We validate the model using data from the literature, from ASH activation by stimuli of several strengths and durations. Finally, we demonstrate how our model can be used to predict the ASH Ca²⁺ response to stimulation pulses that are challenging to achieve experimentally (stimuli sequences of varying durations/lengths, or ramp stimuli). Our model includes for the first time the changes in ASH cytoplasmic Ca²⁺ flux observed both upon delivery and withdrawal of the stimulus (i.e., the "on" and "off" responses). This effort is the first to propose a quantitative dynamic model of the Ca²⁺ transients generating mechanism in a *C. elegans* neuron, based on essential biochemical pathways of the Ca²⁺ homeostasis machinery.

**854A The discovery of neuroactive small molecule tools using a high-throughput egg laying screening pipeline.** Sean Harrington1,2, Aaron Au1,3,4, Maximilliano Giuliani1,3, Chris Yip1,3,4,5, Peter Roy1,2,6 1) Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Canada; 2) Department of Pharmacology & Toxicology, University of Toronto, Toronto, Canada; 3) Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Canada; 4) Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Canada; 5) Department of Biochemistry, University of Toronto, Toronto, Canada; 6) Department of Molecular Genetics, University of Toronto, Toronto, Canada.

The neuromuscular circuit that governs *C. elegans* egg-laying is well understood and the majority of regulatory elements within it are highly conserved. The egg-laying system has been exploited previously to probe the polypharmacology of established neuromodulators such as clozapine (Karmacharya et al. (2011), *Brain Res.*, **1393**(1), 91-99) and imipramine (Weinshenker et al. (1999), *J of Neurosci.*, **19**(22), 9831-9840). Findings from these studies translated to a mouse model demonstrating the conserved nature of components of the egg-laying system. Here, we aim to identify novel small-molecule modulators of neuromuscular function by screening for those that modulate egg-laying behaviour in the worm. To do so, we have developed a
high-throughput screening pipeline that takes advantage of the amenability of the egg-laying phenotype to computational counting. Using this screening pipeline, we have screened through 4642 molecules and identified 83 molecules that either stimulate or suppress egg laying behaviour at a concentration of 60µM or less. Many of these molecules have no reported relevant bioactivities in the literature. Several of our hits are bioactive in a vertebrate model (Danio rerio) suggesting the possibility of a conserved mechanism of action (Burns et al. (2015), Nature Comm., 6(1), doi:10.1038/ncomms8485). We are currently characterizing the mechanism of action of these molecules using four parallel approaches: 1) testing their interaction with a panel of human neuronal targets (NIH Psychoactive Drug Screening Project – Besnard et al. (2012), Nature, 492(7428), 215-220); 2) thermal proteome profiling using quantitative mass spectrometry to identify candidate targets in worms (Franken et al. (2015), Nature Protoc., 10(10), 1567-1593); 3) forward genetics screens to identify candidate targets (Kwok et al. (2006), Nature, 441(7089), 91-95); & 4) understanding their interaction with a panel of C. elegans egg laying mutants. We hope that our hits will become useful tools for future biological investigations, and that a subset might have therapeutic potential to modulate neuromuscular activity in more complex systems.

855B Screening bioactivity of virus surface glycoprotein peptides using C. elegans electropharyngeograms. J. C. Hincks1,2, E. House1, B. E. Taylor2, M. Harris3, K. Hueffer4  1) Department of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, AK; 2) Department of Chemistry, University of Alaska Fairbanks, Fairbanks, AK; 3) Department of Biology, California State University Long Beach, Long Beach, AK; 4) Department of Veterinary Medicine, University of Alaska Fairbanks, Fairbanks, AK.

The rabies virus surface glycoprotein contains an amino acid sequence similar to neurotoxin peptides known to bind to and inhibit the function of nicotinic acetylcholine receptors (nAChR). In preliminary experiments, we have shown that a peptide fraction of the neurotoxin-like-domain of the rabies virus inhibits signaling of central nervous system nAChRs. In the nematode, Caenorhabditis elegans, defined motor neuron MC acts as a neurogenic pacemaker for pharyngeal pumping, and nAChRs are critical to excitation of the pharynx. We propose to use pharyngeal pumping in C. elegans as a screening assay for bioactivity of variants of rabies virus glycoprotein peptides. We first conducted a study to confirm various elements of our method such as treatment administration, treatment incubation time, and whether time spent in the microfluidic chamber influenced pharyngeal pumping activity. In the present study we formed 3 treatment groups from these 29 amino acid long variants along with a PBS control. C. elegans were incubated at a concentration of 1 mM for each group for 60 minutes each. Following treatments, we assessed pharyngeal pumping frequency during 2-minute observation periods using a non-invasive electropharyngeogram (NemaMetrix Katalyst 100). Pharyngeal pumping was absent or greatly attenuated in animals incubated in active peptide compared to controls. Results indicate that internal and external exposure to a peptide proposed to inhibit nAChR attenuate pharyngeal pumping in C. elegans in a manner expected by nAChR antagonism. Results support the bioactivity of this virus peptide fraction and the utility of C. elegans electropharyngeogram as a screen for bioactivity of variants of this peptide.

856C Long-term imaging of circuit-wide neural dynamics during unconstrained locomotion. Ni Ji, Steven Flavell  Brain and Cognitive Sciences, Massachusetts Institute of Technology, CAMBRIDGE, MA.

Mapping neural activity across the entire brain in a freely behaving animal represents one of the most ambitious and exciting challenges in neuroscience. In an effort to attain such ideal datasets in C. elegans, recent studies have established key technologies to image large numbers of neurons across the brain of C. elegans during locomotion. To allow this exciting approach to be robustly and routinely applied to circuit interrogation in C. elegans, we tackle a number of open technical challenges. These include 1) removing mechanical constraints to the animal during imaging, 2) improving light efficiency to minimize phototoxicity and bleaching, 3) reducing motion artifacts by improving behavioral tracking, and 4) developing computationally efficient software to automate the segmentation of neurons. We will present progress towards these goals and demonstrate the application of circuit-wide imaging during unconstrained locomotion.


Recent advances in bio-imaging have made large scale recordings of neural activity at cellular resolution possible; providing the unprecedented opportunity to observe the dynamic activity of the entire nervous system in Caenorhabditis elegans. While software to analyze stationary data taken on immobilized animals is both publicly available and straightforward enough to develop, imaging immobilized animals has clear scientific limitations. In order to study the full scope of the complex transformation of sensory inputs to appropriate behaviors, the sample must be allowed to freely behave under a variety of controlled stimulations, at which point the challenge of detecting of moving objects is encountered?an active field in computer vision research. We have developed a lightweight software package capable of detecting and quantifying calcium dynamics in freely navigating C. elegans. A frame-by-frame static detection of intensity maxima is performed on a rapidly scanned volumetric dataset, followed by a variety of inference methods to establish the continuous identities of neurons and calculate their respective activity (measured by delta F/F0). Data taken with strain QW1217 was used for the development of the software, however only the dynamic GCaMP6 channel was used for the analysis and static nuclear markers such as RFP are not required for neuron detection. We also make no presumption on the hardware setup of the user with the hopes of increasing accessibility, compatibility and applicability for any future neurodynamic investigations. Experimental results have shown sufficient neural identification and tracing with minimal manual proofreading, so long as the volumetric acquisition rate is high compared to the worm’s translation through the field of view, such that the movement of a typical neuron will not exceed its diameter in the time between image volumes.
858B  Neural Interactome: Interactive Visualization of a Neuronal System.  J. Kim¹, W. Leahy², E. Shlizerman¹ ²  1) Electrical Engineering, University of Washington, Seattle, WA; 2) Applied Mathematics, University of Washington, Seattle, WA.

Both connectivity structure and dynamical biophysical processes determine the functionality of neuronal networks. We therefore develop a real-time framework, called Neural Interactome, to simultaneously visualize and interact with the structure and dynamics of such networks. Neural Interactome is a cross platform framework implemented in Python and is also a Web interface. It combines graph visualization with simulation of neural dynamics, or experimentally recorded multi neuronal time series, and allows application of stimuli to neurons for examining responses of the network. In addition, Neural Interactome supports structural changes, such as disconnection of neurons from the network (ablation feature), as typically done in experiments. Neural dynamics can be explored on a single neuron level (using a zoom feature), back in time (using a review feature) and recorded (using presets feature). We implement the framework to a model of the nervous system of Caenorhabditis elegans (C. elegans) nematode, and show that it can assist in studying neural response patterns associated with locomotion and other stimuli. In particular, we demonstrate how stimulation and ablation help to identify neurons, which play critical role in dynamics related to experimentally studied touch response circuit, and explore new scenarios that did not undergo extensive experimental studies.

859C  Pan-behavioromics: comprehensive analysis of worms' behavior using machine learning methods and its application to animals in general.  Kotaro Kimura¹, Shuhei Yamazaki¹, Ichiro Takeuchi²³, Takuya Maekawa⁴  1) Graduate School of Science, Osaka University, Osaka, Japan; 2) Nagoya Institute of Technology, Nagoya, Japan; 3) Center for Advanced Intelligence Project, RIKEN, Tokyo, Japan; 4) Graduate School of Information Science and Technology, Osaka University, Osaka, Japan.

A brain activity can be measured as a large number of neural activities using simultaneous optical monitoring, which is described as a time series vector data of many neural activities. Behavior, the final output of neural activities, however, is still poorly described, often just with one or a few parameters chosen subjectively. This huge asymmetry in data of neural activity and behavior is caused by the difficulty in analysis of behavior: Which aspects of a behavior such as speed, direction, their duration and changing rate, are related to animal's brain activities like sensory perception, memory, and decision-making?

Behavioral analyses using machine learning have been reported to classify animal's behavioral states or behavioral patterns (e.g., Brown et al., PNAS 2013), although they have not yet provided clues to understand the causal relationship between stimuli and behavioral response. To explore new methods for a comprehensive analysis of animals' behavioral response to environmental stimuli in collaboration with data science, we applied 3 classic and cutting-edge machine learning methods—decision tree, deep neural network (DNN), and pattern mining—on worms' repulsive odor learning (Kimura et al., J Neurosci 2010). (1) In decision tree analysis, researchers specify a number of features, and the algorithm chooses the ones that effectively classify two groups. We found that naive worms stop straight migration upon sensing a slight increase in the repulsive odor concentration (dC/dt > 0), although learned worms do not respond to it and continue the migration, suggesting that they ignore the "yellow signal". In addition, multiple mutant strains were classified based on the characteristic patterns of component change. (See Yamazaki et al. this meeting.) (2) Using DNN for time series data analysis, we found that, although the speed of naive worms is relatively constant, that of learned worms changed more vigorously in windows of tens of seconds even though the average speeds were the same. The result may suggest that the learned worms avoid the odor using "accelerator and brake" more effectively. (See Maekawa et al. this meeting.) (3) In discriminative pattern mining, we represented odor concentration change and behavioral responses per second as 30 combinations, and found prominently discriminative behavior patterns between wild-type and mutant worms from an extremely large number of possible patterns by using a new sparse estimation technique. In summary, each machine learning method efficiently provided novel and critical knowledge that cannot be obtained from traditional analyses. Such objective comprehensive study of behavioral response, or "behavioromics," allows us to concentrate on key features of behavior. Currently, we intend to apply the above methods for the behavioral analysis of other animals, such as long-travelling sea birds, disease model mice, and humans.

860A  An inverse-type of fluorescent Ca²⁺ indicator for detecting neuronal inhibition.  S. Kuge¹ ², T. Nishihara¹, T. Matsuda⁴, T. Teramoto¹ ², T. Takeharu², T. Ishihara¹ ²  1) Department of Biology, Kyushu University, Fukuoka, Jp; 2) CREST, Tokyo Jp; 3) The institute of Scientific and Industrial Research, Osaka University, Osaka Jp.

Fluorescent Ca²⁺ indicators have been improved for the last several decades and now are indispensable tools for cell biology, especially neuronal science, because they enable us to monitor neuronal activities. However, these fluorescent Ca²⁺ indicators including GCaMPS are ideal only for monitoring the activation of neurons. To understand precise functions of the neuronal network, a fluorescent Ca²⁺ indicator that is suitable for monitoring the neuronal inhibition would be necessary. We previously reported "inverse-pericam", which has a unique property; the fluorescence intensity gets 7-fold dimmer upon Ca²⁺ binding. We improved "inverse-pericam" by iterative error-prone PCR and got a variant, IP2.0, which shows nearly 20-folds dynamic range in vitro. When IP2.0 was expressed in HeLa cells, the fluorescence of IP2.0 was greatly quenched responding to histamine stimulation, indicating that IP2.0 can be used to detect change of the intracellular concentration of Ca²⁺. Then we expressed IP2.0 in AWcON neuron of C. elegans and monitored the decrease of Ca²⁺ concentration during the stimulation of isomyl alcohol, as well as the increase of Ca²⁺ concentration after removing isomyl alcohol. While the fluorescence of GCaMPS has known to be reduced slightly during the stimulation, we observed the increase of fluorescence of IP2.0 during the stimulation. These results suggest that odor stimuli decrease Ca²⁺ concentration in AWcON neurons. Next, we succeeded in expressing both of IP2.0 and RCaMP2.0 in AWcON neurons to monitor neuronal activation and inhibition simultaneously. Furthermore, we analyzed the neuronal activities of ASE neurons responding to the concentration of NaCl by expressing IP2.0 and RCaMP2.0. The fluorescence of IP2.0 increased by the decrease of NaCl, indicating that Ca²⁺ concentration in ASE neuron
is decreased by the decrease of NaCl concentration. These results suggest that IP2.0, which can be used with various red fluorescent Ca^{2+} probes is suitable for sensitively detecting neuronal activities, even which cannot be sensitively detected by GCaMPs.

Now, we are analyzing local Ca^{2+} dynamics in a whole single neuron by a wide-field imaging system by expressing IP2.0 in AWC^{OH} and ASEI neurons and we hope that this analysis makes clear that each sensory neuron has unique properties for informational processing within the neuron.

We hope that our imaging system makes it possible to analyze precise Ca^{2+} dynamics of sequential rapid neuronal activation and inhibition in a whole single neuron and that these results are helpful for understanding the informational processing in the whole neuronal circuit.

861B An automated high-throughput functional screen of neuromodulators for neuropsychiatric disease models. Ross Lagoy1, Heesun Kim2, Dirk Albrecht1  1) Worcester Polytechnic Institute, Worcester, MA; 2) University of Massachusetts Medical School, Program in Molecular Medicine, RNA Therapeutics Institute, and Howard Hughes Medical Institute, Worcester, MA.

Mental health disorders, like autism, depression, and epilepsy, affect millions of people worldwide. Some of these illnesses are highly associated with channelopathies, or dysfunction in ion channels, that affect neural activity in the brain. To accelerate the discovery of compounds that modulate neuronal activity in a living whole-organism, we are developing functional screening methods and establishing novel C. elegans models of human channelopathies.

To rapidly and cheaply screen many compounds in C. elegans, we developed two automated approaches based on calcium imaging in a single neuron with precise odor or optical stimulation. Our first method adapts a high-throughput microfluidic device that allows for automated serial drug exposure (seconds-minutes) to animals while recording neural responses when stimulated by odor or light. Our second method is an automated 384-well plate-based screen that allows for increased drug exposure (hours-days) and records neural responses by optogenetic stimulation. To address drug bioavailability, we are using these methods to observe dynamics of drug effects in various mutants. For example, we found nemadipine-A (an L-type calcium channel blocker) to inhibit calcium responses time-dependently in wild-type animals over 14 hours of exposure. Currently, we are scaling-up this process to screen hundreds of FDA approved small-molecules to detect other compounds that modulate neuronal activity in C. elegans for secondary screens.

In parallel, we are establishing various C. elegans models of channelopathies in the orthologus voltage-gated calcium channel (VGCC) EGL-19/CACNA1C. We used CRISPR-mediated homologous recombination to generate the first C. elegans model of Timothy syndrome (TS), a human disorder caused by a missense VGCC mutation that results in autism, arrhythmia, and developmental disorders. Shown at the cellular level in various model systems, this mutation leads to decreased channel inactivation. In our whole-organism TS model, we observe a severe developmental phenotype; animals are arrested immediately post-hatching stage, while heterozygous animals develop as wild-type. With our high-throughput microfluidic pulse device, we can quantify residual calcium, channel activation and inactivation rate, and detect altered channel kinetics in various EGL-19 channelopathies. Altogether, we aim to rescue these phenotypes with novel compounds derived from our screens and suggest their use for mammalian models.

862C Development of a SLM-based sheet illumination microscope for rapid, large-scale, video-rate 3-D neurodynamic observation in C. elegans. Blake Madruga1,2, Javier Carmona1, Steve Mendoza1, Joe Thatcher1, Katsushi Arisaka1,2, Elegant Mind Club 1) Physics and Astronomy, UCLA, Los Angeles, CA; 2) Electrical Engineering, UCLA, Los Angeles, CA.

Sheet illumination microscopy has made a large impact on the microscopy community due to its many inherent advantages. Increased photonic efficiency allows for lower power light sources, which in turn reduce phototoxic damage to the sample while providing an increased signal to noise ratio. To take advantage of such technique, a type of phase modulator, known as a Spatial Light Modulator (SLM) is used to generate a deep-penetrating, extremely long and narrow Bessel beam interference pattern. Through the use of an SLM, one can easily modulate the phase characteristics of an illumination beam in real time. This property enables greater flexibility and aberration compensation at the sample. The Bessel beams are generated through a bitmap image of the beam’s modulation transfer function, which is then displayed on the SLM with a prism phase rotation. This provides a much longer region of micron-order uniformity along the beam axis compared to conventional Gaussian geometries, while shifting the modulated beam away from the higher diffraction orders. The beams are then mapped onto the readout of two scientific CMOS cameras for rapid multi-channel imaging. A piezoelectric objective collar is used to enable rapid z-scanning, thereby creating 4D image volumes with adequate time resolution to characterize and observe active neural dynamics in C. elegans. A long working distance, high numerical aperture (NA), refractive-corrected objective lens is used to study neurobiology and participate in ratiometric calcium imaging. Tools of such flexibility will enable the study of whole-brain-scale neuronal activity and structure under various controlled conditions in C. elegans, over a variety of temporal and spatial scales.


Biological studies in general investigate behavioral differences between two different groups by observing a number of trajectories and/or extract manually designed statistical features from trajectory data to find statistical differences between the two groups. However, observation of a number of trajectories places large burdens on the researchers. In addition, manually designed statistical features can result in subjective analysis. This study introduces a computational method for automatically
finding characteristic trajectory segments of one group using deep neural networks (DNNs), which have received considerable attention in recent years. The notable characteristic of DNNs is a functionality of automatic feature extraction, and this characteristic permits us to analyze data without designing handcrafted features. In this study, we design a DNN architecture based on recurrent neural networks (RNNs), which are used to analyze time-series data such as trajectories, and train the network to distinguish trajectories of one group from trajectories of another group. Because the trained network is a binary classifier, the inputs of the network is a trajectory, i.e., a series of worm’s coordinates, and the output is a classified class, i.e., which group the input trajectory comes from. Because the network is trained to distinguish between trajectories of one group and trajectories of another group, artificial neurons in DNN are trained as detectors of distinguishing segments between one group and another group. Therefore, using activations of the trained artificial neurons, we can find segments in a trajectory that are characteristic of one group (or another group). When we provide the results of our proposed method to the biologists, we highlight the found segments in a trajectory and this helps the researchers proposing a new hypothesis based on the characteristic segments in trajectories. Because the inputs of our network is a series coordinates, to be exact a series of speed and angular speed, the proposed method can be easily applied to observed trajectories from other animals such as birds, mice, fishes, and humans. We also develop a web site for analyzing uploaded animal trajectory data by researchers. When a researcher uploads trajectory data of two different groups to the site, the data are analyzed by our DNN-based method and then the researcher can browse each uploaded trajectory where characteristic segments in the trajectory are highlighted.


The ability to monitor neural activity in freely behaving animals is important in determining neural mechanisms responsible for behavior. Real time worm tracking with neuronal observation has been used to study worm behavior in freely moving C. elegans. However, few setups have attempted to use worm tracking in conjunction with behavioral stimulations. The difficulty being that most behavioral stimulations used to study C. elegans such as electrotaxis and thermotaxis are difficult to incorporate into existing worm tracking platforms. We present a novel microscope platform, W-TEM (Worm Tracking Epifluorescence Microscope), to bridge this gap in our understanding of worm behavior. The microscope is a standard wide field epi-fluorescence microscope placed on top of a stable platform to move the microscope as it tracks along the sample plane, accommodating most C. elegans behavioral stimulations. This microscope can incorporate a stimulation system that covers 20 cm x 20 cm of worm area movement, 50 cm x 50 cm of total size, and a height of up to 30 cm, which is larger than most other stimulation systems. It can take data at video rate 30 fps, for cameleon ratiometric imaging, as well as monitor whole worm behavior. In addition, our platform can be moved easily between different experiments, using a system of rails that is attached to the microscope platform. Using this system, we have been able to study both Electrotaxis and Thermostatic behaviors using the same system. In the case of Electrotaxis, we have found that high voltages corresponding to 8V/cm create a dampening effect of worm motion, possibly due to paralyzing the lower half of the worm body. We also conduct investigations into the AFD and AY neurons during isothermal behavior and find a phase lag of 0.8 seconds between neural activity and head location during isothermal tracking.

864C In vivo optogenetic acidification of synaptic vesicles in C. elegans. J.P. Merchant, M.T. Palfreyman, E. Hujber, R. Hobson, E. Jorgensen, G.G. Ernstrom, 1) Department of Biology; 2) Program in Neuroscience; 3) Program in Molecular Biology and Biochemistry; 4) Middlebury College, Middlebury, VT; 5) University of Utah, Salt Lake City, UT; 6) Howard Hughes Medical Institute.

Reliable chemical neurotransmission requires that neurotransmitters are loaded in regular quantities into each synaptic vesicle; however, the mechanisms that control synaptic vesicle filling are not known. We are testing the hypothesis that the acidic pH created inside synaptic vesicles during the loading process regulates synaptic vesicle fusion. We have previously found that fusion is decreased when vesicles are poorly acidified. To test whether acidification is sufficient for vesicle fusion, we designed a construct that encodes a light-activated proton pump targeted to synaptic vesicles. We hypothesized that light stimulation of strains expressing this construct would show an increase in vesicle acidification and corresponding increase in synaptic vesicle fusion. pH-dependent changes in fluorescence from vesicle-targeted pHluorin were measured before and after light stimulation using confocal microscopy in immobilized, dissected worm preparations. We found that light stimulation of strains expressing the vesicle-targeted proton pump enhances vesicle acidification in situ. Additionally, by analyzing the baseline vesicle acidification in situ, we found that the relative extent of the acidification defect caused by proton pump mutations unc-32(e189) and vha-12(n2915) correlates with the severity of known physiological and behavior phenotypes in these mutants. Relative to vha-12(n2915), which shows a locomotion defect, a reduced vesicle fusion rate, and a vesicle acidification defect, unc-32(e189) mutants exhibit a stronger locomotion defect, a larger decrease in vesicle fusions, and a greater acidification defect.

866A Labeling of active neural circuits by the calcium probe CaMPARI. K. Mori, Y. Toyoshima, Y. Iino Graduate school of science, The University of Tokyo, Tokyo, JP.

To understand the information processing by the nervous system, it is necessary to reveal the functional connectivity of component neurons. In C. elegans the wiring diagram of all its neurons has been reported. However, the functional networks that are used for particular behaviors such as sensory perception or goal-oriented behaviors, have not yet been fully clarified. Thus, we are currently working on mapping the functional circuits to the connectome data.

Here, we constructed an experimental system to extract the functional neural circuits by using a new type of genetically encoded Ca2⁺ indicator called CaMPARI (1). CaMPARI fluorescence changes from green to red irreversibly only when high calcium concentration and ultraviolet (UV) light are present simultaneously. Since this probe has been used only in other model
organisms such as *D. melanogaster*, zebrafish and mouse, we tested whether this probe works efficiently in the neurons of *C. elegans*. Several CaMPARI variants which show different dissociation constant (Kd) for Ca$^{2+}$ have been reported. We tested some of these variants to find which one would be suitable for this new experimental system. We used ASER neuron and NaCl downstep stimulus for the verification of the CaMPARI function in *C. elegans*. We next made a strain that expresses CaMPARI in all neurons. We localized CaMPARI at nuclei of each neuron so that we can identify each neuron automatically and annotate them (2). By using this strain, we labeled the neurons that are active after the decrease of NaCl concentration. Our laboratory has previously shown that pairing starvation with exposure to NaCl causes salt avoidance learning in *C. elegans*. We are currently searching for neurons that respond differently before and after the formation of memory by using this system.


867B **Cold-tolerance is a fast and easy method to identify neuronal dysfunction in *C. elegans***. Cole Tomberlin1, Nichole Liachko2, Chris Link1 1) Integrative Physiology, Univ Colorado, Boulder, CO; 2) Geriatrics Research Education and Clinical Centers, Veterans Affairs Puget Sound Health Care System, Seattle, WA; 3) Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Washington, Seattle, WA.

Wild-type *C. elegans* raised at cooler temperatures (16°C) can survive a sudden shift to a cold temperature (4°C). However, animals raised at warmer temperatures (20-25°C) die following a similar sudden cold stress. A single pair of neurons, ASJL and ASJR, are required for sensing temperature. Active ASJ neurons initiate a signaling cascade that results in lipid changes in the gut and decreased cold tolerance. In the absence of signaling from these neurons, worms maintain a cold-resistant lipid profile. Mutants that affect neuronal signaling broadly or ASJ signaling specifically are resistant to cold temperatures (Ohta et al., 2014). Testing *C. elegans*’ ability to survive a sudden drop in temperature is a rapid and robust method to detect changes in signaling from the ASJ nerve pair, and may provide a surrogate screen for broader neuronal dysfunction. We have used this assay to test a broad range of transgenic strains and single gene mutants, particularly those with relevance to neurodegenerative disease. Transgenic strains with neuronal expression of neurodegeneration-related proteins (e.g., TDP-43, Abeta) display cold resistance, while equivalent strains with muscle expression of these same proteins do not. This assay has been particularly useful in detecting neuronal dysfunction in strains lacking obvious movement or morphological defects [e.g., tdp-1(ok803), sut-2(bk741), trx-1(ok1449)]. We suggest that cold resistance can be used as a simple method for characterizing new strains or screening for mutants with neuronal dysfunction.

868C **Laser axotomy and optogenetics for functional analysis of ipsilateral neuronal connection at synaptic level**. Y. Tsukada, I. Mori Neuroscience Institute and Group of Molecular Neurobiology, Graduate Sch Sci, Nagoya Univ, Nagoya, JP.

Functions of neural circuits arise from structural ensembles of synaptic connections that are composed of abundant parallel synapses in a connection-specific manner. However, it remains unclear whether each of the parallel synapses differently functions to the signal transmission among neurons. Here, we dissect ipsilateral synapses between AFD thermosensory and AIY interneurons. A pair of AFDs and a pair of AIYs are important for thermotaxis behavior of *C. elegans* because the disruption of either AFD or AIY by laser ablation or genetic manipulation resulted in severe impairment of thermotaxis. AFD and AIY neurons are connected each other with 6 to 10 chemical synapses along nerve ring but the significance of the connection manner remains unknown.

We utilized femtosecond laser axotomy and optogenetic inhibition to analyze the effect of perturbing synaptic connections between AFD and AIY. In a laser axotomy approach, red and green genetically encoded fluorescence calcium indicators R-CaMP2 and GCaMP3, respectively, were expressed to distinguish closely located thin axonal processes of AFD and AIY. We verified that laser cuts of AFD axonal regions in L4 stage did not cause severe damage to AIY neurites. In an optogenetic approach, we expressed a genetically encoded singlet oxygen generator called miniSOG specifically at AFD pre-synaptic region and using gcy-8 AFD-specific promoter and making a fusion protein with synaptobrevin SNB-1, a synaptic vesicle protein orthologous to human vesicle-associated membrane protein 1. Because intact AFD and AIY neurons show synchronized neural activities in response to thermal stimulus of sinusoidal increase, simultaneous monitoring of the AFD and AIY neural activities after the laser cut of AFD axon or optogenetic inhibition with miniSOG elucidates the connectivity strength between these neurons as a result of the perturbation. We are trying to categorize AIY neuronal activity after perturbation of ipsilateral connections at synaptic level. Given that sensory neurons have been shown to be polymodal and neural circuits consisting of only 302 neurons enable to give rise to a variety of complex sensory behaviors, these approaches should uncover the function of individual synapses of *C. elegans* nervous system.

869A **cGAL and split cGAL for precise transgene control in *C. elegans***. H. Wang1,2, J. Liu1,2, S. Gharib1,2, C.M. Chai1,2, A. Hill1,2, K.P. Yue1,2, E.M. Schwarz1, N. Pokola1, P.W. Sternberg1,2 1) Biology and Biological Engineering, California Institute of Technology, Pasadena, CA; 2) Howard Hughes Medical Institute, California Institute of Technology, Pasadena, CA; 3) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY; 4) Department of Life Sciences, New York Institute of Technology, Old Westbury, NY.

Genetic tools that allow tight control of gene expression in desired spatiotemporal patterns are critical for dissecting neural circuits and elucidating gene function. The bipartite GAL4-UAS system, in which a transcriptional activator protein (Gal4p) is driven in specific cells to bind its target sequence (UAS) upstream of an effector and drives expression of the effector gene, has become a powerful tool for manipulating gene expression in several model organisms. However, this system has not been successfully adopted for *C. elegans* research, due to its weak performance at *C. elegans* cultivation temperatures (15-25°C). By
systematically engineering the three major components, we have established cGAL, a robust GAL4-UAS system for *C. elegans*. First, we replace the endogenous Gal4 transcriptional activation domain with a synthetic strong activator VP64; second, to improve the binding of Gal4p to the effector construct, we put 15 copies of UAS sites upstream of the effector gene; lastly, to enhance the performance of GAL4-UAS system at low temperatures, we adopt a Gal4p DNA-binding domain (DBD) from a cryogenic yeast strain *S. kudriavzevi*. Unlike *S. cerevisiae* (grown best at 30-34°C) from which the canonical Gal4p DBD is derived, *S. kudriavzevi* has the optimal growth temperature at 23-24°C, much closer to *C. elegans* cultivation temperatures. The optimized cGAL system displays temperature robustness of driving GFP effector across the 15-25°C range. We further demonstrate the utility of the cGAL system using reporter expression in multiple tissues, site-of-action experiments, and gain-of-function channelrhodopsin experiments. We have also built a basic cGAL toolkit with cGAL strains and plasmids for genetic labeling, cell ablation, visualization and manipulation of neural activity. However, for many cell types, especially neurons, there are not known DNA regions that direct expression only in that cell type. We thus develop a novel split cGAL system using intein, in which VP64 and Gal4p DBD are driven by two distinct promoters. The two components are reconstitute into a functional transcriptional activator to trigger the expression of the effector gene only in those cells where both promoters are active. We expect that cGAL and split cGAL will significantly aid *C. elegans* research by providing precise transgene control.

870B  Learning-dependent behavioral modulation of sensory behavior revealed by machine learning and optophysiological analysis in a virtual environment.  S. Yamazaki1, Y. Ikejiri1, F. Hiramatsu1, A. Yamazoe-Umemoto1, K. Fujita1, Y. Tanimoto1, K. Hashimoto2, T. Maekawa3, K. Kimura1 1) Graduate School of Science, Osaka University, Osaka, Japan; 2) Graduate School of Information Sciences, Tohoku University, Miyagi, Japan; 3) Graduate School of Information Science and Technology, Osaka University, Osaka, Japan.

Animals modify their behavior based on experiences as learning, although identifying component(s) of behavior modulated by learning has been difficult. In contrast to neural activities, which can be monitored in large numbers of cells simultaneously recently, behavior in general is still analyzed in classic ways and insufficiently studied using simple measures, such as velocity, migratory distance, and/or the probability of selecting a particular goal. Comprehensive classifications of animals' behavior by using machine vision and machine learning methods have been achieved (Gomez-Marín et al., Nat Neurosci, 2014; Brown et al., PNAS, 2013). However, these methods have not been applied to animals' sensory behavior because of technical limitations in measuring sensory signals that animals receive during the behavior. To overcome this problem and effectively identify behavioral components modulated by learning, we used machine learning aiming to detect changes in navigation of worms in a measured odor gradient. We have previously reported that, after experiencing the repulsive odor 2-nonanone for 1 h, worm's odor avoidance behavior is enhanced, and that they move away from the odor source more efficiently (Kimura et al., J Neurosci, 2010). We have also quantified the dynamic changes in the odor concentration during odor avoidance behavior (Yamazoe-Umemoto et al., Neurosci Res, 2015). In the present study, we used decision tree, a machine learning algorithm, to extract features of the animal's sensorimotor response during navigation modified by odor learning. During the migration down the odor gradient, naive worms responded to slight increases in the repulsive odor concentration by stopping forward movements and initiating turns. In contrast, the probability of response was lowered after learning, suggesting that the learned worms ignore "a yellow light". Consistently, by calcium imaging of ASH neurons, whose activation causes turns under a virtual odor gradient (Tanimoto et al., this meeting), we found that the ASH response to a small increase in the odor concentration was reduced after learning. Furthermore, by applying the decision tree analysis, multiple mutant strains were categorized into several groups based on behavioral features. Thus, the integrative machine learning analysis of sensory information and behavioral response is a powerful tool to obtain comprehensive understanding of dynamic activities of neural circuits and its modulation by learning.

871C  Whole-Brain Imaging with Single-Neuron Identity in *C. elegans*.  E.I. Yemini1, V. Venkatachalam2, A.D. Samuel2, O. Hobert1 1) Department of Biological Sciences, Columbia University, Howard Hughes Medical Institute, New York, NY, USA; 2) Department of Physics and Center for Brain Science, Harvard University, Cambridge, MA, USA.

Stereotypy of the *C. elegans* nervous system affords single-neuron registration across animals, and consequently, robust statistics for neurobehavioral coding and transcriptomics. Fast methods of whole-brain imaging in worm exist, but unfortunately determining the identity of neurons within these volumes remains a bottleneck – requiring a long, difficult, ad hoc process. We have developed a landmarked strain for whole-brain neural identification, NeuroPAL (a Neuronal Polychromatic Atlas of Landmarks). Each neuron is assigned an invariant fluorescent barcode such that color and position specify unambiguous neural identity via the unique 3-tuple (color, position, ganglion). The GFP channel is preserved for reporters of neural activity (GCaMP) and transcriptomics (GFP). Our landmark strain employs 5 fluorophores. To visualize this strain we have used a new microscope, developed by the Samuel lab, with the capability of imaging 9 unique fluorescence channels generated by 4 excitation lines and 4 emission bands. This microscope acquires whole-brain volumes, of 4 fluorophores, simultaneously, at 10Hz. Together, these two innovations permit fast whole-brain imaging, with single-neuron identity and neuronal registration, across an animal populace.

Neurobiology - Regeneration and Degeneration

872A  Stress leads to neurodegeneration in *C. elegans* SOD-1 knock-in models for Amyotrophic Lateral Sclerosis.  S. N. Baskoylu, J. Yersak, P. O’Hern, J. Simon, S. Grosser, A. Hart  Neuroscience Graduate Program, Brown University, Providence, RI.

Amyotrophic lateral sclerosis (ALS) is an adult-onset, progressive disease of the nervous system marked by loss of cortical glutamatergic and spinal cholinergic motor neurons. A common cause of familial ALS (fALS) is mutation of Cu/Zn superoxide dismutase.
dismutase 1 (SOD1). SOD1 containing patient mutations (fALS SOD1) leads to intra-neuronal aggregates and neurodegeneration. Overexpression of fALS SOD1 in C. elegans results in synaptic dysfunction and dramatic SOD1 aggregation (PMID:19165329), consistent with a gain of toxic function in fALS. However, overexpression models may obscure the potential impact of diminished fALS SOD1 activity in early stages of disease. Thus, it remains unclear how altered fALS SOD1 activity affects neuronal function prior to neurodegeneration. Here, we examine the impact of fALS SOD1 mutations in single-copy, fALS SOD-1 knock-in models in C. elegans. Using MosSCI and CRISPR mediated homologous recombination, we edited the endogenous sod-1 gene to incorporate the ALS mutations corresponding to A4V, H71Y, L84V, G85R and G93A. The wild-type SOD-1 enzyme protects the cells from oxidative damage. To test fALS sod-1 function in vivo, we subjected animals to oxidative stress. fALS sod-1 alleles resulted in neurodegeneration, to varying degrees, in cholinergic and glutamatergic neurons after exposure to oxidative stress. Furthermore, oxidative stress lead to degeneration of glutamatergic neurons in animals lacking sod-1 as well as in a subset of fALS sod-1 animals, suggesting that decreased sod-1 function may contribute to neuronal degeneration in fALS. Intriguingly, oxidative stress lead to degeneration of cholinergic motor neurons only in fALS sod-1 animals but did not affect neuronal survival in animals lacking sod-1, consistent with a gain of toxic role in fALS SOD-1. We found that all fALS sod-1 alleles increased aggregation of human SOD1 in motor neurons. Combined, our results suggest that exposure to exogenous stressors may unmask or aggravate the defects associated with fALS SOD1. Also, our findings show that both loss and gain of toxic SOD-1 function may be involved in disease pathogenesis, albeit to differing extents for different fALS SOD1 alleles.

873B Characterization of Alzheimer’s disease risk factors in a C. elegans model of tauopathy.  S.J. Benbow1,2,  B.C. Kraemer1,2,3,4 1) Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Washington, Seattle, WA 98104, USA; 2) Geriatric Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA 98108, USA; 3) Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, Washington 98195, USA; 4) Department of Pathology, University of Washington, Seattle, Washington 98195, USA.

Aberrant aggregation, misfolding, and mislocalization of the microtubule-associated protein tau characterize several neurodegenerative diseases, termed tauopathies. Alzheimer’s disease (AD) is the most common tauopathy and most prevalent form of dementia within the global aging population. AD is distinguished from related dementias by the presence of two aggregant protein pathologies: intracellular neurofibrillary tangles (NFTs) composed of aggregated tau protein and extracellular amyloid-ß (Aß) plaques.

While heritable mutations in amyloid precursor protein (APP) and presenilins 1 and 2 (PSEN1, PSEN2) have considerably advanced our understanding of AD pathogenesis, they account for only a small portion of total AD cases. The majority of cases are classified as sporadic late-onset Alzheimer’s disease and present without known causative mutations. However, human genomic studies have revealed a number of genetic variants contributing significant risk for AD. Genetic analysis by genome wide association studies (GWAS) has led to the identification of several novel genetic risk factors for AD (e.g. ABCA7, PICALM, DSG2, INPP5D, MEF2C, PTK2B, SLC24H4-RIN3, and AB13). We hypothesize that a subset of these gene variants may modulate the severity of tau pathology, thereby contributing to the risk of developing AD. To test this hypothesis, we will explore whether these genes affect tau pathology in our C. elegans model of tauopathy. In this model, pan-neuronal expression of human tau recapitulates several features of human disease including accumulation of detergent-insoluble phosphorylated tau aggregates, abnormal behavior, neurodegeneration, and shortened lifespan. Tau transgenic C. elegans will be crossed with worms carrying a loss-of-function mutation in one of the homologous gene to incorporate the ALS mutations corresponding to A4V, H71Y, L84V, G85R and G93A. The gene to be edited the endogenous sod-1 gene to incorporate the ALS mutations corresponding to A4V, H71Y, L84V, G85R and G93A. The wild-type SOD-1 enzyme protects the cells from oxidative damage. To test fALS sod-1 function in vivo, we subjected animals to oxidative stress. fALS sod-1 alleles resulted in neurodegeneration, to varying degrees, in cholinergic and glutamatergic neurons after exposure to oxidative stress. Furthermore, oxidative stress lead to degeneration of glutamatergic neurons in animals lacking sod-1 as well as in a subset of fALS sod-1 animals, suggesting that decreased sod-1 function may contribute to neuronal degeneration in fALS. Intriguingly, oxidative stress lead to degeneration of cholinergic motor neurons only in fALS sod-1 animals but did not affect neuronal survival in animals lacking sod-1, consistent with a gain of toxic role in fALS SOD-1. We found that all fALS sod-1 alleles increased aggregation of human SOD1 in motor neurons. Combined, our results suggest that exposure to exogenous stressors may unmask or aggravate the defects associated with fALS SOD1. Also, our findings show that both loss and gain of toxic SOD-1 function may be involved in disease pathogenesis, albeit to differing extents for different fALS SOD1 alleles.

874C A C.elegans model to study LDL-Related Proteins involvement in Alzheimer’s disease. Carla Bertapelle1,2, Federica Cocco3, Elia Di Schiavi3, Claudio Russo1 1) Department of Health Sciences, Università del Molise, Campobasso, IT; 2) Institute of Bioscience and BioResources, CNR, Naples, IT.

The Alzheimer’s disease (AD) is the most common neurodegenerative disorder and its symptoms include dementia, loss of memory and motor dysfunction. The main neuropathologic hallmarks of AD are the extracellular depositions of β-amyloid (Aß) plaques, insoluble aggregates of beta-amyloid peptide, and the intraneuronal fibrillary tangles, resulting from the hyperphosphorylation of the microtubule associated protein Tau. These aggregates both contribute to the loss of synapse functions and the consequent neuronal death. The major genetic risk factor in sporadic AD is represented by the E4 isofrom of Apolipoprotein E (ApoE), ligand of the Low Density Lipoprotein-Related Proteins Receptors (LRPs), involved in a variety of functions, including cholesterol metabolism and cell signaling. LRPs intramembrane proteolysis is regulated by the γ-secretase cleavage and similarly the beta-amyloid peptide is the product of the Amyloid Precursor Protein (APP) γ-secretase cleavage. Moreover, ApoE affects Aß production and clearance and colocalizes with Aß in amyloid plaques. C.elegans presents the orthology of human APP, γ-secretase presenilins and Tau (respectively apl-1, sel-12, hop-1 and ptf-1), and it has been extensively used to study AD, with several neurodegeneration models generated to investigate APP processing, Aß aggregation and Tau hyperphosphorylation. Neurodegeneration results in defects progressively increasing with aging, such as neuronal abnormalities (inclusions and varicosities in cell body), axonal degeneration (bulges, dilatation and collapse of axonal membrane), altered neurotransmission and uncoordinated movement (Kraemer et al., 2003). Considering the lack of informations about LRPs and its involvement in AD, we propose a new C.elegans model to study the correlation of human
aged worms, and lifespan. These results allow us to hypothesize an involvement of LRP8 in neuronal function and to investigate preliminary results suggest that huLRP8 overexpression affects nematode locomotion, a defect that progressively worsens in aged worms, and lifespan. These results allow us to hypothesize an involvement of LRP8 in neuronal function and to investigate huLRP8 processing and its genetic interaction with APP.

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by a progressive degeneration of upper and lower motor neurons. The majority of ALS cases are sporadic but approximately 10% of cases have a familial origin. Among the genes identified are transactive response DNA Binding Protein 43 (TARDBP) and Fused in Sarcoma (FUS) that account for 5% of familial cases. However, the most prominent animal model remains the SOD1 transgenic mouse. So, there is a unmet need to develop new animals models to better represent the vast genetic spectrum of this disease. In our laboratory, we use Caenorhabditis elegans as a powerful genetic model to study the cellular and molecular changes which lead to a diseased state. Previously, we have made transgenic worms expressing human mutant and wild-type FUS and TDP-43 in motor neurons, resulting in a progressive paralysis and neurodegeneration. However, these models are overexpressing the transgenes and may not properly recapitulate human pathology. With the development of CRISPR/Cas9 techniques, we have generated new ALS models by creating point mutations mimicking human disease-causing mutations in the C. elegans orthologue of FUS (fust-1) and TDP-43 (tdp-1). Our goal is to fully characterize these new models and determine if they can recapitulate key aspects of the disease at physiological expression levels. These models would provide better disease relevance and would open the possibility to generate new CRISPR/Cas9 models for other ALS-causing genes.

876B Developing a C. elegans model system to screen for novel gene mutations causing inherited peripheral neuropathy.  
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Inherited peripheral neuropathies (IPNs) are a group of diseases causing progressive, length-dependent, axonal degeneration of the peripheral nerves. Clinically, patients present with muscle wasting and weakness starting in the hands and/or feet, sensory loss and gait abnormalities, leading to lifelong disability. Although over 80 genes have been identified as a cause of IPN, up to 50% of families still remain unsolved. Whole exome sequencing (WES) has greatly facilitated the screening of IPN families for gene mutations. However, multiple variants of unknown significance (VOUS) are often identified in the remaining unsolved small nuclear families. VOUS are rare, non-synonymous protein-coding variants that segregate with the disease. Identification of multiple VOUS hinders genetic diagnosis due to the absence of genetic power in the family to resolve the pathogenic status of these variants. Additional functional evidence is therefore required. We have developed an in vivo model system where candidate gene variants, identified through WES studies, will be overexpressed in C. elegans to determine which variants cause neurotoxicity within the organism.

We are optimising and validating this model system using the PDK3 R158H mutation, which we recently identified in patients with an X-linked inherited peripheral neuropathy (X-linked Charcot-Marie-Tooth neuropathy subtype CMTX6). We have generated C. elegans strains overexpressing the human PDK3 R158H mutation specifically in the GABA motor neurons (Punc-25::PDK3R158H). Visual assessment of the GABA motor neurons in adult day 1 C. elegans revealed that twice as many animals exhibit abnormal axon axon commissure morphology when overexpressing R158H PDK3 compared to animals overexpressing wild-type PDK3. Additional assays are being performed to determine whether this difference observed between C. elegans overexpressing wild-type and mutant PDK3 is 1) maintained at older ages, 2) translates into locomotive defects, and 3) is observed in C. elegans overexpressing benign polymorphic PDK3 (K114T and Y334S PDK3).

Correct identification of the disease causative mutation is imperative for both providing accurate genetic counselling and developing effective treatments. We predict this model system will be an invaluable tool that complements the gene discovery research undertaken in our laboratory.

877C BTBD9, a restless legs syndrome associated protein, regulates manganese-induced toxicity in Caenorhabditis elegans.  
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Restless Legs Syndrome (RLS) is a common neurological disorders seen in ~10% of the US population. RLS-associated sleep deprivation can seriously impact life quality, causing anxiety, depression and attention-deficit/hyperactivity disorder (ADHD) symptoms. Moreover, RLS may portend hypertension, heart disease and stroke. RLS exhibits both familial and non-familiar (idiopathic) forms, with ~60% of cases having a family history of the disease. BTBD9 is one of the genetic risk factors, associated with decreased serum iron (Fe) level. Interestingly, lymphocytes from RLS patients have an altered Fe management protein profile, which also regulates manganese (Mn) homeostasis. This raises the question as to whether the symptoms
inherent to RLS patients are the result of Fe deficiency or elevated concentrations of another metal that opportunistically increases when Fe levels are low. Here we present novel data that BTBD9 functions to regulate Mn homeostasis in *Caenorhabditis elegans* (*C. elegans*). A blast search identified *hpo-9* as the BTBD9 homolog in *C. elegans*, with ~75% sequence similarity. A mutant strain tm3719 (*hpo-9*/-) carrying 761 bp deletion of *hpo-9* was obtained. We found that *hpo-9*/- worms were more sensitive to Mn exposure. Upon Mn treatment, *hpo-9*/- worms showed a significantly lower survival rate and more severe DAergic neurodegeneration compared with wild type worms. Interestingly, no difference was seen when worms were exposed to Fe. However, a low level of Fe (0.1 mM) pretreatment was able to protect Mn-induced lethality. To better characterize HPO-9 protein, a transcriptional fusion construct was created with green fluorescent protein (GFP) driven under *hpo-9* promoter. We found that GFP was present high in the head and pharynx and low in the intestine and seam cells. Using a confocal microscopy, we found that *hpo-9* was expressed in dopaminergic neurons, indicating that HPO-9 might play a role in dopamine signaling. To confirm that, we over-expressed HPO-9 in DAergic neurons of *hpo-9*/- worms and found that it rescued Mn-induced DAergic neurodegeneration. Together, our results suggest a novel role for *hpo-9/-BTBD9 in regulating Mn homeostasis and possibly dopamine signaling in *C. elegans*.

878A  Screening for small molecule therapeutics in a *C. elegans* model of tauopathy.  K.S. Chickering1,2, A. Saxton1, J. Wheeler1, B. Kraemer1,2,4,5  1) Geriatric Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA; 2) Undergraduate Student, Department of Biology, University of Washington, Seattle, WA; 3) Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Washington, Seattle, WA; 4) Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA; 5) Department of Pathology, University of Washington, Seattle, WA.

Accumulation of pathological tau protein in the brain occurs in many neurodegenerative diseases. Alzheimer’s disease and frontotemporal lobar degeneration (FTLD) are the most common diseases with pathological tau. In order to identify potential therapeutics for Alzheimer’s disease and related disorders, we screened 700 compounds from one of the National Institute of Health’s Clinical Compound Library for modifiers of the disease associated phenotype seen in tau transgenic *Caenorhabditis elegans*. The compound library consists of drugs with a history of use in humans that we can assess for other potential uses, a process known as drug repurposing. Observing *C. elegans* disease models for specific drug effects may ultimately lead to identifying ways to alter or prevent accumulation of tau aggregates in the brain.

Populations of tau-expressing *C. elegans* were cultured in the presence of a single library compound and assessed for modification of disease phenotype at day one of adulthood, roughly 3 days after eggs were placed on the media. Animals were scored for improvement or deterioration of coordination as well as growth rate, brood size, mortality, and general health and size of the population as compared to control populations without drug. In the initial screen, eight compounds appearing to partially suppress the tauopathy Unc phenotype were selected for dose response analysis. Further characterization of these compounds is ongoing and will include quantitative analysis of behavior and biochemical analysis of tau protein. We hope that this drug repurposing work could lead new therapeutic strategies for ameliorating dementia in patients with pathological tau deposition.

879B  Tamalin/GRAS-1 is a Candidate Link between Glutamate Receptors and the Insulin/IGF Signaling Cascade in Neuroprotection in a Nematode Model of Excitotoxicity.  Ayesha Chowdhury1,2,3, Itzhak Mano1,2,3, Shavanie Pershad4  1) Molecular, Cellular and Biomedical Sciences, City College of New York CUNY, New York City, NY; 2) Behavior and Cognitive Neuroscience Program, Graduate Center of CUNY, New York City, NY; 3) Cluster on Neural Development and Repair, City College of New York CUNY, New York City, NY; 4) Undergraduate Program in Biochemistry, City College of New York CUNY, New York City, NY.

In brain ischemia, excitotoxic neurodegeneration is caused by the over-activation of glutamate receptors (GlRs), an influx of calcium into the neuron, and activation of necrotic neurodegenerative signaling pathways. Surprisingly, excitotoxic over-activation of GlRs involves both death-promoting and pro-survival signaling. FoxO/DAF-16 is a transcription factor that regulates neuronal survival, but the study of its role in neuroprotection from excitotoxic necrosis in mammals is complicated by its pro-apoptotic roles in other paradigms. It is also not clear if and how FoxO/DAF-16 is regulated by the GlR hyper-activation seen in excitotoxicity. We study the role of FoxO/DAF-16 and the Insulin/IGF signaling (IIS) cascade in excitotoxicity in *C. elegans*. We have recently shown that in a nematode model of excitotoxic necrosis (*glr-3; nusl5*) inhibition of the IIS cascade (known to increase FoxO/DAF-16 nuclear activity) reduces neurodegeneration. Furthermore, we have shown that a protein complex containing Cytohesin/GRP-1 is an important upstream regulator of IIS cascade in excitotoxicity. However, it is not yet clear if Glu signaling regulates this IIS-mediated protection. We suggest that the scaffolding protein Tamalin/GRAS-1 links the GluR and IIS cascades through the Cytohesin/GRP-1 complex. We hypothesize that Tamalin/GRAS-1 is an upstream effector responsible for regulating IIS cascade in excitotoxicity and is able to link the pathway to the activity of GlRs. I have recently shown that elimination of Tamalin/GRAS-1 reduces neurodegeneration levels in excitotoxic conditions. I now continue to study the possible interaction between GluRs, Tamalin, and the Cytohesin complex. These studies could provide a link between the activity of GluRs and IIS cascade and subsequently establish a novel neuroprotective pathway regulated by GluRs in excitotoxicity.

880C  Parkinson’s disease mutations in pdr-1 and pink-1 cause the accumulation of damaged mitochondria leading to activation of the mitochondrial unfolded protein response.  Jason Cooper, Emily Machiela, Dylan Dues, Megan Senchuk, Jeremy Van Raamsdonk  Center for Neurodegenerative Disease, Van Andel Institute, Grand Rapids, MI.

Parkinson’s disease (PD) is the second most common neurodegenerative disease affecting approximately 10 million people worldwide. While the pathogenesis of PD is incompletely understood a crucial role for the mitochondria is suggested by the fact
that PD patients exhibit deficits in the mitochondrial electron transport chain, that mitochondrial toxins have been used as a model of the disease, and that genes involved in mitochondrial function have been shown to cause monogenic forms of PD. In this work, we use *C. elegans* to study the effect of three mitochondria-related genes, which have been implicated in PD (*pdr-1, pink-1* and *djr-1.1*), on mitochondrial morphology, mitochondrial function, dopamine neuron survival, and dopamine neuronal function. We found *pdr-1* mutants exhibit deficits in dopamine-dependent behaviors, including basal slowing and ethanol avoidance, but no loss of dopamine neurons. In contrast, we did not observe any significant differences in *pink-1* mutants, while *djr-1.1* mutants only showed an increased sensitivity to oxidative stress. In examining mitochondrial morphology and function, we found that *djr-1.1* mutants exhibit increased mitochondrial fragmentation leading to decreased rate of oxidative phosphorylation and decreased ATP levels. *pdr-1* and *pink-1* mutants show an accumulation of mitochondria with age. In *pdr-1* mutants this leads to an increase in oxidative phosphorylation but decreased levels of ATP, suggesting an increase in damaged mitochondria. Consistent with this conclusion, we observed a marked activation of the mitochondrial unfolded protein response (mitoUPR) in both *pdr-1* and *pink-1* mutants. To determine if the activation of mitoUPR is acting to protect the dopamine neurons from dysfunction and death, we crossed *pdr-1* and *pink-1* mutants to an *afs-1* deletion mutant, which is unable to activate the mitoUPR. Our preliminary results show a trend towards increased neuronal loss in the *pdr-1* and *pink-1* mutants when the mitoUPR is blocked, suggesting that the upregulation of the mitoUPR acts to protect the dopamine neurons from degeneration and death. Overall, our results suggest that mutations in *pdr-1* and *pink-1* cause the accumulation of damaged mitochondria, which activates the mitoUPR to mitigate the detrimental effect of these mutations on the dopamine neurons thereby resulting in only mild PD-like deficits in these mutants.

**881A  C. elegans chemical genetic screen to counter Progranulin deficiency.** James Julian Doyle1,2,3, Claudia Maios3, Céline Vrancx5, Andrew Bateman1,2, Hugh Bennett1,2, Alex Parker3,4  1) Metabolic Disorders and Complications, Research Institute of the MUHC, Montreal, QC; 2) Division of Experimental Medicine, McGill University, Montreal, QC; 3) Axe Neurosciences, Centre de Recherche du CHUM, Montreal, QC; 4) Deportement de Neurosciences, Universite de Montreal, Montreal, QC; 5) Institute of Neurosciences, Catholic University of Louvain, Louvain, Belgium.

Progranulin (PGRN) is a widely-studied neurotrophic factor known for its ability to reverse toxicity induced by amyloid β, TDP-43, and Huntingtin, and is an attractive therapeutic target for its role as a neuronal survival factor. Mutations in the encoding gene result in either frontotemporal dementia or neuronal ceroid lipofuscinosis. Despite the important role of this protein in the maintenance of neuronal health, there are no approved therapeutics capable of modulating the downstream molecular action of PGRN. Since performing large-scale chemical screens in vivo are costly and unfeasible in rodents, screening in *C. elegans* is an attractive approach to address this issue. Characterization of *pgrn*-1 mutant worms indicated they displayed an age-dependent paralysis, an effect which can be accelerated when they are placed in liquid culture allowing for large scale, rapid screening. We screened ~4000 commercially-available small molecules in liquid culture for their ability to rescue *pgrn*-1 mutant worms and validated them by their ability to also rescue age-dependent paralysis. In total, we have identified 12 compounds as hits capable of specifically restoring *pgrn*-1 deficiency, and we will further assess the preclinical efficiency of these compounds in zebrafish and cell culture models. This screen represents the first step in a collaborative effort to translate compounds from the bench to the clinics.

**882B  C. elegans model of spinal muscular atrophy.** James Julian Doyle1,2,3, Céline Vrancx5, Claudia Maios3, Kessen Patten5, Alex Parker3,4  1) Metabolic Disorders and Complications, Research Institute of the MUHC, Montreal, QC; 2) Division of Experimental Medicine, McGill University, Montreal, QC; 3) Axe Neurosciences, Centre de Recherche du CHUM, Montreal, QC; 4) Deportement de Neurosciences, Universite de Montreal, Montreal, QC; 5) Institut Armand-Frappier, INRS, Laval, QC; 6) Institute of Neurosciences, Catholic University of Louvain, Louvain, Belgium.

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder which is the leading genetic cause of infant death. SMA is characterized by a progressive degeneration of lower motor neurons resulting in muscle atrophy, and ultimately death. At present, there are no clinically approved treatments for SMA, therefore, a better understanding of the molecular mechanisms involved in the regulation of the degenerative process is essential for developing new therapeutic treatments. Here, we investigate the effects of a point mutation in the *C. elegans* *smn-1* gene, orthologous to the human disease-causing *SMN1* (Survival Motor Neuron 1) and *SMN2* genes, which models the same mutation that leads to SMA in humans. We observe that *smn-1* mutant animals recapitulate key aspects of the human disease, including accelerated paralysis and death. Furthermore, we show that interference of ER stress, which is elevated during the course of the disease, exerts a protective effect on mutant animals. Accordingly, our results indicate that our novel SMA model is a relevant tool to study this disease, and can be useful to discover new therapeutic treatments.

**883C  Rescue of ATXN3 Neuronal Toxicity in C. elegans by Chemical Modification of ER Stress.** Yasmin Fard Ghassemi1,3, Arnaud Tauffenberger1, Sarah Gosselin1, Alex Parker1,2,3  1) Neuroscience, CRCHUM, Montreal, Quebec, CA; 2) Neuroscience, University of Montreal, Montreal, Quebec, CA; 3) Biochemistry, University of Montreal, Montreal, Quebec, CA.

Polyglutamine expansion diseases are a class of dominantly inherited neurodegenerative disorders that develop when a CAG repeat in the causative genes is unstably expanded above a certain threshold. The expansion of trinucleotide CAG repeats is increasing and there are no pharmacological therapies available that successfully treat this disease. Therefore, the development of novel therapeutics for MJD is urgently needed.

Polyglutamine expansion diseases are a class of dominantly inherited neurodegenerative disorders that develop when a CAG repeat in the causative genes is unstably expanded above a certain threshold. The expansion of trinucleotide CAG repeats is increasing and there are no pharmacological therapies available that successfully treat this disease. Therefore, the development of novel therapeutics for MJD is urgently needed.
In this study, we generated transgenic Conchans strains expressing wild type or mutant human ATXN3 genes and tested them for recovery of motility defects, decreased lifespan, and neurodegeneration phenotypes upon treatment with compounds known to modulate ER stress and having neuroprotective roles. We observed differences between both transgenic lines and found that the motility defects, the reduced lifespan and neurodegeneration were rescued by compounds that have been previously identified in our laboratory. These compounds were also able to prevent the oxidative stress and the ER stress response induced by mutant ATXN3 in transgenic worms. These promising results prompted us to expand our approach to perform a comprehensive, blind drug screen of ~3600 compounds in our transgenic ATXN3 lines. The aim of this screen is to identify new compounds that allow us to directly gain insights into the mechanisms underlying MJD.

We introduce novel Conchans models for MJD based on the expression of full-length ATXN3 in GABAergic motor neurons. Using these models we discovered that chemical modulation of the ER unfolded protein response reduced neurodegeneration and could be a new therapeutic approach for the treatment of MJD. Also, using Conchans to study MJD in conjunction with well-characterized compounds, we may identify underlying mechanisms that could also be used to develop novel therapeutic approaches.

884A  A Potential Non-Canonical Activation of CREB in Excitotoxicity-Induced Neuroprotection.  K. Genevieve Feldmann 1,2, Jessi Becker 3, Itzhak Mano 1 2 1) Molecular, Cellular and Biomedical Sciences, Center for Discovery & Innovation, Cluster on Neural Development and Repair, The CUNY School of Medicine at City College, The City University of New York; 2) The Graduate Program in Biology (Neuroscience), The Graduate Center, The City University of New York; 3) Undergraduate Program in Biology, The City College of New York.

Excitotoxicity is the principal mechanism that causes extensive neuronal damage in stroke and other neurodegenerative diseases. In excitotoxicity, over-accumulation of Glutamate (Glu) in the synapse causes hyperstimulation of Glu receptors (GluRs) and excessive calcium influx in the postsynaptic cell, leading to cell death. Failure of GluR antagonists in clinical trials provides strong evidence that Glu signaling can also activate neuroprotective pathways, one of which involves the activation of the transcription factor, CREB. At least 300 stimuli can lead to CREB activation and CREB in turn can exert its transcriptional effects on 4,000 target genes in many tissues, making CREB a fairly promiscuous molecule. It therefore seems that the precise outcome of CREB stimulation depends on the exact context of its activation. We aim to elucidate the specific mechanism of CREB activation necessary to trigger excitotoxicity-induced neuroprotection by examining canonical and non-canonical models of activation under this specific scenario. We use a Conchans model of excitotoxicity, and we examine the role of CRTC, and other molecules in the mechanism of CREB activation under neuroprotection. Thus far, our results suggest that it is a non-canonical mode of CREB activation that is important for excitotoxicity-induced neuroprotection. Discovering the exact mechanism of CREB activation important for neuroprotection and further examining CREB-mediated changes in gene transcription may allow us to find candidate genes for future therapeutic treatments in stroke patients.

885B  Transcriptome analysis of Conchans smn-1 mutant.  X. Gao, W. Gan, S. Hu, J. Xu, L. Ma  State Key Laboratory of Medical Genetics, School of Life Sciences, Central South University, Changsha, CN.

Spinal muscular atrophy (SMA) is caused by mutations in the highly conserved gene survival motor neuron 1 (SMN1).

Although many efforts have been made to elucidate the molecular functions of SMN1, mechanisms underlying the pathogenesis of SMA are not well understood. SMN1 plays important roles in several biological processes including the assembly of U snRNPs and pre-mRNA splicing. The nematode Caenorhabditis elegans carries a single ortholog of SMN1, smn-1, and has been used as a model for studying the functions of SMN. To understand how SMN-1 affects mRNA splicing and expression, we determined the transcriptomes of wild type, smn-1(ok355), uaf-1(n4588) and smn-1(ok355); uaf-1(n4588) mutants on day 2 post L1, when the deleterious phenotypes of smn-1 mutants are less obvious. We found that mutations in smn-1 or uaf-1 cause widespread alterations of gene expression. KEGG analysis suggests that the differentially expressed genes (DEGs) in smn-1 mutants are enriched in neuronal ligand-receptor interactions, phagosomes and cancer-related signaling pathways, implying that widespread alternations of gene expression. KEGG analysis suggests that the differentially expressed genes (DEGs) in smn-1 mutants are enriched in neuronal ligand-receptor interactions, phagosomes and cancer-related signaling pathways, implying that widespread alternations of gene expression.


Regenerative capacity of adult nervous system is limited by the fact whether the injured axon can reach their target or not. We have previously shown that neurons for gentle touch sensation in Conchans show robust regrowth after axotomy. Conserved Calcium signalling and Dual Leucine Zipper kinase-1 pathways were identified as cell intrinsic factors for regrowth. Several other players regulating regrowth potential of touch neuron have been identified using candidate-screening approach.

It is highly important to study neuronal regeneration at functional level because significance of regrowth lies in its behavioural recovery. Using two femtosecond lasers simultaneously, we could scan and sever posterior touch receptor neurons (Posterior Lateral Microtubule) on both sides of worm. We showed that axotomy of both PLMs leads to a dramatic loss of posterior touch sensation. During the regenerative phase, only those axons, which fuse to their distal counterparts, contribute to functional recovery. Moreover, the mutants such as ced-7(0) or eff-1(0) which specifically impair fusion fail to show functional recovery.
The ability to establish cytoplasmic continuity between proximal and distal ends declines with age and recovery becomes poor. This is overcome by the loss of lethal-7 microRNA. The enhanced functional restoration due to loss of let-7 depends on the axon fusion pathways, involving eff-1 and ced-7. We found that let-7 negatively regulates expression of fusogen molecules. Our data reveal a functional property of regenerating neurons.

887A  **The toxicity associated with a routinely prescribed antidepressant drug is due to one of its metabolites.**  K.J. Knudsen1,2, J.N.K. Nyarko1, R. Heistad1, J. Greer1, C.E. Carvalho2, D.D. Mousseau1  1) Psychiatry, University of Saskatchewan, Saskatoon, Saskatchewan, CA; 2) Biology, University of Saskatchewan, Saskatoon, Saskatchewan, CA.

An association is seen between early-life depression and the risk for developing Alzheimer disease. Population studies suggest that some of the risk for Alzheimer disease might be more associated with the type of antidepressant drug being used. The Selective Serotonin Reuptake Inhibitors (SSRIs), which include drugs such as fluoxetine (Prozac®), are associated with the greatest risk. We tested whether fluoxetine, or its major metabolite, norfluoxetine, could exacerbate an Alzheimer-related phenotype using the *Caenorhabditis elegans* strain GMC101. This strain expresses the human β-amyloid1-42 peptide, which is known to aggregate in the form of plaques in the brains of patients with Alzheimer disease. Treatment with fluoxetine resulted in mixed outcomes (i.e. some worms showed more accumulation/aggregation; some showed less), whereas treatment with norfluoxetine increased the rate of accumulation. Parallel studies using human cell cultures overexpressing the human APP gene (encodes the precursor molecule that yields β-amyloid) revealed that treatment with fluoxetine blocked the secretion of β-amyloid from the cell into the culture medium. This is the first evidence that the serotonin transporter, that mediates serotonin reuptake, might play a role in β-amyloid clearance. This has major clinical relevance as fluoxetine has been progressively prescribed for off-label purposes, which might be exposing far more individuals to an unanticipated increased risk of developing Alzheimer disease.

888B  **Loss of bas-1 suppresses tau-induced toxicity in tau transgenic *Caenorhabditis elegans*.**  R.L. Kow1,2,3, J.M. Wheeler*, C. Sikkema4,6, C. Wilkinson1,6, B.C. Kraemer1,2,6,7  1) Geriatrics Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care, Seattle, WA; 2) Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Washington, Seattle, WA; 3) Division of Medical Genetics, Department of Medicine, University of Washington, Seattle, WA; 4) Seattle Institute for Biomedical & Clinical Research, Seattle, WA; 5) Mental Illness Research Education and Clinical Centers, Veterans Affairs Puget Sound Health Care System, Seattle Division, Seattle, WA; 6) Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA; 7) Department of Pathology, University of Washington, Seattle, WA.

The microtubule-associated protein tau can accumulate into toxic aggregates seen in multiple neurodegenerative diseases such as Alzheimer’s disease and frontotemporal lobar degeneration (FTLD). Mutations in the human gene encoding tau, MAPT, directly cause FTLD. To aid in identifying genetic modifiers of human tau-induced toxicity, we used a *Caenorhabditis elegans* model of tau toxicity. Overexpression of human tau in the neurons of *C. elegans* causes significant motor dysfunction, progressive loss of neurons, and shortened lifespan. Additionally, these worms accumulate abnormally phosphorylated and detergent insoluble aggregated forms of tau protein. We previously found that loss of both *dop-2* and *dop-3*, the D2-like dopamine receptors, significantly ameliorated tau transgene-induced phenotypes. To better understand how loss of D2-like dopamine receptors led to suppression of tau-induced toxicity, we screened mutations in over 40 genes related to dopamine signaling for effects on tau-induced motor dysfunction. We identified *bas-1* as a suppressor of tau-induced motor dysfunction, neuronal loss, and phosphorylated and insoluble tau accumulation. Loss of function in dopamine and/or serotonin synthesis pathway genes such as *cat-2*, *tph-1*, and *cat-4* did not alter tau-induced toxicity but did reduce *bas-1* suppression of tau-induced toxicity. Additional loss of *dop-2* and *dop-3* did not further improve motor function in tau transgenic *C. elegans*. This indicates that there is a shared pathway for tau suppression by *bas-1* and the D2-like dopamine receptors, which also requires intact dopamine and serotonin synthesis pathways.

889C  **Modulation of Gut Microbiota Rescues Paralysis and Neurodegeneration in *C. elegans* ALS Models.**  Audrey Labarre1,2, J Alex Parker12  1) The University of Montreal Hospital Research Center (CRCHUM), University of Montreal, Montreal, Quebec, Canada; 2) Department of neurosciences, University of Montreal, Montreal, Quebec, Canada.

Microbiota is known for its various effects on the human body with implications in chronic intestinal diseases, asthma and allergies. Some evidence is beginning to link microbiota to neurodegeneration. Moreover, microbiota is known for its impact on serotonergic neurons, especially in serotonin biosynthesis regulation, and these neurons are the most vulnerable in ALS. However, there is no available date for ALS. In 2014 we partnered with Lallemand Health Solutions to develop an assay in *C. elegans* to screen probiotic strains for their effects on fat accumulation. We were curious if these probiotics had additional effects so we tested them in a variety of assays, including lifespan, stress resistance, as well as in our worm ALS models. We were surprised to discover that two strains suppressed motility defects and motor neuron degeneration in our *C. elegans* models of ALS. But what accounts for this neuroprotective effect? We used a combination of genetics and gene expression profiling to identify genes and pathways that are influenced by microbiota and are responsible for neuroprotection in our worms. So far, we demonstrated that our *C. elegans* ALS models, when fed with specific probiotics, show a rescue of neurodegeneration and adult-onset age-dependent paralysis. The neuroprotection provided by these probiotics are not dependant on classic metabolic/stress resistance pathways in *C. elegans*, like *daf-16* and *hsf-1*, but seems to be linked to fat metabolism. Finally, these findings may confirm a link between microbiota and ALS, and can lead the way to future therapies perhaps through modulation of the intestinal environment.
**890A rab-27 inhibits axon regeneration in vivo.** A. T. Lin-Moore, M. Hammarlund

Axon regeneration is a conserved property of neurons, but the cellular mechanisms that control regeneration are incompletely understood. We have found that **rab-27** is a novel, cell intrinsic inhibitor of regeneration. Loss of **rab-27** improves regeneration initiation and regrowth in GABAergic DD/VD neurons. Re-expression of **rab-27** in the GABA neurons of null animals restores normal regeneration. **rab-27** encodes a small GTPase known to regulate vesicle tethering and fusion. **rab-27** was previously shown to act redundantly with **rab-3** through the common effector Rabphilin to promote synaptic vesicle transmission (Mahoney et al., 2006). We find that loss of either **rab-3** or Rabphilin does not affect regeneration, and that loss of either **rab-3** or Rabphilin suppresses the high regeneration phenotype of **rab-27** mutants. These data suggest that the common function of **rab-27**, **rab-3**, and Rabphilin in synaptic vesicle tethering facilitates regeneration. At the same time, these data suggest a unique role for **rab-27** independent of **rab-3** and Rabphilin in to inhibit regeneration, possibly by regulating fusion of dense core vesicles or other vesicle types. Together, our findings point to a complex role for **rab-27** as a regulator of regeneration. Understanding the cell biological functions of **rab-27** in regeneration may identify specific mechanisms that limit the ability of neurons to respond to injury.


**891B Role of RtcB-mediated RNA ligation in axon regeneration.** X. Liu, S. Kosmaczewski, S. M. Han, M. Hammarlund

RNA processing modifies the flow of genetic information and increases the coding potential of the genome. A critical form of RNA processing, RNA ligation, is mediated by the conserved RNA ligase RtcB. We identified a novel function for RtcB-mediated RNA ligation in the nervous system. We found that RtcB inhibits axon regeneration after neuronal injury. Intriguingly, this inhibitory effect on the neuronal injury response is dependent on RtcB’s catalytic activity, but is independent of its two known substrates. This suggests that RtcB ligates additional, novel RNA substrates for its neuronal function. Using genome-wide RNA-seq, we are working to identify novel substrates of RtcB. We are also using genetic and cellular approaches to determine how RtcB-mediated RNA processing contributes to neuronal function. Our work will expand our understanding of RNA processing in the nervous system, and indicate how RNA ligation can be harnessed to affect axon regeneration. It will also shed light on the mechanisms that affect the ability of the nervous system to restore its structure and function after injury.

References:


Familial Amyloid Polyneuropathy (FAP) is a lethal autosomal dominant systemic amyloidosis caused by the aggregation of transthyretin (TTR), a tetrameric protein secreted primarily by the liver. The most common FAP mutation -V30M TTR- affects primarily peripheral nerves, resulting in loss of pain- and thermo-sensation, and sensory-motor function. In FAP, the V30M TTR tetramer dissociates post-secretion, leading to monomer misfolding, and aggregation around peripheral neurons. The non-native TTR species (i.e. oligomers, aggregates) have been hypothesized to result in peripheral neurodegeneration, but not tested due to the lack of models faithfully recapitulating the cell non-autonomous neuronal phenotypes. To investigate the mechanisms of neurodegeneration in FAP, we generated *C. elegans* models expressing human V30M TTR exclusively in the body-wall muscle. Using immunofluorescence and small molecule fluorogenic sensors, we confirmed secretion of the V30M TTR tetramer, which is then endocytosed into degradative macrophage-like coelomocytes. The V30M animals display impaired pain sensation and uncoordinated locomotion, phenotypes that we show depend on the expression of TTR, and that resemble FAP patient relevant neuronal symptoms. To test whether modulating TTR levels alters TTR proteotoxicity, we first genetically ablated coelomocytes in V30M TTR expressing animals, and observed that levels of TTR oligomers were significantly increased, followed by decreased pain sensation response. We also used RNAi to reduce TTR protein levels, and treated V30M TTR animals with tafamidis, a regulatory agency approved drug that binds strongly to the native TTR tetramer and stabilizes it and dramatically slows tetramer dissociation. Both approaches resulted in reduced TTR oligomer formation and in
significant rescue of the uncoordinated phenotype in V30M TTR animals. Thus, we showed that in our FAP disease models that exhibit TTR-mediated patient relevant phenotypes, modulation of TTR levels can be used as a potential therapeutic opportunity.

893A  Alaskan Blueberry attenuates alpha-synuclein over expression and improves movement in Parkinson’s like model of Caenorhabditis elegans.  Malabika Maulik1-4, Swarup Mitra1,4, Skyler Hunter2,4, Elena Vayndorf2,3, Barbara Taylor5, Abel Bult-Ito2 1) Department of Chemistry and Biochemistry University of Alaska Fairbanks, Fairbanks, AK; 2) Department of Biology and Wildlife, University of Alaska Alaska Fairbanks, Fairbanks, AK; 3) Biomedical Learning and Student Training (BLaST); 4) IDeA Network of Biomedical Excellence (INBRE); 5) California State University at Long Beach.

Parkinson’s disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic neurons and aggregation of alpha-synuclein (AS) protein leading to motor and cognitive impairment. The current study investigates the role of Alaskan bog blueberry (Vaccinium uliginosum), on alpha synuclein aggregation using a transgenic model of Caenorhabditis elegans expressing human alpha-synuclein [OW13 (P(unc-54)::alpha-synuclein::YFP+unc-119)]. The current study also examines the role of Sir2 in the worm, in reducing the toxicity of alpha-synuclein aggregates and whether this effect is mediated via expression of other downstream molecular targets. The Alaskan bog blueberry was chosen for its high phenolic content, because phenolics have been shown to modulate sirtuin-mediated molecular pathways. Our experiments showed that the crude extract of low bog blueberry (100 and 400 ug/ml) reduced alpha-synuclein aggregation and improved motility in the worm model. The study also highlights the molecular mechanism through which the botanicals are exerting this beneficial effect. These findings encourage further studies on these Alaskan botanicals as possible therapeutic agents for Parkinson’s disease, specifically of interest are the identification of active ingredients within the extracts and their optimal doses.

894B  Identification of novel genes and epigenetic mechanisms in C. elegans models of Parkinson’s disease.  R Nass1, J. Trinidad2, J. Dobbs1 1) Pharmacology & Toxicology, Indiana Univ Sch Med, Indianapolis, IN; 2) Department of Chemistry, Indiana University, Bloomington, IN.

Background: Parkinson’s disease (PD) is the second most prevalent neurodegenerative disorder, and is characterized by the selective loss of DA neurons in the substantia nigra. Sir2s are members of the histone deacetylase family of proteins that play a role in a variety of cellular functions including cell cycle regulation and promotion of longevity. Although the vast majority of vertebrate studies suggest that the activation of sirtuins contributes to cell viability and is neuroprotective, recent studies indicate that respect to PD and Alzheimer’s disease, suppression of sirtuins may inhibit the neurodegeneration. The molecular basis of sirtuin-associated neurodegeneration is largely unknown. Aims/Objectives: In this study we asked what are the genes, molecular pathways, and mechanism involved in sirtuin-associated neuroprotection. Methods: We utilized reverse genetics, biochemical assays, immunofluorescence, transgenic C. elegans, RT-PCR, Western analysis, ICP-MS, and neuronal morphology analysis to characterize expression, localization and the role that sirtuin and post-translational modifications play in PD-associated neuronal death. Results: In this study we demonstrate that a sirtuin mutation inhibits DA neuron vulnerability to PD associated neurotoxicants up to 15-fold more relative to WT. We show that the sirtuin is expressed in DA neurons, and overexpression results in a 2-fold increase DA neuron vulnerability. We also show that post-translational modifications involving these and other proteins identified utilizing a whole genome RNAi screen, Western analysis, and ICP-MS affects DA neuron vulnerability. Conclusions: This study identifies novel genes and molecular pathways involved in PD-associated DA neuron vulnerability in PD. Support: NIEHS, Alzheimer’s Disease Foundation, and IUF support to RN.

895C  Using C. elegans to investigate natural variation in Alzheimer’s disease and related protein mis-folding diseases.  Y. Nie, L. Byrne, S. Harvey  Canterbury Christ Church University, CANTERBURY, GB.

The nematode Caenorhabditis elegans has been important in discovering several aspects of the basic biology of protein misfolding diseases such as Alzheimer’s disease (AD). This work has however nearly all been done in a single genotype of the worm and no systematic attempt has been made to look at the effects of nematode genetic variation on these diseases. This work is designed to address this by using C. elegans to investigate natural variation in the onset and progression of disease in worms carrying human genes involved in Alzheimer’s disease. The main C. elegans AD model involves the inducible expression of human amyloid (Aβ1-42) in the body wall muscle of wild type N2. The excessive expression of amyloid causes systemic pathology in terms of locomotion, stress responses, lipid disorders and protein mis-folding. In the current project, we have introgressed the AD transgene from an N2 genetic background (line CL2659) into nine different genetic backgrounds (lines SCH0001-SCH0009). We have also introgressed a separate transgene that results in the expression of Aβ1-42 in worm neurons from an N2 genetic background (line CL2355) into four different genetic backgrounds (lines SCH0010-SCH0013). The progression of Aβ-associated pathology in these new lines will be assessed by measurement of development and life span, of liquid thrash activity, and of chemotaxis. To assess the interaction with other traits we are also assessing lipid content, and the stress response potential in these lines. Protein misfolding disease associated pathology will also be further linked to the amyloid dynamics in vivo. In combination with analysis of variation in native protein aggregation in a panel of sequenced and genotyped recombinant inbred lines derived from four wild isolates, we seek to identify genomic regions associated with variation in native protein aggregation and protein mis-folding disease associated pathology.

896A  Using C. elegans for amyotrophic lateral sclerosis (ALS) drug discovery.  S. Ore Rodriguez1, C. Maios2, A. Parker1,2 1) Université de Montréal, Montréal, Québec, Canada; 2) Centre de recherche du CHUM, Montréal, Québec, Canada.

Introduction: ALS is an agressive neurodegenerative disease characterized by the loss of motor neurons in the brain and the spinal cord, leading to a progressive degeneration of the motor system, culminating in paralysis and the patient’s death within 1 to 5 years following diagnosis. Although the onset of the disease still remains to be further elucidated, multiple genes
have been linked to ALS, including Fused in sarcoma (FUS). No cure is known for ALS, and riluzole is administered only as a palliative treatment, extending the patient’s lifespan by only 3 months. Hence, it is urgent to find a treatment to considerably increase patient’s survival.

**Aim:** Our objective is to use a FUS *Caenorhabditis elegans* model of ALS to rapidly identify new neuroprotective drugs and to gain insight into the mechanisms underlying neurodegeneration in ALS.

**Methods:** A drug screen of ~4000 compounds allowed us to identify hits that suppress the ALS motor-deficient motility problem phenotype of FUS-(S57?) mutant worms. To better assess these drugs, we will further investigate whether they restore neuronal morphology and/or neuronal connectivity and if they extend the lifespan of the mutant worms. Afterwards, various double mutants of FUS-(S57?) and genes participating in proteostasis, e.g., insulin / IGF-1 signaling pathway (daf-2, daf-16), heatshock response (hsf-1), protein degradation (uba-1), or the unfolded protein response mediated by the ER (ire-1) will be generated to assess any phenotypic changes in the mutant FUS worm.

**Results:** The drug screen identified 5 promising drugs that increase the motility and suppresses the progressive paralysis in FUS-(S57?) worms. Further characterization will allow for a better assessment of these drugs for potential ALS treatment.

**Conclusion:** Because of its short life cycle, *C. elegans* is a great tool to rapidly discover molecules for ALS drug discovery and development. Additionally, the elucidation of the mode of action of the compounds may allow for a better understanding of the neuronal dysfunction of the disease.

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**897B Evaluating contributions of DAT-1 and FKH-8 to dopaminergic neuron degeneration through oxidative stress. C.M. Paton, B.L. Nelms**  
Biological Sciences Department, Fisk University, Nashville, TN.

Dopamine is a neurotransmitter involved in motor control, reward pathways, pleasure, and addiction; misregulation of dopamine signaling is associated with conditions such as Parkinson’s disease, schizophrenia, and ADHD. An auspicious potential treatment for such disorders is the cultivation of dopaminergic neurons for transplantation; but damaging physiological conditions such as high oxidative stress pose a formidable challenge. Dopaminergic neurons are particularly susceptible to degeneration through oxidative stress, but these mechanisms of degeneration are not fully understood. Two molecules that may mediate such a mechanism in *C. elegans* dopamine neurons are DAT-1 (dopamine transporter) and FKH-8 (a forkhead domain transcription factor). To test the contribution of these proteins to oxidative stress susceptibility or resistance, I have used four experimental conditions: exposure to manganese, juglone, 6-OHDA, or the natural aging process. I then quantified the number of worms in each group that exhibit neuron degeneration (shown by blebbing and loss of GFP fluorescence in the cephalic neurons) to test the differential susceptibility between genotypes. My manganese, juglone, 6-OHDA, and aging studies suggest that mutants lacking the dopamine transporter are less susceptible to neuron degeneration than wildtype worms; but worms lacking FKH-8 may only be significantly different from wildtype in susceptibility to degeneration upon 6-OHDA treatment.

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**898C Effects of manganese exposure on *Caenorhabditis elegans* models of fluoroescently tagged polyglutamine repeats. T. V. Peres, W. Salgueiro, A. Bowman, D. S. Avila, M. Aschner**  
1) Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY, USA; 2) Laboratorio do Grupo de Pesquisa em Bioquimica e Toxicologia em Caenorhabditis elegans (GBToxCe), Universidade Federal do Pampa, Uruguaiana, RS, Brazil; 3) Department of Pediatrics, Neurology and Biochemistry, Vanderbilt University Medical Center and Vanderbilt University, Nashville, TN, USA.

Sporadic cases of neurodegenerative diseases may arise from environmental exposures or even a combination of genetic factors and environmental influence. Neurotoxic metals including manganese (Mn) have been associated with neurodegeneration and modulation of the age of onset and severity of various neurodegenerative diseases. Mn is a naturally occurring heavy metal and is essential for various metabolic processes. However, chronic exposure to high doses may lead to Mn accumulation in the central nervous system (CNS). Alteration in metal homeostasis may contribute to degeneration through protein binding and formation of protein aggregates, mitochondrial dysfunction and oxidative stress. Huntington’s disease (HD) is an autosomal dominant neurodegenerative disorder predominantly afflicting the corpus striatum. Expansion of a glutamine encoding CAG repeat in the *huntingtin* (HTT) gene is the hallmark of HD. Expression of mutant HTT is associated with resistance against Mn toxicity in mice and striatal cell cultures and Mn has been shown to alter protein levels and phosphorylation of wild-type (WT) HTT. Here we aim to determine by which mechanism mutant HTT modulates Mn toxicity. We used a *Caenorhabditis elegans* model of fluorescently tagged polyglutamine (polyQ) repeats. We exposed WT (N2) and mutants *rms133* (unc-54p::Q40::YFP) (AM141, expression of polyQ protein in body wall muscles) and *rms110* (F25B3.3p::Q40::YFP) (AM101, polyQ expression in neurons) to Mn (25 mM, for 30 min) in the L1 stage. Survival assay revealed mutants to be more sensitive to Mn. Lifespan was not altered in the tested conditions. Locomotion, tested by number of body bends per 3 min intervals at days 6, 9, 12 and 15 post-treatment was not altered. Mn exposed mutant strains presented a reduction in the number of protein aggregates at days 9 and 12, observed by fluorescent microscopy. Our findings provide evidence that HTT polyQ40 exacerbates Mn toxicity in worms, however in the presence of excess Mn, fewer protein aggregates are observed at the tested periods. Mn levels and neuronal integrity in polyQ mutants exposed to Mn remain to be determined in order to establish a disease-toxicant interaction between Mn exposure and HD.

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**899A A natural product inhibits the initiation of α-synuclein aggregation by displacing it from lipid membranes and suppresses its toxicity in cells and in *C. elegans*. Michele Perni, Celine Galvagnion, Alexander S Maltsev, Martin BD Muller, Pavan K Challia, Julius B Kirkegaard, Roberta Cascella, Ryan Limbrocker’s, Pietro Sormanni, Nunilo Cremades, Fabrizio Chiti, Ellen AA Nollen, Tuomas PJ Knowles, Michele Vendruscolo, Ad Bac, Michael Zasloff, Christopher Dobson, Centre For Misfolding Diseases**  
1) Chemistry, University of Cambridge, Lensfield Road, CB2 1EW, Cambridge, GB; 2) Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-2560, USA; 3) University of Groningen, University Medical Centre Groningen, European Research
Among them, we have focused on mutations cause: (1) a loss-of-function effect in a yeast model system; and (2) dominant aberrant axonal morphological defects investigate three missense mutations in alanyl-tRNA synthetase (AARS); two mutations cause axonal CMT disease (G102R and R329H) and one is a common non-pathogenic variant (G931S). We present data showing that the disease-associated mechanism of how membrane contact site protein may play an important role in axon regeneration.

ESYT-2, which may represent a change in membrane contact sites in response to injury. We are currently investigating the membrane contact sites between the endoplasmic reticulum (ER) and plasma membrane. We found that ESYT-2 localizes to interorganellar membrane contacts. Loss of function in these genes display differential effects on PLM axon regeneration.

Loss of function in these genes display differential effects on PLM axon regeneration.

Axons are vital for the function of the nervous system as they transmit neuronal signals to their targets. During development of the nervous system, axons readily grow and make precise connections, but that ability is lost as animals mature into adults. It is unclear which signaling pathways promote and restrain axon growth throughout development into adulthood. Through a systematic genetic screen using laser axotomy of PLM neurons, we identified several genes that are predicted to function at interorganelar membrane contacts. Loss of function in these genes display differential effects on PLM axon regeneration. Among them, we have focused on esyt-2, a member of the extended synaptotagmin family, which have been shown to maintain membrane contact sites between the endoplasmic reticulum (ER) and plasma membrane. We found that ESYT-2 localizes to putative ER-plasma membrane contact sites in neurons. Inducing injury by laser axotomy causes an immediate realocalization of ESYT-2, which may represent a change in membrane contact sites in response to injury. We are currently investigating the mechanism of how membrane contact site protein may play an important role in axon regeneration.

Investigating the roles of membrane contact site proteins in axon regeneration. C.A. Piggott1, Z. Wu1,2, Y. Jin1,2 1) Division of Biological Sciences, University of California San Diego, La Jolla, CA; 2) Howard Hughes Medical Institute, University of California San Diego, La Jolla, CA.

Axons are vital for the function of the nervous system as they transmit neuronal signals to their targets. During development of the nervous system, axons readily grow and make precise connections, but that ability is lost as animals mature into adults. It is unclear which signaling pathways promote and restrain axon growth throughout development into adulthood. Through a systematic genetic screen using laser axotomy of PLM neurons, we identified several genes that are predicted to function at interorganelar membrane contacts. Loss of function in these genes display differential effects on PLM axon regeneration. Among them, we have focused on esyt-2, a member of the extended synaptotagmin family, which have been shown to maintain membrane contact sites between the endoplasmic reticulum (ER) and plasma membrane. We found that ESYT-2 localizes to putative ER-plasma membrane contact sites in neurons. Inducing injury by laser axotomy causes an immediate realocalization of ESYT-2, which may represent a change in membrane contact sites in response to injury. We are currently investigating the mechanism of how membrane contact site protein may play an important role in axon regeneration.

A model system for assessing ARS-mediated neurotoxicity in Charcot-Marie-Tooth disease. H. Prior1, H. McLaughlin2, S. Oprescu1, A. Antonellis1, A.A. Beg2 1) Neuroscience Program, University of Michigan, Ann Arbor, MI; 2) Pharmacology, University of Michigan, Ann Arbor, MI; 3) Human Genetics, University of Michigan, Ann Arbor, MI.

Charcot-Marie-Tooth (CMT) disease is a group of heterogeneous inherited peripheral neuropathies that cause sensory loss and muscle weakness. CMT is the most commonly inherited peripheral neuropathy, affecting approximately 1 in 2,500 people. There are two major classes of CMT: 1) CMT1 (demyelinating) is due to a primary defect in myelinating Schwann cells, and 2) CMT2 (axonal) is due to defects in nerve cell axons. It is predicted that over 100 genes play a role in CMT disease and, to date, ~80 loci have been identified. Aminoacyl-tRNA synthetases (ARSs) are a ubiquitously expressed, essential class of enzymes responsible for ligating amino acids to cognate RNA molecules. Four ARS loci are mutated in patients with axonal CMT disease and additional alleles of unknown significance are being identified at a rapid pace. Mutational analysis of human ARS genes continue to produce novel variants with unknown pathogenic consequences. Localization, aminoacylation and viability assays are incapable of determining the effects of ARS mutations in the context of an axon, which is problematic as each disease-associated ARS mutation has been implicated in inherited peripheral neuropathy with an axonal pathology. These obstacles present the need for a rapid, tractable, in vivo model system capable of distinguishing between pathogenic and non-pathogenic variants in axons.

We developed a C. elegans model as a resource for studying the pathogenicity of ARS mutations in neurons. Here, we investigate three missense mutations in alanyl-tRNA synthetase (AARS): two mutations cause axonal CMT disease (G102R and R329H) and one is a common non-pathogenic variant (G931S). We present data showing that the disease-associated mutations cause: (1) a loss-of-function effect in a yeast model system; and (2) dominant aberrant axonal morphological defects and motor behavioral deficits in the worm. Importantly, both models distinguish between pathogenic and non-pathogenic variants thus providing a key resource for assessing the pathogenicity of newly identified alleles.

Novel translational products encoded by disease-associated GC-rich repeat expansions cause toxicity in C. elegans. Paige Rudich1,2, Carley Snoznik1, Todd Lamitina1,2 1) Department of Cell Biology, University of Pittsburgh, Pittsburgh, PA; 2) Department of Pediatrics, University of Pittsburgh, Pittsburgh, PA.

Expanded GC-rich repeats cause many age-onset neurodegenerative diseases. An emerging mechanism that may underlie disease pathology is an unusual type of protein translation called Repeat Associated non-ATG (RAN) translation, which
specifically targets these GC-rich repeats. RAN translation requires extended GC-rich repeats and occurs independent of a canonical start codon, allowing translation in all three reading frames. Antisense RNA from these GC-rich repeats also gives rise to RAN protein products causing up to six distinct protein products from one repeat expansion. Most of these RAN proteins have never been studied in any biological system. Defining the role of RAN proteins in neurodegenerative diseases and their potential mechanisms of toxicity represents an exciting new translational research opportunity.

We developed RAN protein models in C. elegans for two GC-rich expansion repeat diseases – C9orf72-associated Amyotrophic Lateral Sclerosis (ALS) and Huntington’s Disease (HD). To separate RAN protein toxicity from RNA-derived toxicity, we generated ‘pure’ protein models by synthesizing codon-varied constructs that preserve RAN protein sequence but eliminate repeat-derived RNA structures. We used canonical ATG-dependent protein translation to bypass RAN translation and a C-terminal GFP or RFP tag to examine the subcellular localization. In our C. elegans ALS model, the arginine-containing dipeptides Pro-Arg (PR) and Gly-Arg (GR) exhibited age-onset toxicity when expressed in multiple cell types, including motor neurons. PR and GR exhibited nuclear localization that was necessary for toxicity. PR and GR did not form protein aggregates. Age-onset toxicity of PR, but not GR, was influenced by mutations that alter the rate of ageing, suggesting differences in the toxicity mechanisms of these related RAN peptides. In our HD model, initial studies suggest that HD RAN proteins exhibit distinct cell biological properties from the canonical polyGln (Q) HD protein. Currently, we are comparing the toxicity of these new HD RAN protein products to the well-described toxicity of polyQ. Understanding how different RAN peptides contribute to HD and to ALS is vital for developing appropriate treatments for these diseases. Moreover, comparing RAN proteins within a single experimental paradigm may reveal common themes that facilitate RAN protein toxicity and generate a more integrated understanding of the role of RAN-derived proteins in neurodegenerative diseases.

903B  Phenotypic effects of combined neuronal expression of toxic peptides associated with neurodegenerative diseases.  J. Russell1, K La1, J Lee1, B Ma2, A Mendenhall2, M Kaeberlein1  1) Pathology, University of Washington, Seattle, WA; 2) Interlake High School, Bellevue, WA.

With the dramatic increases in life expectancy over the past century, millions of people are living long enough to develop age-related neurodegenerative diseases associated with cognitive decline and dementia. Neurodegenerative diseases are thought to be largely proteinopathies, or diseases associated with misfolded proteins. Ab and Tau protein aggregation are key hallmarks of Alzheimer’s disease (AD), while aggregated a-synuclein is a strong indicator of Parkinson’s disease (PD). Yet, this categorization is not definitive by any means; many patients with dementia exhibit neurons with combined Ab, Tau, and a-synuclein aggregation. Some recent murine and cell culture studies have shown that Ab, Tau, and a-syn have synergistic effects resulting in enhanced pathologies. Another recent report in Drosophila showed that these toxic peptide interactions could be studied by looking at the effects on fly development. While genetically tractable, the model is not neuronal based. Therefore, we have developed C. elegans as a model for studying interacting neurotoxic proteins in vivo in neurons. To do this we have generated transgenic worm lines with neuronal expression of different alleles of different neurodegenerative-disease associated toxic proteins (Ab, Tau, and a-synuclein), and crossed the animals together to generate animals with different dosages and combinations of toxic peptides. Additionally, we have developed new ways for quantifying neuropathologies in these animals. Using these assays, we can discriminate between the physiological effects of neuronal Ab, Tau, and a-synuclein, alone, or in combination with one another. We hope that these animals will become a platform for investigating the combinatorial effects of distinct toxic peptide species in vivo.

904C  Sex-specific response of the cytoplasmic thioredoxin system (trxr-1/trxr-1) in methylmercury-treated Caenorhabditis elegans.  J.A. Russkiewicz1, A. Miranda-Vizuete1, A. Bowman2, M. Aschner1  1) Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY, United States; 2) Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío, Sevilla, Spain; 3) Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN, United States.

Methylmercury (MeHg) is an environmental contaminant linked to many neurological defects. Disrupted renox homeostasis is a crucial event in MeHg-induced neurotoxicity. The thioredoxin system is a key renox regulator affected by MeHg, however the mechanism and consequences of this process are not completely understood. Caenorhabditis elegans (C. elegans) is a valuable tool in studying the thioredoxin system, since in worms, unlike in other model organisms, thioredoxin null mutants do not lead to embryonic lethality. Therefore, we used this model to evaluate the role of the thioredoxin system in MeHg-induced neurotoxicity. Synchronized worms at different developmental stage (L1, L4 and adult) were exposed for 1hr to MeHg in M9 buffer. The survival of wild type (wt) and mutant worms with disrupted homologs of the thioredoxin system (trxr-1, trxr-1, trxr-2, trxr-2, trxr-3) was evaluated 24hr after exposure. Deficiency of the cytoplasmic thioredoxin system led to higher susceptibility to MeHg, as trxr-1 and trxr-1 mutants showed significantly higher lethality in response to MeHg than wt worms, especially when treated at L4 stage. Susceptibility of other mutants (trxr-2, trxr-2, trxr-3) remained unaffected. Still, similarly to the wt, the lifespan of mutants (trxr-1, trxr-1, trxr-2, trxr-2, trxr-3) was unchanged for worms which survived MeHg treatment when at L1 or L4 stage. The expression of thioredoxins TRX-1, TRXR-1, TRX-2, TRXR-2 was evaluated 24 and 48hr after MeHg exposure in L1 and L4 worms with corresponding GFP constructs. MeHg downregulated the expression of TRXR-1 in hermaphrodites treated at L1 and L4 stage, both 24 and 48hr after exposure, but not in L4 males. The expression of TRX-1, TRX-2 and TRXR-2 remained unaffected by MeHg, however the TRX-1, which is exclusively expressed in a pair of ASJ sensory neurons, was significantly higher in males when compared with hermaphrodites. Sex-specific differences in response of the trxr-1/tr xr-1 system were previously reported in mice brain and were linked to differential susceptibility to developmental MeHg exposure. Therefore, further studies addressing the role of the trxr-1/tr xr-1 system in sex-specific response, especially neurodegeneration upon MeHg treatment, might help to understand this effect. To date, our findings indicate that the cytoplasmic thioredoxin system trxr-1/trx-1
is not only a sex-specific target, but also an important protectant against MeHg toxicity in C. elegans. Supported by NIEHS R01 ES07331 to MA and ABB.

905A  Synergistic patterned neurodegeneration caused by APP and APOE4 in C. elegans model of Alzheimer's disease.  W. Sae-Lee, L.S. Scott, A.J. Encanacion, P. Satarasinghe, J.T. Pierce  Molecular Biosciences, University of Texas at Austin, Austin, TX.

  Alzheimer's disease (AD) is a progressive neurodegenerative disease that cannot be prevented, cured, or slowed down. Genetic and epidemiological studies have linked several genes to AD, notably the amyloid precursor protein (APP) and the e4 allele of apolipoprotein E (APOE4). APOE4 confers the highest risk for developing AD compared to the other isoforms (APOE3 and APOE2) contributing to a 91% lifetime chance of developing AD for individuals homozygous for APOE4. Moreover, APOE4 represents the most common genetic risk factor for AD; over 40% of current AD patients carry an APOE4 allele. At present, how APP and APOE4 functionally interact at the cellular and molecular level to trigger neurodegeneration in AD remains elusive. Progress has been slow using common mouse models of AD that do not display neurodegeneration. Here, we show that C. elegans can model important aspects of AD including age-related, patterned neurodegeneration that is modified by APOE. In our worm model of AD, we found that a certain subset of neurons is vulnerable to death, while other neurons survive. This is reminiscent of localized degeneration observed in the hippocampus and entorhinal cortex of brains in AD patients. Furthermore, in our worm model of AD we found that APOE4, but not its neutral risk isoform APOE3, acts synergistically with APP to hasten and expand the pattern of neurodegeneration. By combining genetic, molecular, and cell-specific proteomic approaches, we aim to uncover how these molecules synergize to kill some neurons while leaving others unaffected.

906B  Cellular and molecular mechanisms of smn-1-mediated neuron-specific degeneration.  P. Santonicola1, I. Gallotta1, N. Mazzarella1, A. Donato1, A. Esposito1, G. Zampi1, H. Lu2, E. Di Schiavi1  1) Institute of Bioscience and BioResources, CNR, Naples, Italy; 2) Georgia Institute of Technology, Atlanta, USA.

  Smn1 is the gene responsible for Spinal Muscular Atrophy (SMA), a devastating disease characterized by progressive degeneration and death of a specific subclass of motor neurons. The lethality associated with loss of function mutations in Smn1 has made the study of its function hard to investigate in any animal model. In C. elegans, two mutants in Smn1/smnl-1 are available, which present some limitation for manipulation and no neurodegeneration (Briese et al., HMG, 2009; Sleigh et al., HMG, 2010). To overcome these limits and investigate the role of smn-1 in the nervous system, we used a neuron-specific RNAi strategy (Esposito et al., Gene, 2007) to silence smn-1 selectively in the GABAergic motor neurons. These animals, viable and fertile, presented an altered backward movement and an age-dependent degeneration specifically in these motor neurons, detected as disappearance of presynaptic and cytoplasmic fluorescent markers, and as positive reactivity to genetic and chemical cell-death markers (Gallotta et al., HMG 2016). We then determined that homologs of Smn1 modulators identified in other species, such as Plastin3/plst-1, genetically interacts with smn-1 for neuron survival and that valproic acid, a drug successfully used in SMA mouse models, partially reverses this phenotype. We also demonstrated that genes of the classical apoptosis pathway, and not of the necrotic pathway, are involved in the smn-1-mediated neuronal death. Remarkably, this phenotype was rescued by the expression of human Smn1, indicating a strong functional conservation between the two genes. To determine the neuron-specific effects of smn-1 depletion, we silenced it in the touch receptor neurons, revealing cell-specific phenotypes and molecular machineries activated by lack of smn-1. Finally, by forward and reverse genetics approaches we identified several genes that interact with smn-1 to either worsen or rescue the degeneration. Some of these genes impair the RNAi pathway (e.g. rde-1), others are able to prevent the apoptotic death but not the degeneration, while others fully protect neuronal function, integrity and survival (e.g. Y105E8A.8). Some of the neuroprotective genes identified have been tested for suppressing different neurodegenerative models (e.g. axon degeneration or a-syn overexpression) and we found that a subset of them has a general protective role, while others are specific to our SMA model, providing a unique new framework to elucidate the molecular mechanisms that underlie neuron degeneration.

907C  Loss of SEL-12 function leads to aberrant Ca²⁺ homeostasis and mitochondrial metabolism resulting in neurodegeneration.  Shaanika Sarasija, Jocelyn Laboy, Kenneth R. Norman  Regenerative and Cancer Cell Biology, Albany Medical College, Albany, NY 12208.

  Maintenance of mitochondrial structure and activity is integral to cellular function and survival. Mitochondrial dysfunction and subsequent metabolic deregulation is observed in neurodegenerative diseases and aging. Mutations in the presenilin (PSEN) encoding genes (PSEN1 and PSEN2) cause most cases of familial Alzheimer’s disease (AD); however, the underlying mechanism of pathogenesis remains unclear. Using genetic, cell biological and pharmacological approaches, we have found that the Caenorhabditis elegans PSEN homolog, SEL-12, is required for Ca²⁺ homeostasis, mitochondrial function and neuronal health. In SEL-12 deficient animals, aberrant Ca²⁺ transfer from the ER to the mitochondria alters mitochondrial morphology and function. Furthermore, sel-12 mutations lead to neuronal structural defects in the form of ectopic sprouting, axonal lesions and breaks in the mechanosensory neurons and concomitant functional loss. Interestingly, these mitochondrial and neuronal defects are independent of SEL-12’s role in the β-secretase proteolytic complex and Notch signaling. Conversely, we find that reduction of ER-Ca²⁺ release or mitochondrial Ca²⁺ uptake rescues the mitochondrial changes and the neuronal structural and functional defects observed in sel-12 mutants. Moreover, we demonstrate that the treatment of sel-12 mutants with a mitochondrial targeted antioxidant also rescues these neurodegenerative phenotypes. Thus, we hypothesize that loss of SEL-12/presenilin function leads to aberrant ER-Ca²⁺ release and mitochondrial Ca²⁺ uptake, which increases mitochondrial oxidative phosphorylation leading to excessive superoxide production. Consistent with this hypothesis, we detect an increase in ATP levels, oxygen consumption and ROS levels in sel-12 mutants. Additionally, we find that reducing ER Ca²⁺ release or reducing mitochondrial Ca²⁺ uptake normalizes oxygen consumption and ROS levels in sel-12 mutants. Thus our data argues that loss of
SEL-12/preselinin function leads to neuronal abnormalities by altering mitochondrial metabolic activity, which leads to toxic levels of superoxide production. Our results provide evidence for a novel gamma-secretase independent role of PSEN in mediating neuronal structural and functional integrity via regulation of ER-mitochondrial Ca²⁺ transfer.

**908A  A UBQLN2 transgenic C. elegans model of ALS.** A. Saxton¹, ², B. Kraemer¹, ³, ⁴, ⁵  1) Geriatric Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA; 2) Online non-thesis Master's in Biology Program, Department of Biology, University of Nebraska Kearney, Kearney, NE; 3) Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Washington, Seattle, WA; 4) Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA; 5) Department of Pathology, University of Washington, Seattle, WA.

Amyotrophic lateral sclerosis (ALS) is a debilitating, fatal neurodegenerative disease causing rapid muscle wasting. It shares common neuropathological features and a spectrum of cognitive symptoms with frontotemporal lobar degeneration (FTLD). UBQLN2 encodes a ubiquitin-like protein involved in the ubiquitin-proteasome degradation pathway. Mutations in the PXX domain of UBQLN2 cause familial ALS with or without concomitant FTLD. Expression of ALS mutated human UBQLN2 in rat neurons causes protein aggregation and neurodegeneration. To explore the mechanism of UBQLN2 in disease, we have generated a transgenic C. elegans model with pan-neuronal expression of a human UBQLN2 cdDNA carrying either wild-type sequence or with ALS causing mutations P497H or P506T driven by the rgef-1 promoter. Characterization of the transgenic lines revealed a decrease in motor function and a reduced lifespan along with an increase in neurodegeneration.

In disease, mutated UBQLN2 aggregates in skein-like inclusions with other ALS and FTLD associated proteins including TDP-43 and ubiquitin. TDP-43, TAR DNA binding protein 43, is an essential RNA and DNA binding protein that is the major aggregating protein in ~90% of sporadic ALS cases. Furthermore, mutations in TDP-43 cause ALS in ~5% of familial cases. Given the clear disease relationship between abnormal UBQLN2 and pathological TDP-43, transgenic C. elegans lines of both UBQLN2 and TDP-43 models have been crossed in order to examine this interaction in vivo. Dissection of the interplay between TDP-43 and UBQLN2 will help to further elucidate mechanisms of disease in ALS.

**909B  Neuronal nicotinamide-N-methyltransferase controls metabolism, behavior, and aging by regulating neuronal autophagy.** Kathrin Schmeisser, Alex Parker  CRCHUM, University of Montreal, Quebec, CA.

The enzyme nicotinamide N-methyl-transferase (NNMT) is an essential contributor in various important epigenetic, reproductive, and metabolic processes. It plays a role in healthy aging, cellular stress response and resistance, and it regulates weight gain and the prevalence of obesity, among others. Although it is expressed and functionally active in the brain, its role in neurons remains obscure. This is surprising given that in vitro studies have shown NNMT to be a potential risk factor for psychiatric diseases like schizophrenia and neurodegeneration, especially in dopaminergic neurodegeneration typical of Parkinson’s disease. Neurodegeneration is a pervasive major risk for increased mortality in the elderly.

Here, we studied the role of neuronal NNMT in vivo using C. elegans. The nematodal ortholog of human NNMT is ANMT-1. In contrast to most in vitro studies, we found neuronal ANMT-1/NMNT overexpression to be neuroprotective in wild type and various disease models as the animals age. ANMT-1/NMNT uses methyl groups from S—adenosyl-methionine (SAMe). As the most important methyl group donor in the cell, SAMe also provides methyl groups for the methyltransferase B0285.4, the C. elegans ortholog of the human leucine carboxyl methyltransferase 1 (LGMT1). ANMT-1/NMNT titrates methyl groups from B0285.4/LGMT1, reducing its catalytic capacities.

B0285.4/LGMT1 targets NPR-L-2 (human NPR2-like, GATOR1 complex subunit), a known regulator of autophagy and cell growth. Autophagy is activated by neuronal overexpression of ANMT-1/NMNT, which acts by “clearing up” misfolded, dysfunctional and aggregated proteins within the neurons, maintaining proteostasis and healthy neuronal function in age. Furthermore, neuronal expression of ANMT-1/NMNT mediates increased neurotransmitter release, which may act as an endocrine-like signal in the whole animal to control metabolism, behavior, and extend lifespan. This study shows for the first time that ANMT-1/NMNT may contribute to neuronal health in vivo based on its direct or indirect regulation of cellular processes such as the induction of autophagy and endocrine-like signalling, which are critical for neuronal homeostasis, ultimately curtailing neurodegeneration, all the while promoting healthy aging.

**910C  Discoidin domain receptor DDR-2 modulates the Met-like RTK-JNK signaling pathway in axon regeneration.** T. Shimizu, Y. Nagamori, K. Matsumoto, N. Hisamoto, S. Pastuhov  Nagoya University, Nagoya Chikusa-ku, Aichi, JP.

The ability of specific neurons to regenerate their axons after injury is governed by cell-intrinsic regeneration pathways. However, the signaling pathways that orchestrate axon regeneration are not well understood. In Caenorhabditis elegans, initiation of axon regeneration is positively regulated by SVH-2 Met-like growth factor receptor tyrosine kinase (RTK) signaling through the JNK MAPK pathway. Here we show that SVH-4/DDR-2, an RTK containing a discoidin domain that is activated by collagen, and EMB-9 collagen type IV regulate the regeneration of neurons following axon injury. The scaffold protein SHC-1 interacts with both DDR-2 and SVH-2. Furthermore, we demonstrate that overexpression of svh-2 and shc-1 suppresses the defect in axon regeneration observed in ddr-2 mutants, suggesting that DDR-2 functions upstream of SVH-2 and SHC-1. These results suggest that DDR-2 modulates the SVH-2-JNK pathway via SHC-1. We thus identify two different RTK signaling networks that play coordinated roles in the regulation of axonal regeneration.

**911A  Analysis of KillerRed-mediated neurodegeneration.** C. Shoben, L. Young, D. Williams  Department of Biology, Coastal Carolina University, Conway, SC.

Aging is the primary risk factor for many neurodegenerative diseases and oxidative stress can induce degeneration of neurons. However the cellular mechanisms of neurodegeneration and the association between aging and oxidative stress that contribute to neuronal pathology are not well established. Our lab uses activation of KillerRed in GABA neurons to produce
reactive oxygen species and trigger neurodegeneration. Like other toxic conditions, KillerRed neurodegeneration is dependent on \textit{crt-1} and \textit{itr-1}, indicating a role for calcium signaling in ROS-mediated neurodegeneration. The effects of KillerRed are similar in wild-type and \textit{unc-68} mutants suggesting different ROS-sensitivities of calcium release channels. We have begun exploring the association between ROS-mediated neurodegeneration and aging by activating KillerRed in larval and adult animals and scoring for loss of GABA function. Preliminary results indicate L4 worms are more susceptible to the effects of KillerRed than adult animals, suggesting adult onset mechanisms to protect against ROS-mediated neurodegeneration.

912B  \textbf{C. elegans} glia regulate neuron shape and function through multiple novel mechanisms including engulfment of neuronal endings. \textit{A. Singhvi}, A Bae, S Shaham  Laboratory of Developmental Genetics, Rockefeller University, New York, NY.

Mammalian glia prune neuronal synapses, a process that is thought to regulate the number and fidelity of functional neuronal endings and neuron-neuron connections. Glia-mediated pruning is implicated in the control of synapse numbers in normal development, learning and memory, and aging. Disrupted pruning is often seen in neurodevelopmental disorders such as autism, and in age-dependent neurodegenerative disorders such as Alzheimer’s disease. Molecular mechanisms regulating pruning are poorly understood.

We discovered that \textit{C. elegans} glia engulf sensory-neuron receptive-ending fragments. Thus, glia-dependent pruning is an evolutionarily conserved phenomenon. Specifically, animals expressing GFP targeted to the receptive-ending of AFD thermosensory neurons also display GFP fluorescence in punctate structures within AMsh glia, which wrap around these receptive-endings. Ablation of the ipsilateral AFD neuron abrogates glial GFP accumulation, suggesting that glia take up material from the ensheathed AFD neuron. We find that glial engulfment rates track with temperature, but not with age, suggesting that glia are responding to AFD activity in a context-dependent manner throughout animal life.

CED-1/MEGF10 is required for phagocytic engulfment of apoptotic corpses in \textit{C. elegans} and for glia-dependent pruning in mammals. Surprisingly, we find that CED-1 is not required for glial engulfment of AFD fragments. However, our analyses implicate the CED-2/5/10/12 ternary complex in glial engulfment, and show that CED-10/Rac GTPase function is required cell-autonomously in glia. Thus, glial engulfment employs components of the apoptotic cell engulfment machinery acting downstream of unidentified receptors. Our preliminary data suggest roles for the phosphatidyly-serine receptor, PSR-1 and FIG-1, a glial thrombospondin-domain protein, in this process. We will also present identification and characterization of additional mutants obtained from a genetic screen for disruption of glia-dependent engulfment.

In a previous study (Singhvi et al, Cell, 2016), we identified a novel mechanism whereby glia regulate the shape and function of neuronal receptive-endings by regulating the ionic microenvironment, and described the underlying molecular pathway. We also showed that glia molecularly discriminate between associated neurons to differentially regulate each one. Together, our studies reveal that a single glial cell modulates the shape and function of its associated neurons through multiple cellular and molecular mechanisms.

913C  \textbf{N-glycosylation is required for axon regeneration in Caenorhabditis elegans through modification of discoidin domain receptor.} \textit{M. Takeo}, Y. Kato, K. Matsumoto, N. Hisamoto  Division of Biological Science, Nagoya University, Nagoya, JP.

In \textit{Caenorhabditis elegans}, initiation of axon regeneration is positively regulated by JNK MAP kinase (MAPK) pathway. Previously, we have identified \textit{svh} genes involved in JNK-mediated signaling. Two of these, \textit{svh-1} and \textit{svh-2}, encode a HGF-like growth factor and Met-like receptor tyrosine kinase (RTK), respectively. In addition, the \textit{svh-4/ddr-2} gene encodes another RTK carrying a discoidin domain activated by collagen, which functions upstream SVH-2. In this study, we investigate the roles of the \textit{svh-10} and \textit{svh-11} genes encoding components of Golgi glycosylation in axon regeneration. We find that frequencies of axon regeneration are decreased in \textit{svh-10} and \textit{svh-11} mutants. Genetic analyses indicate that \textit{svh-11} functions in the JNK pathway in a cell-autonomous manner. Furthermore, we show that overexpression of \textit{svh-2}, but not of \textit{ddr-2}, is able to suppress the \textit{svh-11} defect in axon regeneration, suggesting that DDR-2 is a candidate for glycosylation target. Consistent with this, we find that DDR-2 is N-glycosylated in the extracellular domain. N-glycosylation of DDR-2 is required for the efficient axon regeneration. These results highlight the biological importance of N-linked glycosylation in the control of axon regeneration in \textit{C. elegans}.

914A  \textbf{A conserved RNA binding protein regulates axon regeneration and integrity.} \textit{Ngang Heok Tang, Andrew Chisholm}  Section of Neurobiology, Division of Biological Sciences, University of California, San Diego, La Jolla, CA.

Axonal regeneration after injury involves a suite of signaling pathways and cytoskeletal regulators such as DLK-1 and EFA-6. Intriguingly, DLK-1 has also been implicated in maintenance of axon integrity in the mammalian nervous system (1). Conceptually, maintenance of axonal integrity is crucial to prevent neurodegeneration whereas axon regeneration provides potential therapeutic routes to recovery from neurodegeneration. From protein interaction screens with known regeneration regulators we have identified a conserved RNA binding protein that regulates axon regeneration. Loss of function in this protein results in increased regeneration, if injury occurs prior to adulthood. Longitudinal analysis of axon regeneration suggests this protein controls the axon extension phase of regrowth. Intriguingly, upon entry into adulthood, we observe progressive axonal breakage in mechanosensory and GABAergic motor neurons in this mutant. Importantly, the axonal breakage observed in this mutant can be reversed by loss of function in an axonal transport protein. Our data suggest this protein acts as a double-edged sword in controlling axon regeneration and integrity. We will describe our efforts to dissect the biochemical and functional roles of this pathway in axon regeneration and integrity.

1. A. S. Ghosh \textit{et al.}, DLK induces developmental neuronal degeneration via selective regulation of proapoptotic JNK activity.
915B  **Hyperphosphorylation of TDP-43 and Tau by TTBK1 and TTBK2 drives neurodegeneration.**  
* L.M. Taylor1,2, P.J. McMillan3,4,5, N.F. Liachko1,2, B. Ghetti6, T.D. Bird1,6,7, C.D. Keene8, B.C. Kraemer1,2,4,8  
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Progressive neuron loss in the frontal and temporal lobes of the cerebral cortex typifies frontotemporal lobar degeneration (FTLD). FTLD sub types are classified on the basis of neuronal aggregated protein deposits, typically containing either aberrantly phosphorylated TDP-43 or tau. Our recent work demonstrated that tau tubulin kinases 1 and 2 (TTBK1/2) robustly phosphorylate TDP-43 and co-localize with phosphorylated TDP-43 in human postmortem tissues of FTLD. Both TTBK1 and TTBK2 were initially identified as tau kinases and TTBK1 has been shown to phosphorylate tau epitopes commonly observed in Alzheimer’s disease. To further elucidate how TTBK1/2 activity contributes to both TDP-43 and tau phosphorylation in the context of the neurodegeneration seen in FTLD, we examined the consequences of human TTBK1/2 kinase expression in transgenic animal models of disease. We show that *C. elegans* co-expressing tau/TTBK1, tau/TTBK2, or TDP-43/TTBK1 transgenes exhibit significant behavioral abnormalities, increased protein phosphorylation, aberrant neuronal architecture, and neuron loss. Additionally, we observed an increase in TTBK1/2 expression in human FTLD tissue. Our findings support a kinase-driven mechanism of neurodegeneration in FTLD implicating TTBK1/2 hyperactivity, and may explain the etiology of FTLD-TDP and FTLD-tau disease subtypes.

916C  **Investigating the DLK-1 axonal regeneration pathway in *C. elegans* models of neurodegeneration.**  
* G. Tossing1,2, A. Parker1,2  
1) CRCHUM, Université de Montréal; 2) Département de Neurosciences.

The nervous system is highly dependent on their axonal structures to function properly. The axon’s long morphological structure make them particularly fragile to several conditions, such as injury, aging or neurodegenerative processes which can induce damage, axonal degeneration and neuronal dysfunction. To oppose the axonal degeneration, the neurons possess diverse and complex regeneration pathways to induce axonal regeneration. *C. elegans* has been used as a powerful model to investigate these pathways after mechanical injury in young worms, but currently there is a limited knowledge about their implication in aging and disease.

In this study, we are investigating if an up-regulated DLK-1 pathway, which regulates the axonal regeneration response to injury, can repair axonal damage observed in a neurodegeneration context. We have used transgenic *C. elegans* models of amyotrophic lateral sclerosis and Huntington’s disease, both of which recapitulate some early stages of disease progression including age-dependant axonal degeneration and paralysis. We have tested if pharmacological or genetic inhibition on negative regulators of the DLK-1 pathway, such as *fsn-1* or *rpm-1*, can reduce the axonal degeneration phenotype and improve the worm’s condition.

Our preliminary data supports the hypothesis that stimulating axon regeneration can be a tool to reduce axonal damage in models of neurodegenerative diseases and to slow down pathological progression.

917A  **Strategies for identifying neuroprotective molecules in the bacterial diet of *Caenorhabditis elegans*.**  
* Arles Urrutia1, Ornella Realini1, J. Carlos Caris1, Víctor García-Angulo2, Mauricio Cano3, Andrea Calixto1  1) Universidad Mayor, Santiago, Santiago, CL; 2) University of Chile, Santiago, Santiago, CL.

Diet is the main supplier of amino acids (AA), and in recent years their role in the modulation of cellular signaling and metabolic processes has been studied. Diets supplemented with glutamate or tryptophan enhance lifespan of worms1, and the imbalance in GABA and tryptophan metabolism modulates the development of neurodegenerative diseases2,3. We previously observed that in the mec-4d model of neurodegeneration in *C. elegans*, the rate of neuronal death depends on the type of bacterial diet. Feeding worms with *Escherichia coli* B strain OP50 does not prevent neuronal death at 72 hours post hatching (<2% wild type (wt) AVM morphology), instead *Comamonas aquatica*, *Comamonas testosteroni* and *Bacillus megaterium* diets provide modest neuroprotection levels (10-20%). Surprisingly, *Escherichia coli* K12 strain HT115 induced almost 45% of wt AVM neurons, which doubles the neuroprotective effect described with other diets6. To decipher which metabolic pathways were involved in neuroprotection, *E. coli* OP50 and HT115 were selected for transcriptomic, genetic and biochemical comparison as diets for mec-4d worms. Transcriptomic approaches discovered differential expressed genes between both bacteria. The glyceraldehyde 3-phosphate dehydrogenase (gap), involved in the glycolysis pathway, and glutamate decarboxylase (gad), required for GABA production, are unique and highly expressed genes in *E. coli* HT115. *gadA* mutant HT115 bacterial diet showed significant differences in neuroprotection in mec-4d worms, highlighting the relevance of a GAD product. We are currently investigating the role of GABA in neuroprotection by supplementation of *E. coli* OP50. Additionally, we assessed the neuroprotective role of other molecules. Lipids of the HT115 strain were separated from the polar molecules, and the fractions were used for diet supplementation. Worms fed with the polar fraction showed 25% wt AVM morphology7. The described
approaches will provide a robust evidence to describe the responsible AA and metabolites in neuroprotection, and the synergistic and synchronized mechanisms from nutrients signaling. This study has potential relevance in understanding the bacterial and microbiome contribution in the modulation of neurodegenerative disease.

918B Transgenic activation of the endoplasmic reticulum unfolded protein response protects against pathological tau. S.M. Waldher,1,2, B.C. Kraemer1,2,3,4 1) Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Washington, Seattle, WA; 2) Geriatric Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA; 3) Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA; 4) Department of Pathology, University of Washington, Seattle, WA.

Efficient protein folding and prevention of abnormal aggregation in neurons relies on the protein homeostasis network. Cellular stress can increase misfolded proteins in the endoplasmic reticulum (ER), which activates the ER unfolded protein response (UPRER) to restore homeostasis. Although it is well-characterized in non-neuronal cells, current studies have revealed a key role for the UPRER in normal neuronal function during aging and neurodegenerative diseases. Neurodegenerative tauopathies are a group of age-related disorders classified by accumulation of hyperphosphorylated and aggregated tau protein in the cytoplasm. Recent work indicates the UPRER transcription factor XBP-1s might play a critical role in tau pathology. We previously generated a transgenic C. elegans tauopathy model. This model recapitulates hallmarks of pathology, such as neuronal loss and accumulation of abnormal tau species. Our results show that loss of xbp-1 exacerbates tau-related phenotypes, including accumulation of abnormal tau protein and uncoordinated movement. Conversely, we found transgenic expression of constitutively active xbp-1s ameliorates pathological tau-induced phenotypes, including uncoordinated movement, motor neuron loss, and abnormal tau protein accumulation. The UPRER is composed of three branches which can initiate independent and overlapping stress response pathways. To understand which UPRER stress sensor branches participate in tauopathy, we also investigated loss of atf-6 and pek-1. Our analysis suggests neither atf-6 nor pek-1 loss of function mutations modify tau toxicity. However, the ATF-6 branch is necessary for xbp-1s-mediated amelioration of pathological tau-induced movement defects and abnormal tau protein accumulation. Aging is the greatest risk factor for developing neurodegenerative diseases. Interestingly, others have demonstrated xbp-1s activation can also extend lifespan in C. elegans via xbp-1s-mediated neuronal signaling events (Taylor and Dillin, 2013). While the mechanistic nature of xbp-1s effects on aging have yet to be dissected, we hypothesize the same xbp-1s regulatory target genes mediate both lifespan extension and suppression of tauopathy. Because tau is a cytoplasmic protein, we propose target genes of xbp-1s include those involved in protein homeostasis mechanisms outside of the ER, such as ER-associated degradation.

919C The predicted CED-4 Ca2+-binding domain inhibits axonal regrowth in Caenorhabditis elegans. G. Wang1, L. Sun2, C. Reina2, C. Gabel2, M. Driscoll1 1) Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ; 2) Department of Physiology and Biophysics, Boston University School of Medicine, Boston, MA.

Apoptosis proteins CED-3 and CED-4 are needed for efficient neuronal regeneration or axonal fusion [1]. In apoptosis, CED-4/APAF1 is an essential activator of the death executor caspase CED-3. In the regrowth consequent to laser axotomy, however, positive roles for ced-3 and ced-4 in repair are evident. Calcium changes at the cut site and later throughout the neuron also influence regeneration. Since modest CED-3 overexpression can counteract the deficiency of axonal regeneration in the calreticulin mutant crt-1(9k948), calcium is inferred to act upstream of CED-3/CED-4. The relationship between apoptotic executioner proteins and Ca2+-signaling pathways is still not well understood, but it is interesting that the CED-4 protein features two consensus sequences for the Ca2+-binding EF-hand domain (EF-hand I (EFH-I) and EF-hand II (EFH-II)). To test the potential role of Ca2+-binding in CED-4-mediated axonal regeneration, we mutated the potential Ca2+-binding residues of CED-4 via genome editing. Interestingly, substitutions engineered into the EFH-I and EFH-II sites did not alter apoptosis affecting the touch neuron lineages. In contrast, we find that the EFH-I mutant exhibits a significant increase in axonal regrowth, but neither the EFH-II mutant nor EFH-I&II double mutant is effected for regeneration. Using the caspase activated green fluorescent protein (P_mec-4CA-GFP) we can optically measure caspase activity in response to laser-induced neuron damage. We find that while ced-4/ced-3 mutations reduce caspase activity, the specific ced-4 EFH-I mutation does not. We examined the two predicted EF-hand domains in the published CED-4 crystal structure [2], and found that only the EFH-I (but not EFH-II) has a helix-loop-helix structure that is similar to a typical EF-hand Ca2+-binding domain. We suggest a model in which the CED-4 EFH-I Ca2+-binding domain functions to negatively regulate regeneration, possibly by turning off CED-3 caspase. Alternatively, the EFH-I disruption might induce a structural change that enhances CED-3 activation and subsequent elevation of axonal regrowth. We are investigating the role of CED-3 in the enhanced regeneration of EFH-I mutant, and seeking an in vitro method to test the Ca2+-binding properties of EFH-I.

References:


Amyotrophic Lateral Sclerosis (ALS) results in the selective degeneration of motor neurons. Approximately 10% of ALS cases
have a familial inheritance pattern (fALS) and 20% of fALS cases result from mutations in superoxide dismutase 1 (SOD1), an enzyme that catalyzes the breakdown of superoxide radicals. One of the many patient alleles in SOD1 is G85R, which converts a glycine to arginine at position 85 in the SOD1 protein. Although decades of research have advanced our understanding of the role of SOD1 in fALS, the mechanisms behind motor neuron degeneration in fALS remain unclear. Identifying suppressors should provide insight into the molecular mechanisms underlying the selective degeneration of motor neurons in SOD1 ALS models. We are undertaking a classical forward genetic screen to identify suppressors of glutamatergic neurodegeneration observed in a knock-in SOD-1G85R model. The first ALS models of SOD1 in C. elegans overexpressed human SOD1 and showed increased aggregation in neurons and neuromuscular dysfunction, but no neurodegeneration was reported. Using MosSCI, our lab has generated knock-in models for SOD-1G85R and other alleles, which are described in a presentation by Baskoylu et. al at this meeting. The SOD-1G85R knock-in mutant animals exhibit neurodegeneration in glutamatergic PHA/PHB neurons after stress. Using EMS, we mutagenized SOD-1G85R mutant animals and then screened roughly 5000 F2 lines for suppression of neurodegeneration after exposure to stress. We identified suppressor lines and are now using whole genome sequencing to identify candidate genes. Once candidate genes are identified, we will further characterize the genes by assessing cholinergic motor neuron death, neuromuscular function, and survival. Additionally, to determine if common pathways underlie neurodegeneration in fALS, we will test candidate and established ALS suppressor genes in other SOD1 and fALS models. Understanding the suppressors in fALS models may lead to the development of treatments and illuminate some of the molecular mechanisms behind ALS. Support for this project was provided by ALS Finding a Cure.

Neurobiology - Synaptic Function and Circuits

**921B Investigating Molecular Mechanisms Underlying Cholinergic Synapse Remodeling in the C. elegans Motor Circuit.**  K. Alexander¹, D. Oliver¹, A. Philbrook¹, C. Lambert¹, A. Thackeray¹, M. Doitsidou², C. Bénard¹, M.M. Francis¹ ¹) UMASS Medical School, Worcester, MA; 2) University of Edinburgh, United Kingdom.

The nervous system comprises complex neural circuits that require precise patterns of connectivity. To accomplish this, there is often considerable remodeling of connectivity during development, typically an initial over proliferation of synapses that is followed by activity-dependent refinement or remodeling. We have only a limited molecular understanding of how this is achieved. To address this question, we have been investigating synaptic remodeling of GABAergic Dorsal D class (DD) motor neurons. Initially, DD motor neurons form neuromuscular synapses ventrally and receive synaptic inputs dorsally. By late larval stages, the polarity of these connections is reversed. DD motor neurons receive synaptic inputs from cholinergic motor neurons in the ventral nerve cord and form neuromuscular synapses to dorsal body wall muscles. Prior work has focused on the remodeling of presynaptic components in DD neurons and identified several genes involved. My work is focused on the remodeling of postsynaptic components using a specific marker (ACR-12::GFP) to label synaptic inputs to DD neurons. Interestingly, only a subset of genes implicated in presynaptic remodeling also affect postsynaptic remodeling. For example, disruption of the heterochronic gene hbl-1 similarly affects both processes [1]. In contrast, the caspase-3, involved in the removal of pre-synaptic components during remodeling [2], is not required for removal of dorsal ACR-12::GFP clusters. We are carrying out a forward genetic screen to identify novel genes involved in the remodeling process. To date, we have obtained two candidate mutants in which dorsal ACR-12 receptor clusters persist into adulthood. Through our ongoing characterization of these candidates and additional mutants identified from the screen, we aim to uncover conserved molecular mechanisms underlying synaptic refinement and remodeling.


Neural networks have arisen in Animalia over vast evolutionary time spans as a means to sense environmental stimuli and take effective navigational action. However, a critical issue lies in the fact that sensory neurons are only able to detect temporal variations caused by external stimuli at the specific location of the receptors, which contain no inherent spatial information of external environment. This raises the question of how such a collection of signals in time is converted into a sufficient understanding of the surrounding space to inform an appropriate reaction. There must be a mechanism for the conversion of signals in time into a distribution in space, and we argue this “conversion factor” must be supplied by the animal’s own motion. We propose a novel concept that such time-to-space conversion can be effectively achieved by utilizing a periodic rhythm of brain activity, Central Pattern Generator (CPG) as a universal clock to synchronize neural networks. This hypothesis has been applied to the C. elegans nervous system to establish the “dynamic connectome” which builds on the well established static connectome by predicting the dynamic function of these circuits especially as they pertain to sensorimotor integration and consequent perception of space. We identify particular groups of motor neurons exhibiting cross inhibitory connections as the prime candidates to be CPGs. We predict the sequence of neural activity for the process of sensory signals arriving via interneurons to the motor neurons while spatial context is provided by the current state of the rhythmic activity of the proposed CPG motor neurons separately controlling body and head orientation. Several experimental evidences supporting this concept will be presented.
Volatile anesthetics produce all stages of general anesthesia, including unconsciousness, amnesia, analgesia, and muscle relaxation. In this state, the experience, memory, and physical response to pain are all lost, yet patients can be returned to consciousness, making it an essential tool in modern medicine. However, the mechanism by which neuronal systems are disrupted to cause such effects remains a mystery. Mammalian models are limited by their experimental resolution, with fMRI and EEG measurements reporting the mean activity of millions of neurons. Previous research has developed C. elegans as an effective model for volatile anesthetics demonstrating that they display the same behavioral response to increasing levels of anesthesia as humans and identifying numerous genetic mutants that alter anesthetic susceptibility. Taking advantage of C. elegans simple neuro-anatomy and its comprehensive capabilities in multi-neuron imaging, we are defining the mechanism of anesthesia on a circuit level with single neuron resolution. Employing calcium based neuronal reporters (GCaMP) we can measure activity of the command neurons within the well-defined touch circuit with and without anesthetic. In worms under higher levels of anesthetics (~8% isoflurane) that are largely quiescent, we observe a reduction in individual neuronal activity mimicking the reduced activity observed in EEG measurements from humans at a similar level of anesthetic. By contrast, in worms under moderate levels of anesthetics (~4% isoflurane) that are unresponsive to external stimuli (touch), activity of individual neurons is not abolished, but the normal correlation between neurons (i.e. coordinated activity between neurons controlling reversal movement that is anti-correlated with those controlling forward movement) is disrupted. From these observations, we hypothesize that the effects of anesthesia causing analgesia, i.e. the lack of response to external stimuli, are due to the desynchrony of neurons in distinct behavioral circuits. We are currently expanding these measurements to pan-neuronal imaging of C. elegans, in order to understand the relationship between individual neuron activity and measurement of mean activity across entire ganglia (that are akin to EEG measurements in humans). In this way we seek a comprehensive understanding of the system wide effects of volatile anesthetics.

specific mRNAs for expression. Our data show that our mutation behaves as a recessive, gain-of-function mutation and mediates suppression cell-autonomously in the cholinergic neurons. We propose a model where EIF-3 modulates interactions between the translation machinery and mRNAs of key genes that adjust synaptic activity, thereby restoring balance to the motor circuit. We are testing this idea using a fusion of genetics, genomics, and live-cell imaging approaches to identify the downstream effectors of eif-3.g, its specific mRNA-binding targets, and its effect on their translation in the motor neurons.

**926A Modulation of food-dependent sensory integration in C. elegans.** F. Calahorro, F. Keefe, J. Dillon, L. Holden-Dye, V. O’Connor  Biological Sciences, University of Southampton, Southampton (UK), Southampton, GB.

Multisensory inputs are integrated before generating the decision making that drives appropriate behavioural outputs leading possibly a processing and a final integration. Dysfunctional behaviour associated with a number of disorders including autism spectrum disorders, schizophrenia, and anxiety appears to disrupt decision making at the level of the post sensory integrating circuits. The molecular and cellular mechanisms underlying the neural processing underlying decision-making behaviours are beginning to be elucidated. In the nematode C. elegans decision-making is mediated by layers of interneurons controlling the interplay of synergistic and antagonistic multimodal inputs from sensory neurons. Food is a potent modulator of decision-making and context-dependent feeding and locomotion are underpinned by a number of well understood microcircuits. We have investigated food-dependent behaviours in mutants deficient in nlg-1, the homologue of the human neuroligin implicated in autism. We identify that deficiency of this gene indirectly impacts on the feeding and other food dependent behaviours. These findings add to the understanding of the pharyngeal and extra pharyngeal circuits that integrate the decision to feed. The cellular basis of this food dependent behaviour reinforces a top down route that imposes regulation of pharyngeal dependent regulation of feeding. These data highlight details of how neuroligin organizes sub-circuits in C. elegans. Further it resolves how the behavioural expression of the molecular determinants of neuroligin supports in function in C. elegans and parallels observations in higher organisms, at level of integrating circuits. Further in this paradigm we highlight the ability to rescue of nlg-1 deficiencies with human homologues thus providing a platform to investigate autism related mechanisms.

**927B Probing receptive fields in c. elegans.** Z. Cecere1,2, T. Sharpee1,2, S. Chalasani1,2  1) University of California San Diego, San Diego, CA; 2) Salk Institute for Biological Studies, San Diego, CA.

The best way to probe receptive fields in a neural circuit is to inject uncorrelated stimulation into the circuit. We have built an optogenetics-based system to do just this. We use a custom Arduino control system to integrate a selective plane illumination microscope with an LED light engine. This system is capable of simultaneously performing full brain calcium imaging at 5 volumes per second and injecting uncorrelated activity into the C. elegans nervous system on the timescale of milliseconds. Here, we show preliminary results on the complexity of C. elegans receptive fields: how they are affected by the level of correlation and the predictability of their inputs.

**928C Breaking the cycle: separating synaptotagmin’s role in endocytosis from exocytosis.** S. Chen1,2, R. Hobson1,2, T. Vu1,2, G. Hoyer1,2, E. Bend3, Q. Liu4, E. Jorgensen1,2  1) Biology, University of Utah, Salt Lake City, UT; 2) HHMI; 3) Greenwood Genetic Center, Greenwood, SC; 4) The Rockefeller University, New York, NY.

Synaptic vesicle fusion is regulated by calcium. The major calcium sensor on synaptic vesicles is synaptotagmin. Synaptotagmin null mutants have reduced neurotransmitter release. In addition, the number of synaptic vesicles present in the synapse is also decreased. Therefore, disrupting synaptotagmin affects not only exocytosis, but also endocytosis. This makes it difficult to study the mechanism underlying synaptotagmin regulated synaptic vesicle fusion. We created a synaptotagmin mutant to separate the role of synaptotagmin in endocytosis from exocytosis in C. elegans. Complexin is also a regulator of synaptic vesicle fusion, but its role remains controversial. Complexin has proposed roles in both priming and inhibiting synaptic vesicle fusion. In C. elegans complexin is required to inhibit tonic fusion and facilitate evoked fusion. Complexin null mutants have increased neurotransmitter release, showing that complexin has a larger role in inhibiting than in facilitating fusion.

We built a synaptotagmin mutant that cannot bind calcium but partially rescues the locomotion defect of the synaptotagmin null mutant, suggesting that calcium binding at these sites is not absolutely required for synaptotagmin function. When crossed into the complexin null mutant, the synaptotagmin calcium binding mutant has increased neurotransmitter release, which is similar to the complexin null, suggesting that synaptic vesicle recycling is rescued in these mutants, but that exocytosis is defective. Using synaptotagmin calcium binding mutant as a tool, we can further study the mechanism underlying the synaptotagmin and complexin interaction in vesicle fusion.

**929A Plexin specifies synapse patterning via RAP-2 GTPase and MIG-15.** Xi Chen, Kota Mizumoto  Life Sciences Institute, Zoology, Vancouver, British Columbia, CA.

Fine motor coordination depends on the precise synaptic connection between individual motor neurons and muscles. Recent studies have revealed the roles of extracellular signals such as Wnt, Netrin and Semaphorin (Sema) in synapse specificity. On the other hand, little is known about their intracellular mechanisms in synapse patterning. We have previously shown that Sema and its receptor, Plexin (plx-1), mediate the inter-axonal interaction between two neighboring DA class of motor neurons (DA8 and DA9), which is critical for the tiled synaptic innervation between these neurons. Plexin is localized at the anterior edge of the DA9 synaptic domain, thereby restricting synapse formation. In the absence of plx-1, synaptic domains of DA8 and DA9 overlap due to the expansion of each synaptic domain. To understand the mechanisms of Plexin-dependent synaptic tiling, we investigated the intracellular components of Plexin signaling.

Structural and biochemical studies have suggested that mammalian Plexin acts as a GTPase activating protein (GAP) for Rap small GTPases. Among three rap genes in C. elegans genome, we found rap-2, but not rap-1 and rap-3, is preferentially
expressed in the nervous system including DA neurons. Consistent with the idea that Plexin negatively regulates Rap GTPase via its GAP domain, overexpression of constitutively active form of rap-2 (G12V) mimicked synaptic tiling defect of plx-1 mutants. Surprisingly, however, loss of function of rap-2 mutants also showed the same synaptic tiling defect, suggesting that Plexin regulates spatial activity of rap-2 along DA9 axon. To test this idea, we generated constitutively GTP-bound (G12V) and GDP-bound (S17A) forms of rap-2 mutants using CRISPR/Cas9 genome editing methods. Both rap-2 (G12V) and rap-2 (S17A) mutants showed synaptic tiling defect, supporting the idea that the cycling of RAP-2 activity plays a critical role in synapse patterning. Genetic experiments using these mutants suggested that rap-2 acts downstream of plx-1 to regulate synaptic tiling. To identify effectors of rap-2 in synaptic tiling, we conducted candidate screening and found that loss of function mutants of mig-15, an ortholog of Traf2 and Nck-interacting kinase (TNIK), showed severe synaptic tiling defect due to the increased synapse number of DA8 and DA9. Strikingly, overexpression of mig-15 eliminated synapses of DA9 and other neurons, suggesting that mig-15 is a key negative regulator of synapse formation. rap-2 (S17A), but not rap-2 (G12V) suppressed the increased synapse number of mig-15 (rh148) hypomorphic mutants, suggesting that RAP-2 inhibits Mig-15 activity. Consistently, overexpression of plx-1, which is a negative regulator of rap-2, also suppressed the mig-15 (rh148). Taken together, we propose that Plexin, which is localized at the specific subaxonal domain, specifies synapse distribution via RAP-2 small GTPase and Mig-15.

930B  Determining the function of the ion channel CLHM-1 in ciliated sensory neurons.  M. Clupper, K. Kervin, E. Miller, J. Tanis  Department of Biological Sciences, Delaware, Newark, DE.

Calcium (Ca²⁺) signaling is involved in mediating signal transduction, transcriptional regulation, neurotransmitter release, muscle contraction, and memory formation. Levels of Ca²⁺ are tightly regulated in the cell and disruptions in Ca²⁺ homeostasis can lead to neurodegenerative disorders such as Alzheimer’s disease (AD). Recent human genetic studies suggest that the P86L polymorphism in Calcium Homeostasis Modulator 1 (Calhm1) has been linked to late-onset AD. CALHM1 is an ion channel and member of the CALHM ion channel family. CALHM1 is regulated by both extracellular Ca²⁺ concentration and voltage and plays a role in mammalian taste perception. However, the functional consequences of CALHM channel activation in neurons are unclear. Furthermore, the proteins that regulate the function of CALHM channels are unknown. There is a single homolog of CALHM1 in Caenorhabditis elegans, CLHM-1, which controls motility. CLHM1 and CLHM-1 exhibit shared biophysical properties when expressed in heterologous systems and conserved functional properties when expressed in vivo. Understanding the roles and regulation of CLHM-1 in C. elegans may give insight into the importance of CALHM channels in other systems. We have determined that CLHM-1 localizes to the ciliated tips of sensory neurons, including the inner labial 2 (IL2) and amphid sensory (AS) neurons. Based on this expression pattern, we hypothesize CLHM-1 may play a role in sensation of environmental stimuli or amplification of the response to these sensations. Our current work is focused on defining a role for CLHM-1 in these neurons and identifying factors that regulate the trafficking, localization, and function of CLHM-1. To isolate CLHM-1 regulators, we performed a forward genetic screen for suppressors of CLHM-1 over-expression induced lethality. We are identifying the causative suppressor mutations using whole-genome sequencing and will characterize these mutants to achieve an understanding of the mechanisms that regulate CLHM-1 signaling in vivo.

931C  Gaq-Rho signaling regulates locomotion through the MPK-1/ERK MAPK pathway.  B.D. Coleman, M. Alion  Biochemistry, University of Washington, SEATTLE, WA.

We performed a forward genetic screen for suppressors of activated Gaq to identify signaling pathways that regulate C. elegans neuronal activity and locomotion. Activating mutations in the G protein EGL-30/Gaq cause hyperactive locomotion with exaggerated body bends. We identified the MAP kinase scaffold protein KSR-1 as a suppressor of activated Gq, and further found that inhibition of the LIN-45/Raf - MEK-2/MEK - MPK-1/ERK kinase cascade suppresses Gq-dependent locomotion. However, we found that the canonical MAPK activator, LET-60/Ras, is not required for this pathway. The small GTPase RHO-1 is one downstream effector of Gq and expression of an activated RHO-1 allele in cholinergic neurons causes exaggerated body bends similar to activated Gq. We found that mutants in the ERK MAPK pathway also suppress activated RHO-1. Through tissue-specific rescue of ksr-1 we found that Gq-ERK signaling acts primarily in the cholinergic neurons in the head to regulate body bend intensity. To test if ERK MAPK pathway activity is sufficient to regulate locomotion, we created transgenic lines expressing an activated form of LIN-45/Raf. We found that Raf over-activation in cholinergic neurons causes exaggerated body bends and locomotion defects similar to activated Rho mutants. Aldicarb assays show that the Gq-Rho-ERK pathway positively regulates acetylcholine release at the neuromuscular junction. Our results suggest that the ERK MAPK signaling pathway regulates locomotor behavior and neuronal activity downstream of Gq and Rho.

932A  The role of cross-inhibition in C. elegans locomotion.  L. Deng¹, V. Marfil¹, C. Doyle², G. Haspel¹  1) Biological Sciences, New Jersey Institute of Technology, Newark, NJ; 2) Bergen County Technical High School, Teterboro, NJ.

To achieve translocation, many locomotor systems produce alternation of antagonist muscle, including axial bending and limb alternation. Cross-inhibition is believed to be key to antagonistic alternation so that when a muscle is activated by the neural circuit, cross-inhibitory elements inhibit a counteracting muscle. Nineteen GABAergic motoneurons in C. elegans have been suggested to provide cross inhibition, based on their morphology and inhibitory effect on muscles. They each receive synaptic input from excitatory motoneurons and provide inhibition to its opposing motoneurons and muscles. However, the activity of D motoneurons during locomotion has not been reported. The locomotion behavior of mutants that are deficient in GABA transmission has been described and their phenotype was generally termed Shrinker. While their forward locomotion was described as normal, shrinker mutants contract their anterior section instead of undulating backward in response to noxious stimuli. However, we show that the deficiencies are not related to the direction of locomotion but rather to its speed and that
while cross-inhibition is not necessary for slow antagonistic alternation, it plays a crucial role in fast alternation. We use a combination of behavior analysis, optogenetics, and calcium imaging to clarify the role of the inhibitory D motoneurons. GABA transmission mutants moved at lower frequency and slower translocation speed in both forward and backward directions. Moreover, we found that both forward and backward undulation frequencies of wild type animals each fall into slow and fast distributions, and that the frequencies of mutants overlap with the low frequency distribution of wild type. When mutant animals were exposed to noxious stimuli that would have induced fast forward or backward undulation in wild type animals, they contracted their posterior or anterior sections, respectively. Similar changes in locomotion behavior occurred when D motoneurons were acutely inactivated with optogenetics. We recorded the neuronal activity of D motoneurons, as well as the activity of excitatory motoneurons and muscle cells, during restricted locomotion using microfluidic channels that mimic natural shape of natural undulation so that all elements can be correlated in the same framework of undulatory phase. We manipulate undulation frequency by changing the ambient viscosity. We hypothesize that cross inhibition sharpens the coordination of fast antagonistic alternation.

933B Understanding how Gαq signaling regulates egg-laying circuit activity and behavior of *Caenorhabditis elegans*. P. Dhakal, K. Collins  Department of Biology, University of Miami, Coral Gables, FL.

Gαq protein signaling modulates many cellular processes including neural circuit activity and synaptom in vivo. Gαq signaling regulates serotonin release from the serotoninergic Hermaphrodite Specific Neurons (HSNs), acetylcholine release from the ventral type A, B, and C neurons, and postsynaptically in the vulval muscles to modulate the response to released serotonon in vivo. In rescue experiments we find that Phospholipase CÎ² (PLCβ) expression in neurons rescued egg laying and aldicarb sensitivity of the *egl-8*(sa47) null mutant indicating EGL-8 function in the presynaptic cholinergic neurons is sufficient. In order to investigate how Gαq and its effectors regulate presynaptic and/or postsynaptic activity, we used GCaMP5 calcium imaging to record presynaptic HSN and postsynaptic vulval muscle activity in freely behaving animals. In this experiment we reveal that *egl-8*(sa47) mutants have similar HSN activity to the wild type and both *egl-30*(*n686*) and *egl-8*(sa47) mutants have persistent vulval muscle activity during infrquent egg-laying events. In contrast, we find a complete loss of vulval muscle activity in animals with the *unc-73*(ce362) mutants have persistent vulval muscle activity during frequent egg-laying events. Taken together, our results suggest a working model for how Gαq signaling regulates egg-laying behavior. We propose that PLCβ regulates acetylcholine release from the VA, VB, and VC neurons, rhythmically excite the vulval muscles during locomotion and for egg laying. Postsynaptic Gαq signaling through UNC-73 and Rho potentiates the vulval muscle response to released acetylcholine, driving their rhythmic excitation that triggers muscle contractility and egg release.

934C Excitatory/inhibitory switch from asymmetric neurons defines postsynaptic tining for rapid response in the C. elegans salt-chemotaxis circuit. M. Kuramochi1,2, M. Doi1,2 1) Biomedical Research Institute, AIST, Tsukuba, Japan; 2) Life Sci. & Bioeng., Grad. Sch. of Life & Env. Sci., Univ. of Tsukuba, Japan.

The nervous systems regulating animal behaviors are encoded as a combination of neuronal circuit activity and inhibition. However, it is not well known how the temporal activity of circuits are dynamically characterized by each excitatory and inhibitory synapse, and how both synapses are well organized for the fine regulation of circuit activities. The salt-chemotaxis behavior in *Caenorhabditis elegans* is regulated by a pair of sensory neurons and their postsynaptic interneurons. Both the ASEL and ASER sensory neurons detect NaCl concentration changes, but these neurons show opposite responses to NaCl. ASEL is activated by the increase of NaCl concentration, whereas ASER is activated by the decrease of NaCl concentration. In addition, these neurons seem to have distinct functions for chemotaxis behavior: the ASEL activation promotes forward run probability, whereas the ASER activation promotes turn probability. In spite of asymmetric property, both neurons use glutamate as a neurotransmitter and connect to the AIB interneurons which is known to regulate reversal/turn behaviors. These facts provoke an intriguing question. What is the synaptic mechanism leading to opposite behavioral outputs by asymmetric responses in the one class sensory neuron? To answer this question, we applied both calcium imaging and molecular genetics analyses to the *C. elegans* salt-sensing neural circuit. we first examined cellular and molecular features of synthetic regulation between the ASEs and AIB by characterizing the calcium responses of AIB in the wild-type and several synaptic mutant animals. From these analyses, we found that the glutamate from the ASEL inhibits AIB through the glutamate-gated chloride channel or unidentified receptors. On the other hand, the glutamate from the ASER activates AIB through both the AMPA-type ionotropic and the G-protein coupled metabotropic glutamate receptors. We also examined how these molecules are involved in the synapse organization between these two neurons. The distribution of excitatory and inhibitory receptors on AIB neurites was shown to be clearly distinguished: the excitatory synapses are mainly formed at left side but the inhibitory synapses are formed at right side of AIB neurites. Therefore, the excitatory/inhibitory switch from asymmetric sensory neurons defines rapid and fine responses in the postsynaptic interneurons to salt concentration changes, using distinct synaptic formation on the neurites.

935A A neural cell adhesion protein code for nervous system connectivity. B. Kim, O. Ivashkiv, S.W. Emmons  Departments of Genetics and Neuroscience, Albert Einstein College of Medicine, Bronx, NY.

Most synaptic connections in animal nervous systems are genetically specified. How this genetic encoding is brought about is
largely unknown. We are addressing this question by focusing on the fully described neural circuits in the *C. elegans* male tail, which subserve copulation. The molecules thought to act as cell-recognition tags for synaptic specificity comprise a largely evolutionarily conserved set of transmembrane or secreted proteins with extracellular protein-protein interaction domains, such as IG, LRR, EGF, cadherin, fibronectin, and so forth. There are 106 such proteins (cell adhesion proteins) encoded in the *C. elegans* genome. We hypothesize that there must be a correlation between the expression of these genes and connectivity. To find such a correlation, using reporter genes we have examined almost the entire set of 106 genes in the 238 neurons and muscles in the male tail neural network. Seventy-seven of the 98 genes tested so far (79%) are expressed. The expression matrix is complex. Most genes are expressed in multiple, unrelated cells and muscles, while most cells and muscles express multiple genes (up to 27 in bodywall muscles). This arrangement is consistent with an abstract combinatorial code for synaptic connectivity. We now seek statistical correlations of individual genes or combinations of genes with connectivity that we hope will reveal the nature of the code. We have validated the use of expression data for identifying genes critical for connectivity by experimentally verifying the roles of 7 genes in formation of a specific set of connections in the male tail (see abstract by Kim and Emmons).

**936B** Subunits that form trimeric DEG/ENaC mechano-electrical transduction channels in touch receptor neurons. *S. Fechner*, F. Loizeau, A.L. Nekimken, B.L. Pruitt, M.B. Goodman

In *C. elegans*, the behavioral response to touch and the underlying molecular actors are thoroughly studied. However, it is still debated which and how many of several co-expressed pore-forming subunits assemble into a functional, multimeric mechano-electrical transduction (MeT) channel in vivo. Two members of the DEG/ENaC channel family, MEC-4 and MEC-10, are important to convert touch into behavioral responses. MEC-4 is required to form native MeT channels, whereas MEC-10 plays a regulatory role. Recently, a third homologous protein and potential subunit, DEGT-1, was identified; its role in touch sensation remains incompletely understood. Given that DEG/ENaC proteins are thought to assemble as trimers, the presence of a third, homologous protein opens new questions regarding the composition of native MeT channels. A key outstanding question is which of these homologous proteins co-assemble to form the channels responsible for touch sensation. With the gentle touch assay, we detected a more severe defect in touch sensation if both MEC-10 and DEGT-1 were removed simultaneously than we did by removing them individually. To investigate this further, we are analyzing the contribution of DEGT-1 to native MeT channels and to channel activity in Xenopus oocytes. Using in vivo patch clamp recordings, we identified that, similar to the behavioral assay, the amplitude of mechanoreceptor current is further reduced by removing both MEC-10 and DEGT-1 simultaneously than individually. This genetic enhancement suggests that DEGT-1 is part of the native MeT channel complex. Using Xenopus oocytes, we are currently analyzing the contribution of DEGT-1 to channel function by expressing it alone and in combination with MEC-4 and MEC-10.


Neurotransmitters signal by activating ion channels or G protein-coupled receptors (GPCRs), and *C. elegans* provides a model for understanding signaling by these highly conserved molecules. Neurotransmitters can diffuse from their synaptic release sites to activate GPCRs on distant neurons. Thus, although the ~5,000 synapses between the 302 neurons of *C. elegans* have been mapped, and the neurons that release each of the 106 such neurotransmitters have also been mapped, it remains unclear what target neurons respond to these neurotransmitters. We are creating an atlas of which cells in *C. elegans* express each of its 27 neurotransmitter GPCRs. To identify each GPCR’s cellular expression pattern, we are making chromosomally-integrated fosmid-based GPCR::GFP reporter transgenes and crossing them into red fluorescent protein marker strains. Some GPCRs have published expression patterns, but in many cases these remain incomplete and contain inaccurate cell identifications. Our results show that a typical neurotransmitter GPCR is expressed in a dozen cells, mostly neurons and muscles, but also some glial and epithelial cells. For example, the serotonin GPCR SERT-4 is expressed in 35 neurons, four muscle cells, and two epithelial cells. Some of the SERT-4 expressing neurons receive direct synapses from serotonin-releasing cells, but most do not, and SERT-4 on these latter neurons could mediate extrasynaptic serotonin signaling. Interestingly, some SERT-4-expressing neurons receive direct synapses from cells releasing other biogenic amines similar in structure to serotonin, suggesting these lower-affinity SERT-4 ligands, may activate SERT-4 on these cells. The Atlas can be used to determine what GPCRs are expressed in any neural circuit. For example, in the egg-laying circuit, our data confirm several published findings, correct the cells that express the GAB2 acetylcholine receptor, and identify additional acetylcholine and octopamine GPCRs expressed in this circuit. Completion of our Atlas, integrated with the synaptic wiring and neurotransmitter maps, will provide a path to understand neurotransmitter signaling through GPCRs in each individual neural circuit and every behavior carried out in *C. elegans*. It should generate insights into how neural GPCR signaling is organized that generalize beyond *C. elegans*.

**938A** Peptidergic modulation of the *C. elegans* escape response. *J. T. Florman*, M. J. Alkema

The escape response is a compound motor sequence in which anterior touch triggers a rapid reversal followed by a ventral head bend which leads to the omega turn. The neural circuit that generates this behavior has been well characterized. The neuropeptide FLP-18 is expressed in the AVA and RIM neurons of escape circuit. To examine how neuropeptides modulate the escape circuit we analyzed the escape response of *flp-18* mutants.

In response to touch, *flp-18* mutants have defects in omega turning and show reduced body bending and velocity during...
escape. In contrast, overexpression of FLP-18 increases omega turning and body bending. We found that repeated stimulation of the escape response reduces fluorescence from a FLP-18::Venus translational fusion in the AVA and RIM neurons, suggesting that FLP-18 is released in response.

FLP-18 can activate the G-protein coupled receptor NPR-51,2. Similar to flp-18 mutants, loss of npr-5 results in omega turning, curvature and escape velocity defects. Furthermore, the increased body bending and reversal frequency observed in FLP-18 overexpressing animals is suppressed by loss of npr-5. NPR-5 is expressed in body wall muscle and is reported to couple to Gαq which can increase intracellular Ca2+. We found that overexpression of FLP-18 caused a significant increase in the fluorescent intensity of animals expressing a muscle specific GCaMP6 Ca2+ reporter, which can be suppressed by loss of npr-5. Together this suggests a model in which touch triggers release of FLP-18 from the AVA and RIM which acts on NPR-5 in muscle to increase intracellular Ca2+ levels. Elevated muscle Ca2+ increases body bending which facilitates the omega turn and increases velocity during the escape response.


**939B** Distributed rhythm generators underlie C. elegans forward locomotion. A.D. Fouad1, S. Teng1, J.R. Mark1, A. Liu1, H. Ji1, E. Comblath1, M. Zhen2, C. Fang-Yen1 1) Department of Bioengineering, University of Pennsylvania, Philadelphia, PA; 2) Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Ontario M5G 1X5, Canada.

Coordinated rhythmic movements are ubiquitous in animal locomotory, feeding, and circulatory systems. In many organisms, a chain of neural oscillators underlies the generation of rhythmic waves. In the roundworm *C. elegans*, proprioceptive feedback plays an important role in wave propagation during forward locomotion. However, it remains unclear whether the locomotor circuit contains more than one rhythm generator. Here, we use targeted optogenetic manipulation and laser ablation experiments to show that multiple sections of forward locomotor circuitry are capable of independently oscillating. When we optogenetically inhibited the muscles, cholinergic neurons, or B-type motor neurons just posterior to the head, we found that the head and tail could simultaneously undulate at different frequencies. We refer to this phenomenon, in which the head frequency decreases while the tail frequency simultaneously increases, as two-frequency undulation (2FU). Worms were capable of 2FU despite ablations of or disruptions to the pre-motor interneurons and most classes of ventral nerve cord (VNC) motor neurons, including subsets of the B-type motor neurons, which are associated with forward locomotion. To confirm that the VNC motor neurons can generate rhythms without synaptic input from head motor circuitry, we developed a method to sever the dorsal and ventral nerve cords in adult *C. elegans*. Worms in which both cords have been severed in multiple locations were capable of generating robust waves in the mid-body and higher frequency waves in the tail, suggesting that multiple units within the VNC motor circuit are capable of independent oscillation.

Finally, we address the decrease in head frequency during 2FU, which is not predicted by models that only consider posteriorward coupling. Using rhythmic optogenetic stimuli, we show that an imposed mid-body pattern is capable of entraining the head to a new frequency. This observation suggests that motor coupling in the VNC motor circuit is bidirectional. Taken together, our results show that like the vertebrate spinal cord, the *C. elegans* forward motor circuit contains multiple oscillators that normally coordinate their activity to generate behavior. Our work opens the possibility of a genetic and neural dissection of how rhythmic locomotion is generated, propagated, and modulated.

**940C** Neuronal circuit underlying cultivation temperature-dependent cold acclimation. Satoko Fujii, Makoto Ioroi, Atsushi Kuhara, Akane Ohta  Graduate school of Natural Science, Konan University, Kobe-Shi, Hyogo, Japan.

Temperature acclimation is important for survival and proliferation of animals. The nematode *C. elegans* can survive at 2°C after cultivation at 15°C, however, the animals cultivated at 25°C cannot survive at 2°C. Interestingly, 25°C-cultivated animals are then exposed to 15°C for 3 hours and it comes to survive at 2°C. Thus, the tolerance against 2°C can be plastically changed after cultivation at 15°C, however, the animals cultivated at 25°C cannot survive at 2°C. Interestingly, 25°C-cultivated animals are then exposed to 15°C for 3 hours and it comes to survive at 2°C. Thus, the tolerance against 2°C can be plastically changed.

To identify the specific cells required for CREB-dependent cold acclimation, we expressed *crh-1* cDNA in various sets of neurons in *crh-1* mutant. We found that delayed-cold acclimation of *crh-1* mutant was rescued by the simultaneous expression of *crh-1* cDNA in ASJ and RMG neurons. However, there is no direct physical connection between these two neurons such as GAP junctions and synaptic connections. We are exploring the neuronal circuits from ASJ to RMG in cold acclimation. We analyzed cold acclimation of the mutants defective in
neuropeptide and serotonin signaling, because RMG expresses NPR-1, a neuropeptide receptor, and HSN neuron upstream of RMG is a serotonergic neuron. We analyzed cold acclimation speed of the mutants defective in npr-1, tph-1 or mod-1. TPH-1 is serotonin synthase expressed in HSN, and MOD-1 is serotonin receptor in RMG. npr-1, tph-1 and mod-1 mutants however showed normal cold acclimation, suggesting that serotonin and NPR-1 are not involved in neural signaling from ASJ to RMG in cold acclimation. We are going on the analysis whether other neurotransmitter receptors in RMG, such as glutamate receptor, are required for cold acclimation.

941A The V-ATPase proton pump genetically interacts with the vesicular acetylcholine transporter. M.S. Greenwald1,2,4, G. Ernstrom1,2,3,4 1) Department of Biology; 2) Program in Neuroscience; 3) Program in Molecular Biology and Biochemistry; 4) Middlebury College, Middlebury VT.

Genes involved with neurotransmitter release can be sensitive to gene dosage. Yook et al. (2001) found several cases where mutations in a single copy of two different synapse genes caused a deficiency in neurotransmitter release. Several cases of this nonallelic noncomplementation were reported between proteins on synaptic vesicles and proteins on the pre-synaptic plasma membrane. We tested whether there were similar genetic interactions between genes expressed primarily on synaptic vesicles, namely the V-ATPase proton pump and the vesicular acetylcholine transporter. To test the hypothesis that the V-ATPase proton pump and vesicular acetylcholine transporter genetically interact, we performed aldicarb sensitivity assays on animals that were heterozygous with a loss-of-function allele in a V-ATPase gene [vha-12(n1915)] and heterozygous with a loss-of-function allele in transporter gene [(unc-17(e245)]. We compared the aldicarb sensitivity of these double heterozygotes to single heterozygotes, single homozygotes, and wild-type animals. Consistent with a genetic interaction, we observed nonallelic noncomplementation of aldicarb sensitivity phenotype in the double-heterozygotes. Because Yook et al. found that nonallelicic complementation was specific to synaptic vesicle trafficking and not neurotransmitter synthesis nor synaptic development, our results support a role of the V-ATPase and the transporter in vesicle transport and fusion pathway in addition to their roles in loading neurotransmitters into vesicles.

942B Identification of novel regulators of GABAergic synaptogenesis. Marine GUEYDAN, Bérangère PINAN-LUCARRE, Aurore-Cécile VALFORT, Jean-Louis BESSEREAU Institut Neuromyogène, Université Claude Bernard Lyon 1 - INSERM U1217 - CNRS UMR5310, Villeurbanne CEDEX, Rhône, FR.

In the central nervous system (CNS), the inhibitory system plays a key role in neuronal network excitability. To identify novel genes and mechanisms involved in the formation and regulation of inhibitory synapses, we use the inhibitory GABAergic neuromuscular junction of the nematode C. elegans as a genetically tractable model. At these synapses, fast neurotransmission is ensured by type A ionotropic GABA receptors (GABA\(_A\)R), which form post-synaptic clusters in front of GABAergic buttons.

We performed an unbiased EMS genetic screen based on the visualization of fluorescently tagged GABA\(_A\)R in vivo in a knock-in strain. A second EMS genetic screen has been performed on this knock-in strain in a sensitized background. We identified 56 (31 + 25, respectively) mutants with abnormal GABA\(_A\)R localization among 3,648 (1,728 + 1,920, respectively) mutagenized haploid genomes. For 36 mutants, we used a novel WGS strategy to simultaneously map and identify causative mutations without any prior time-consuming genetic mapping. We found 7 alleles of genes already known to be involved in synaptogenesis, such as genes encoding master regulators of neuronal identity (the transcription factors UNC-30, a Ptx family member, and UNC-3, a COE motif family member), presynaptic organizers (SYD-2/liprin), postsynaptic scaffold proteins (FRM-3, an ERM domain protein, LIN-2/CASK and the netrin receptor UNC-40/DCC). For 10 mutant strains, we are currently completing the validation of candidate genes, which are known to be involved in various cellular processes, such as transcription factors, motor proteins or extracellular matrix components.

In addition, we undertook the functional characterization of a novel candidate gene, tentatively named nsp-3, which encodes an evolutionarily conserved transmembrane protein. An early step in nsp-3 causes a severe reduction of synaptic GABA\(_A\)R content and the presence of small ectopic punctae in muscle cells. We reproduced this phenotype by generating a complete deletion of this locus using CRISPR technology. We are now investigating NSP-3 expression pattern, subcellular localization and role in GABA\(_A\)R trafficking and localization. Though a few publications showed that other members of this family were involved in cellular adhesion, phagocytosis and immune response in Drosophila, nothing is known about the function of these proteins in the CNS. Our data point to novel functions of these proteins in the traffic or synaptic localization of neurotransmitter receptors in the nervous system.

943C Regulation of the deubiquitinating enzyme USP-46 and the glutamate receptor GLR-1 by WD40 repeat proteins. Molly Hodul1,2, Caroline L. Dahlberg3, Peter Juo1,2 1) Neuroscience, Tufts, Boston, MA; 2) Developmental, Molecular, and Chemical Biology, Tufts, Boston, MA; 3) Biology, Western Washington University, Bellingham, WA.

Ubiquitin-mediated endocytosis and degradation of glutamate receptors controls their synaptic abundance and is implicated in modulating synaptic strength, learning, and memory. Ubiquitination of GLR-1, a C. elegans AMPA-type glutamate receptor, promotes receptor internalization and degradation in the lysosome (1). Ubiquitination can be reversed by deubiquitinating enzymes (DUBs). We previously found that the DUB Ubiquitin-Specific Protease 46 (USP-46) regulates GLR-1 levels (2). USP-46 removes ubiquitin from GLR-1 and likely acts at endosomes to protect GLR-1 from lysosomal degradation. Consistently, usp-46 null mutants have decreased GLR-1 at synapses in the ventral nerve cord (VNC) and corresponding defects in GLR-1-dependent behaviors (2). In contrast, overexpression of usp-46 does not have the converse effect, suggesting that USP-46 activity is tightly controlled in neurons. Very little is known about how DUBs are regulated in vivo.

Two WD-40 repeat proteins, WDR-48 and WDR-20, were previously shown to interact with USP-46 in mammalian cells (3-5). We showed that the C. elegans homologs of these WDR proteins bind USP-46 and stimulate DUB catalytic activity in vitro (6).
Overexpression of \textit{wdr-48} and \textit{wdr-20} in neurons \textit{in vivo} results in increased synaptic levels of GLR-1, and this effect is further enhanced by co-expression of \textit{usp-46} (6). Here we investigate several mechanisms by which the WDR proteins regulate USP-46 and GLR-1. First, we found that overexpression of \textit{wdr-48} and \textit{wdr-20} increase the abundance of USP-46 in HEK cells and in neurons \textit{in vivo}. We will take advantage of point mutations known to disrupt the binding between each WDR and USP-46 to test if their direct interaction is required for the increased levels of USP-46. Second, we have preliminary evidence suggesting that WDR-48 recruits USP-46 to endosomes where it is presumed to act on ubiquitinated GLR-1. Finally, we recently found that \textit{wdr-20} but not \textit{wdr-48} transcription is regulated by chronic synaptic activity blockade, which has previously been shown to increase synaptic GLR-1 (7). Thus, WDR-48 and WDR-20 are emerging as key regulators that appear to use multiple mechanisms to control USP-46 and its effects on GLR-1.

2. Kowsalski et al. (2011) J Neurosci
4. Cohn et al. (2009) JBC
5. Kee et al. (2010) JBC
6. Dahlberg & Joo (2014) JBC
7. Grunwald et al. (2004) PNAS


Caenorhabditis elegans have an ability to exhibit isothermal behavior up to a remarkable precision of a 0.05 degree difference. However, the neural circuitry underlying isothermal behavior remains elusive as it is very difficult to track neuronal activity in freely moving specimen. Furthermore, the computational power of their nervous system is often overlooked due to the small number of neurons - hundreds compared to a mammal’s billions. Isothermal tracking appears to be a deterministic behavior that may be a product of a more complex processing system - a behavior facilitated by a network. There is strong evidence sensorimotor integration is occurring; the C. elegans are tracking temperature differences and coordinating their movements to the local temperature in a highly deliberate and precise manner. In this experiment, we created an assay plate over a thermal gradient and physically tracked the worm’s movements using a high-powered line confocal microscope with a motorized stage and fluorescently labeled neurons. We were able to track the neural activity of the whole nervous system in real time as the worm moved over the thermal gradient. This eliminates the need for laser ablation or immobilization and allows us to observe how a Caenorhabditis elegans would move in its natural form. We conducted this experiment with worms in the L1 stage as well as worms in the L4 stage to observe possible synaptogenesis or synaptic plasticity.

\textbf{945B} Connecting the dots: A multimodal neuronal circuit integrates sensory information to control lipid metabolism. R. Hussey\textsuperscript{1,2}, E. Vanstrum\textsuperscript{2}, S. Srinivasan\textsuperscript{1,2} 1) Dorris Neuroscience Center, The Scripps Research Institute, La Jolla, CA; 2) Department of Molecular Medicine, The Scripps Research Institute, La Jolla, California, USA.

In relation to fat metabolism and energy balance, the central nervous system plays a more intricate role than historically thought. The regulation of energy expenditure is controlled by complex interactions between environmental, genetic, and behavioural factors; through pathways that are not fully understood. We have discovered three neuronal pathways regulating intestinal fat in \textit{C. elegans} that require the secretion of a tachykinin orthologue, the neuropeptide FLP-7, from ASI neurons to relay information to the intestine. Neurons involved in the oxygen-sensing, pheromone-sensing and serotonergic signalling pathways appear to influence each other to ultimately determine the secretion of FLP-7. In the ASI neurons, the neuronal AMPK subunit AAK-2 plays an instructive role in this process. The extent of circulating FLP-7 then controls the utilisation of lipids in the intestinal cells. Thus we believe these neurons are working in a unified circuit that integrates distinct environmental cues that determine the optimal use of fat stores.

To understand the interactions within this circuit we have developed transgenic lines that allow us to chem- and optogenetically activate or silence neurons of choice within our circuit. We will then measure the resulting activity in downstream neurons using live calcium imaging, and secretion of FLP-7 from the ASI neurons using our coelomocyte-uptake assay. Through these studies, we aim to understand, at a mechanistic level, a complex neuronal circuit that integrates multiple graded sensory inputs to ultimately determine the conversion of stored fat to energy.

\textbf{946C} Mathematical methods to analyze imaging data in the whole central nervous system: From noise reduction to causality detection. Y. Iwasaki\textsuperscript{3,7}, T. Teramoto\textsuperscript{2,7}, S. Oe\textsuperscript{2,7}, T. Tokunaga\textsuperscript{3,7}, O. Hirose\textsuperscript{5,7}, S. Wu\textsuperscript{5,7}, Y. Toyoshima\textsuperscript{6,7}, M. S. Jang\textsuperscript{7}, R. Yoshida\textsuperscript{5,7}, Y. lino\textsuperscript{6,7}, T. Ishihara\textsuperscript{2,7} 1) Ibaraki University, Japan; 2) Kyushu University, Japan; 3) Kyushu Institute Technology, Japan; 4) Kanazawa University, Japan; 5) Institute of Statistical Mathematics, Japan; 6) University of Tokyo, Japan; 7) JST CREST, Japan.

\textit{C. elegans} is a useful model organism to study the whole nervous system function because of the following reasons. Firstly, the wiring diagram of all the 302 neurons is anatomically determined. Secondly, recent development in high-speed (3+1)-dimensional imaging technique enables us to measure the neural activity in the whole central nervous system. The neural activities of about 170 neurons in the head region are detected as a worm lives. Function estimated from the imaging data is able to be discussed together with the identified synaptic pathway.
On the basis of statistical models, we carry out time series analysis for our imaging data in the whole central nervous system. The first important step in the process of time series analysis is to remove "noise" in the data. Result of the following time series analysis depends on the noise reduction. We use a Bayesian noise reduction method. Once an information criterion for model selection is given, neuronal signal and noise (level) are separately determined without an artificial judgment. Therefore, the signal-to-noise ratio is calculated to estimate the reliability of each neuronal signal.

The correlation coefficient is frequently used to quantify undirected influence between two neuronal signals. In the central nervous system of C. elegans, there are 395 bidirectional neuron pairs in which two neurons are connected by chemical synapses and/or gap junctions. In addition, there are 925 unidirectional neuron pairs in which chemical synapses unidirectionally transmit the signal from one neuron to another neuron. To determine directional signaling pathways from the neuronal signals, causality analysis is carried out. We apply the Granger causality test to quantify directed influence ("predictive causality") between two neuronal signals. According to a given statistical significance, all pairs of the neuronal signals are classified as unidirectional causality pair, bidirectional causality pair or no causality pair. The causal ("functional") pathways are partially different from the identified synaptic pathways in C. elegans. Some causalities are derived from detour routing through the third neuron but not more neurons. In other words, the neuronal signals are caused by the synaptic pathways within the second nearest neighbor distance. The transfer entropy, which is another causality analysis method based on information theory, is also discussed. This work was supported by JST CREST (No. JPMJCR12W1).

947A  Mechanisms of holding memory of butanone enhancement of C. elegans.  N. Iwase1, E. Sawatari1,2, M. Fujiwara1, T. Ishihara1  1) Graduate School of System Life Science, kyushu University, Fukuoka, JP; 2) Division for Experimental Natural Science Faculty of Arts and Science, kyushu.

Forgetting is important for animals to adapt themselves to changing environments. To investigate mechanisms of the forgetting, we use an olfactory learning in C. elegans. When C. elegans is exposed to butanone under a condition with enough food, the response to butanone is enhanced to show stronger chemotaxis (butanone enhancement). When they are cultivated on food without butanone after the butanone enhancement, the chemotaxis to butanone is much weaker than that before the conditioning. This phenomenon cannot be explained as a model of simple forgetting. Therefore, we hypothesized that the old memory is overwritten by the new memory (overwriting memory).

To test this hypothesis, we observed a change of chemotaxis to butanone after butanone enhancement for 20 hours by using a wild type, a mutant and interneuron ablated animals. The snt-3 mutant and the AIB interneuron ablated animal, in which chemotaxis to butanone is not weakened after butanone enhancement. In the wild type, the chemotaxis index after butanone enhancement was much lower than that before the conditioning. In addition, the chemotaxis to butanone become weak like naïve animals within one day. This result suggests that this phenomenon can be considered as overwriting memory and these animals forget the overwritten memory after 20 hours. In the snt-3 mutant, the enhanced chemotaxis to butanone was slowly weakened than that of wild type. This result suggests that the snt-3 gene affects the regulation of overwriting memory. In the AIB ablated snt-3, chemotaxis to butanone after butanone enhancement was slowly weakened like AIB ablated wild-type animals and snt-3, suggest that AIB neurons and snt-3 may regulate the overwriting memory in the same pathway.

Naïve gcy-28 mutants show weak avoidance of butanone, whereas after the conditioning, they show strong attractive response to butanone. To reveal function of gcy-28 in overwriting memory, we observed a change of the chemotaxis to butanone on food after butanone enhancement for 20 hours by using the gcy-28 mutant. In gcy-28 mutant, the chemotaxis to butanone after butanone enhancement was weakened than that of wild type and that chemotaxis is not recovered after 20 hours. This result suggests that gcy-28 affects forgetting of the overwritten memory.

Our studies on after butanone enhancement suggest that, in C. elegans, some memories can be overwritten after learning when they are exposed to new environments. Our studies will reveal its mechanism.

948B  Identification of neurons and analysis of the neural circuit involved in the learned salt-avoidance behavior in C. elegans.  M.S. Jang1,2, Y. Toyoshima1,2, H. Kunitomo1,2, Y. Iino1,2  1) Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, JP; 2) CREST, Japan Science and Technology Agency, Tokyo, Japan.

Animals flexibly adapt to the environment by modifying their behavior, which depends on the plasticity of neural network. Here, we focused on the functional plasticity of neural circuits of Caenorhabditis elegans that sense salts and generate taxis behaviors.

C. elegans shows a form of associated learning in which availability of food and salt concentration are associatively memorized. Worms are attracted to the salt concentration at which they have been cultured under well-fed conditions, whereas they avoid it if they have been cultivated under starvation, which hereafter we call salt avoidance learning. The ASER gustatory neuron is known to sense salt and has a major function for salt chemotaxis plasticity. Inputs to the ASER gustatory neuron are sufficient for the salt chemotaxis plasticity under well-fed conditions. However, other sensory neuron(s) in addition to ASER neuron were suggested to be required for salt avoidance learning.

To identify the sensory neurons required for salt avoidance learning, we conducted cell-specific rescue experiments using the dysesthesia mutant dyf-11, whose sensory endings are dysfunctional so that these animals are unable to sense water-soluble compounds. As a result, we found that dyf-11 mutants with functionally recovered ASG neurons in addition to ASER showed the salt avoidance learning, but recovery in either ASER or ASG alone did not. Behavioral analyses revealed that the frequency of the turning events upon salt concentration change was recovered in animals whose ASER and ASG were rescued. Moreover, silencing ASG reduced salt chemotaxis after starvation. Unexpectedly, the ASG neurons did not respond to salt stimuli under
any conditions we have tested. These results suggest that the ASG neurons have functions to regulate salt chemotaxis behaviors under starved conditions without responding to the salt stimuli themselves.

949C Molecular determinants of ionotropic GABA receptor desensitization. A.K. Jawad¹, A.A. Beg²

In the nervous system, fast neurotransmission is mediated by ‘cys-loop’ pentameric ligand-gated ion channels. This family of receptors is made up of γ-aminobutyric acid (GABA), nicotinic acetylcholine (nAChRs), glycine (GlyRs), and serotonin type 3 (5HT3R) receptors. Neurotransmitter binding transitions these receptors between resting and open states, where ions selectively flow through an integral membrane pore which causes excitation or inhibition of the postsynaptic cell. Following activation, receptors enter a desensitized state in which the ion channel closes even in the sustained presence of saturating agonist. Receptor desensitization is a fundamental process that can regulate the frequency, duration, and amplitude of synaptic and non-synaptic responses. Importantly, mutations that alter desensitization kinetics are known to be associated with numerous neurological diseases. Structure-function and X-ray crystallographic data have provided valuable mechanistic insight into the properties of agonist binding and receptor activation. However, the molecular determinants regulating ‘cys-loop’ receptor desensitization are poorly understood. Here, we investigate two unique homopentameric GABA receptors (EXP-1 and LGC-35) in C. elegans that exhibit high sequence identity but strikingly different desensitization kinetics. Electrophysiological characterization of heterologously expressed receptor chimerae and point mutants reveal a key residue (I357) within transmembrane domain 2 (M2) of EXP-1 that mediates the differential desensitization kinetics. We used a series of point mutants at this site to gain a better understanding of the role this residue plays in the overall desensitization process. Together, these data will contribute to a better understanding of the molecular determinants that mediate ‘cys-loop’ receptor desensitization.

950A Bacterial diet modulates clonic seizure behavior in a C. elegans model for migraine. W. Kang, M. Alkema

Diet plays an important role to maintaining brain function and mental health. However, how dietary factors affect neural function is still poorly understood. We examined the impact of different bacterial diets on a C. elegans model for voltage-gated calcium channelopathy that causes migraine in humans. Our laboratory has previously identified a gain-of-function (gf) mutation in the unc-2 gene, which encodes the a1 subunit of presynaptic VGCCs in C. elegans. The unc-2(gf) mutants have an increased synaptic transmission and display a clonic seizure behavior characterized by dramatic increase in the number of reversals. We found that Comamonas aquatica diet can suppress the clonic seizure behavior of the unc-2(gf) mutants compared to the standard E. coli diet. Comamonas, in contrast to E. coli, produces vitamin B12. We found that supplementation of vitamin B12 to the E. coli diet decreased the reversal frequency of unc-2(gf) mutants to the same extent as the Comamonas diet, but not that of wild type animals. Conversely, a diet of vitamin B12-deficient mutant Comamonas failed to decrease reversal frequency of unc-2(gf) mutants. Vitamin B12 also suppressed the increased synaptic transmission at the neuromuscular junction observed in unc-2(gf) mutants. These findings show that vitamin B12 produced by Comamonas drives the beneficial effects of this diet on seizure behavior. Interestingly, vitamin B12 supplementation has been reported to also reduce the frequency and severity of headaches in migraine patients. By generating double mutants of unc-2(gf) and several vitamin B12-responsive metabolic genes, we are investigating which of these metabolites mediate the suppression of the unc-2(gf) seizure behavior. This will further reveal the role of these pathways in synaptic transmission and neural function in C. elegans. Our study will provide further understanding of the complex interactions between diet, gut microbiota, metabolism, and brain function.

951B Characterization of chromatin remodeling factors that regulate cholinergic signaling at the neuromuscular junction. K. Kervin, T. Chaya, J. Tanis

Formation and maintenance of the neuromuscular junction (NMJ), regulated synthesis, release and breakdown of acetylcholine (ACh) and precise clustering, and function of AChRs is required for proper neuromuscular transmission. Defects in cholinergic signaling at the NMJ are the underlying cause of muscle weakness observed in myasthenic syndromes. The C. elegans body-wall muscles (BWM) are functionally comparable to vertebrate skeletal muscles and provide an excellent model for the study of molecular mechanisms that regulate post-synaptic signaling at the NMJ. Signaling through ACh and GABA receptors results in contraction and relaxation of the BWM respectively, enabling coordinated locomotion. Exposure to levamisole, a nicotinic AChR agonist, causes time-dependent, hyper-contracted paralysis. A previously reported, forward genetic screen for levamisole resistance identified mutations in 13 different genes⁴, however, mutants with weak resistance or hypersensitivity were not isolated. To identify novel genes required for post-synaptic cholinergic signaling, we performed a genome-wide RNA interference (RNAi) screen for gene knockdowns that cause altered levamisole response. We developed a liquid levamisole swim assay to enable high-throughput screening and screened animals at two time points to identify hypersensitivity and resistance. We screened all 17,469 clones in the RNAi library in duplicate and identified 156 gene knockdowns that caused altered levamisole response. Of these, 138 exhibited hypersensitivity to liquid levamisole. Multiple genes identified in our screen, including epc-1, his-74, lin-53, taf-1, and trr-1, encode proteins that are involved in chromatin modification. We performed time course assays and confirmed that knockdown of these genes results in levamisole hypersensitivity. This led us to hypothesize that epigenetic changes may affect post-synaptic signaling at the NMJ. To ascertain how chromatin remodeling alters neuromuscular transmission, we are establishing the mechanism by which these gene knockdowns regulate post-synaptic signaling. We are gaining insight into the role of altered gene expression induced through life experiences, by determining if chromatin modifications result in changes in NMJ function. Our work aims to establish a novel mechanism of cholinergic signaling regulation at the NMJ.

References:
952C Dissecting the function of acetylcholine in the *C. elegans* egg-laying behavior circuit. R. Kopchock, E. Arvelo, P. Garcia, W. Coffey, K. Collins Department of Biology, University of Miami, Coral Gables, FL.

Egg laying in the nematode *C. elegans* alternates between an active and inactive behavior state. The active phase is initiated by the HSN neurons that release serotonin onto postsynaptic vulval muscles to increase their excitability. The vulval and body wall muscles are also innervated by the cholinergic Ventral Type C (VC) motor neurons whose activity is coincident with vulval muscle contraction and egg laying. Our research aims to dissect the function of the VC neurons and acetylcholine signaling within the egg-laying circuit. To do this, we are using mutants and transgenes to manipulate release of acetylcholine from the VCs and its signaling through both fast-acting nicotinic receptors expressed on the vulval muscles and slow-acting muscarinic receptors expressed on the uv1 neuroendocrine cells. We find that blocking VC neurotransmitter release using tetanus toxin or histamine-gated chloride channels has no gross effect on egg laying. We also find that optogenetic activation of the VCs using Channelrhodopsin-2 fails to induce egg laying, but instead hypercontracts the body wall muscles and stops locomotion. Since active phases of egg laying are accompanied with changes in locomotor activity, the activity of VCs may serve to coordinate locomotor changes with egg release. Worms with silenced VCs also exhibit a ‘kinker’ locomotion phenotype, especially when reversing. To further study this phenotype we looked at *lin-39(n1760)* mutants, in which the VCs die by apoptosis. *lin-39(n1760)* mutants show a locomotion phenotype that resembles the kinked locomotion seen in animals with silenced VCs. We find that individual egg-laying events are accompanied by a slowing of locomotion, a response that is diminished when the VCs are silenced. Recent work has identified a muscarinic receptor, GAR-2, that is expressed in uv1 and predicted to signal through Go to regulate release of tyramine and neuropeptides that inhibit HSN activity. Because egg release mechanically activates the uv1 neuroendocrine cells, we found that acetylcholine released from VCs during vulval muscle contraction primes the uv1s to respond to passage of eggs through the vulva during egg laying. Together, our results suggest that the VCs are involved in coordinating general locomotor behavior in adult animals as well as slowing locomotion at the moment of egg release.

953A The G protein-coupled receptor FSHR-1 is a candidate APC substrate that controls excitatory to inhibitory signaling balance at the *C. elegans* neuromuscular junction. Sarah Olofsson, Alyson Munneke, Amy Godfrey, Julie Kolnik, Erica Damler, David Ryszamp, Jennifer Kowalski Biological Sciences, Butler University, Indianapolis, IN 46208.

Regulation of excitatory to inhibitory synaptic signaling (E/I balance) is critical for nervous system function, and E/I imbalances occur in neurological disorders. G protein-coupled receptors (GPCRs) are common mediators of neuronal signaling, and many GPCRs modulate synaptic transmission. We are investigating a role for the conserved GPCR and glycopeptide hormone receptor FSHR-1 as a regulator of the *C. elegans* neuromuscular junction (NMJ), where a balance of excitatory cholinergic and inhibitory GABA signaling from motor neurons controls muscle activity. FSHR-1 controls several physiological processes in *C. elegans*, including innate immunity, germline differentiation, and stress responses. FSHR-1 also affects cholinergic signaling for muscle excitation and may be a neuropeptide receptor; yet, the mechanism and sites of FSHR-1 activity at the NMJ are unknown. We identified FSHR-1 as a candidate substrate of the anaphase-promoting complex (APC), an ubiquitin ligase that promotes GABA signaling at the NMJ. FSHR-1 has an APC recognition motif and *fshr-1(ok778)* loss of function (lf) mutations, which cause decreased muscle activity, suppress muscle excitation in APC*lf* animals. Re-expression of *fshr-1* in all neurons or only GABA neurons (where APC acts) restores wild type muscle activity to *fshr-1* mutants, as does *fshr-1* expression in cholinergic neurons. FSHR-1 signaling likely involves Gs and adenylyl cyclase, as gain of function (gf) mutations in *gsa-1* (ce81) and *acy-1* (md1756), which promote muscle excitation, suppress the reduced muscle excitation of *fshr-1* mutants. Neuronal activation of this pathway is important, as overexpression of an *acy-1* gf construct only in neurons suppresses *fshr-1* if phenotypes, while *acy-1* gf only in GABA neurons gives partial suppression. Fluorescently tagged synaptic vesicles accumulate at cholinergic presynapses, indicative of decreased acetylcholine release, and at GABA presynapses of *fshr-1* mutants, which may result from decreased cholinergic drive. GABA neuron-specific restoration of *fshr-1* fails to rescue the synaptic vesicle accumulation of *fshr-1* mutants, suggesting rescue of muscle contraction by GABA neuron-specific *fshr-1* may be due to FSHR-1 indirectly influencing muscle activity. Current work will determine FSHR-1 function in each motor neuron class, including using CRISPR to identify sites of endogenous *fshr-1* expression, and evaluate the APC-FSHR-1 relationship and FSHR-1-neuropeptide interactions.

954B Identification of a Plasma Membrane Ca\(^{2+}\) ATPase as a Regulator of Acetylcholine Signaling in *C. elegans*. A. Lam, T. Chaya, J. Tanis Biological Sciences, University of Delaware, Newark, DE.

Motor neurons release the neurotransmitter acetylcholine (ACh) at the neuromuscular junction (NMJ) resulting in skeletal muscle contraction. Defects in cholinergic signaling at the NMJ can lead to the development of neuromuscular diseases. The body wall muscles of *C. elegans* are functionally comparable to vertebrate skeletal muscle and are an excellent model for the study of post-synaptic cholinergic signaling. Coordinated body movements result from excitatory signaling through ACh receptors (ACHRs) and inhibitory signaling through GABA receptors, which causes muscle contraction and relaxation, respectively. Exposure to the AChR agonist levamisole constitutively activates postsynaptic AChRs, leading to hypercontracted paralysis. A forward genetic screen for strong resistance to levamisole identified mutations in 13 different genes. However, mutants with weak resistance or hypersensitivity to levamisole as well as those with sterile and/or lethal phenotypes were not isolated. To identify additional factors that regulate cholinergic signaling at the NMJ, we conducted a genome wide RNAi screen for altered levamisole response and identified 18 gene knockdowns that resulted in resistance and 138 that caused hypersensitivity to levamisole. Knockdown of *mca-3*, which encodes a plasma membrane Ca\(^{2+}\) ATPase required for extrusion of intracellular Ca\(^{2+}\) led to levamisole resistance. This was unexpected, as we would predict that an increase in intracellular Ca\(^{2+}\)
resulting from loss of mca-3 would likely lead to hypersensitivity. A second protein, the Na+/Ca\(^{2+}\) exchanger NCX-2 is also important for efflux of intracellular Ca\(^{2+}\). To determine the effect of loss of mca-3 and ncx-2 on response to levamisole, we knocked down the genes independently and together and performed time-course assays, counting the number of worms moving in 0.4 M liquid levamisole every five minutes for 60 minutes. Knockdown of mca-3 led to significant levamisole resistance, however, loss of ncx-2 resulted in levamisole response indistinguishable from the empty vector control. Loss of both mca-3 and ncx-2 resulted in levamisole resistance not significantly different from the mca-3 knockdown. These results suggest that the mechanism by which loss of mca-3 causes altered levamisole response may be independent of its role in Ca\(^{2+}\) efflux.

References:


955C  Identification of an auxiliary protein that modifies the function of NMDA-type ionotropic glutamate receptors.  Ning Lei, Jerry E. Mellem, Penelope J. Brockie, David M. Madsen , Andres V. Maricq  Department of Biology, University of Utah, Salt Lake City, UT.

The NMDA subtype of postsynaptic ionotropic glutamate receptors (iGluRs) function as molecular coincidence detectors, have critical roles in cellular models of learning and memory, and are associated with a variety of neurological and psychiatric disorders. To date, no NMDA receptor (NMDAR) auxiliary proteins that modify receptor function have been reported. This is in outstanding contrast to the non-NMDA subtypes of iGluRs for which multiple auxiliary proteins have been described. C. elegans expresses only two NMDAR subunits that are required for NMDA-mediated current in vivo, but insufficient to reconstitute current in heterologous cells. We therefore undertook a genetic screen to identify gene products required for NMDAR function. We identified an auxiliary protein that is expressed in presynaptic neurons and associated with postsynaptic NMDARs. In the absence of the auxiliary protein, NMDAR-dependent behaviors are altered and NMDAR-mediated currents are not detected. Heterologous expression studies in Xenopus oocytes demonstrate that NMDA-gated current can only be recorded from oocytes that co-express the auxiliary protein along with the NMR-1 and NMR-2 NMDAR subunits. Our studies reveal a novel mechanism for controlling NMDAR function and synaptic transmission.

956A  The UNC-7 innexin coordinates vulval muscle contraction and egg release.  C. Li, K. Collins  University of Miami, Coral Gables.

Gap junctions are intercellular connections needed for cellular communication and exchange. We have been investigating how gap junctions drive coordinated activity between cells in developing and mature neural circuits using the egg-laying behavior circuit as a model system. Gap junctions are thought to facilitate the coordinated activity of neurons and the muscle cells they innervate. Gap junctions are of particular interest because they are thought to be targets of neuromodulators like serotonin whose dysfunction is implicated in human depression.

We hypothesize that gap-junction proteins regulate egg-laying circuit activity and behavior. Previous work has shown that unc-7 and unc-9 mutants have uncoordinated (unc) locomotion and are resistant to serotonin-induced egg laying. I found that unc-7 or unc-9 mutants have hyperactive egg-laying behavior. The locomotion and egg-laying behavior phenotypes resemble mutants with defective acetylcholine signaling from the presynaptic VC motor neurons that innervate the vulval muscles. To test this hypothesis, I have begun using Ca\(^{2+}\) imaging to quantify changes in cell activity in behaving animals. Wild-type animals show coordinated vulval muscle activity with strong egg-laying Ca\(^{2+}\) transients that allow release of eggs from the uterus within ~1 second. In contrast, unc-7 mutants show uncoordinated vulval muscles, with eggs often getting stuck in the contracting vulva. Eggs are finally released after ~30 seconds of sustained, tetanic muscle contraction. unc-9 mutants do not show as dramatic of an egg-laying or vulval muscle Ca\(^{2+}\) defect. To show where unc-7 and unc-9 function, I will re-express UNC-7 or UNC-9 in the VC neurons and look for a restoration of normal circuit activity and behavior. I hope to also perform dominant-negative experiments to see if inactivation of UNC-7 in specific cells of the egg-laying circuits recapitulates the null mutant phenotype. This work will begin to address how gap junction proteins like UNC-7 promote the proper development and function of neural circuits to execute a coordinated behavior.

957B  Fine tuning of a motor behavior by a dual role interneuron.  Z. Li1, T. Yu1,2, J. Zhou1,2, K. Wani1, BJ. Piggott1, J. Liu1, S. Xu1  1) Life Sciences Institute, University of Michigan, ANN ARBOR, MI; 2) College of Life Science and Technology, Collective Innovation Center for Brain Science and Key Laboratory of Molecular Biophysics of MOE, Huazhong University of Science and Technology, Wuhan, Hubei 430074, China.

Animals produce an incredible repertoire of motor actions. How genes and neural circuits regulate motor behaviors is poorly understood. Using C. elegans locomotion behavior as a model, by employing multidisciplinary approaches including optogenetics and in vivo calcium imaging, we found that a single neuron RIM can both promote and suppress reversals during locomotion. Activation of RIM by optogenetics triggers reversals, and this promotion effect is mediated by the command interneurons AVA/AVE and gap junctions connecting AVA/AVE and RIM. By contrast, blocking chemical transmission in RIM increases reversal frequency, indicating that chemical transmitter release from RIM inhibits reversal initiation. Our findings suggest that motor behaviors are fine-tuned by motor circuits, and a single neuron in the circuitry may have complex roles in the regulation of motor output.
958C Network analyses and experimental approaches for identification of function of an interneuron in *Caenorhabditis elegans*. D.S. Lim1, H. Kang2, J. Kim3, E. Shlizerman4, N. Park2, J. Lee1 1) School of Biological Sciences, Seoul National University, Seoul, Republic of Korea; 2) Department of School of Electrical and Computer Engineering, Seoul National University, Seoul, Republic of Korea; 3) Department of Applied Mathematics and Electrical Engineering, University of Washington, Seattle, WA.

With its neurons and synapses identified, the connectome of *Caenorhabditis elegans* nervous system has become a decent platform to apply network analysis to understand structure and function of a neural system. Analysis of *C. elegans* connectome through community detection algorithm with inclusion of neurobiological information such as neuronal bilateral symmetry, inhibitory synapses and informational significance of gap junctions revealed 11 cluster modules, most of which has been experimentally identified. The analysis also identified PVP neuron, an interneuron with no known behavioral function, as a hub neuron of a cluster module with unidentified function. Simulations through *C. elegans* neural interactome, a time-lapse visualization of neuronal dynamics on *C. elegans* connectome, showed a potential role of PVP neuron in locomotion and oxygen sensing. Currently we seek to experimentally identify the role of the PVP-hubbed neural cluster module by genetic modulation and laser ablation of PVP neurons. Successful observation of related *in vivo* phenotypes of PVP neuron-ablated animals will demonstrate significance of neural network studies in neurobiology.

959A Characterizing *C. elegans* whole brain responses to odor sequences. A. Lin1,2, V. Venkatchalam1,2, W. Hung3, M. Wu3, M. Zhen3,4, A. Samuel1,2 1) Department of Physics, Harvard University, Cambridge, MA; 2) Center for Brain Science, Harvard University, Cambridge, MA; 3) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; 4) Departments of Molecular Genetics and Physiology, University of Toronto, Toronto, ON, Canada.

Complex animal behaviors arise in response to sensory inputs. These inputs from sensory neurons are processed and interpreted by networks of neurons in the brain of the animal. This system then uses motor neurons to command physical behavior. The computational pathway from sensory input to behavioral output is a complex and dynamic one, governed by neuronal activity. To understand these computational processes, studying the activity of a single neuron at a time is insufficient—quantitative brain-wide recording of neuronal activity is required. In this work, we perform brainwide imaging in *C. elegans* housed in a new multichannel microfluidic odor delivery system. This allows us to monitor whole brain responses of individual animals in response to a battery of up to thirteen different individual odors or odor mixtures. Single datasets from these experiments allow us to explore odor coding by sensory neurons, consolidation by interneurons, and fictive motor responses (encoded by command motor neurons). Observing the dynamics of these neurons in response to odors of varying identity and strength have allowed us to build a quantitative and comprehensive picture of the way the olfactory system in *C. elegans* consolidates high-dimensional sensory information into behavioral response.

960B Antidromic-rectifying gap junctions amplify chemical transmission at functionally mixed electrical-chemical synapses. P. Lu1, B. Chen1, R. Mailler2, Z. W. Wang1 1) Department of Neuroscience, UConn Health, Farmington, CT; 2) Department of Computer Science, University of Tulsa, Tulsa, OK.

Neurons communicate through chemical synapses and electrical synapses (gap junctions). Although these two types of synapses often coexist between neurons, little is known about whether they interact, and whether any interactions between them are important to controlling synaptic strength and circuit functions. In *C. elegans* escape circuit, a pair of command interneurons (AVA) contact downstream A-type cholinergic motoneurons (A-MNs) through both chemical and electrical synapses. In this study, we took advantage of our recent success in performing paired voltage- and current-clamp recordings with *C. elegans* neurons and the high degree of genetic amenability of the worm to investigate how electrical and chemical synapses interact to control synaptic transmission and an escape behavior. We found that chemical transmission from AVA to A-MNs is mediated by acetylcholine and an LGC-46 postsynaptic receptor; A-MNs also possess an ACR-14 autoreceptor that facilitates neurotransmitter release; the gap junctions between AVA and A-MNs are formed by UNC-7 innexin in AVA and UNC-9 innexin in A-MNs and only allow antidromic current; and disrupting either the electrical or chemical transmission modality by AVA-specific unc-7 knockdown or A-MN-specific unc-9 knockdown causes defective escape response. Importantly, we found that disrupting the antidromic-rectifying gap junctions inhibits chemical transmission whereas disrupting the chemical synapses has no effect on the electrical coupling. These results show that gap junctions may serve as an amplifier of chemical transmission between neurons with both electrical and chemical transmission modalities. The use of antidromic rectifying gap junctions to amplify chemical transmission is potentially a conserved mechanism in circuit functions.

961C How an asymmetric L1 motor circuit generates symmetrical motor output. Y. Lu1,2, A. Guan1,2, D. Witvliet1,2, B. Mulcahy1, J. Meng1, J. Meng1, Q. Wen1, A. Samuel1, M. Zhen1,2 1) Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON M5G 1X5, Canada; 2) Departments of Physiology and Molecular Genetics, University of Toronto, Toronto, ON MSS 1A8, Canada; 3) Center for Brain Science, Harvard University, Cambridge, MA 02138, USA; 4) School of Life Sciences, University of Science and Technology of China, Hefei, Anhui Province 230027, China.

Throughout development, *C. elegans* maintains serpentine-like dorsal/ventral bending waves for locomotion. In the adult motor circuit, the cholinergic A- and B-type motor neurons innervate and stimulate dorsal and ventral body wall muscles, whereas the D-type GABAergic motor neurons, receiving inputs from A- and B-type motor neurons, innervate and inhibit muscles on the opposing side. Together, they constitute symmetric motor circuit input to the dorsal and ventral body wall muscles that generates balanced muscle activity (f).

The cellular components of motor circuit, however, differ drastically in younger animals. All ventral muscle innervating motor neurons are not born until the mid-first larval stage (L1). Partial EM reconstruction in a previous study (2) showed that in L1 stage, the A- and B-type motor neurons only innervate dorsal body wall muscles, and D-type motor neurons innervate only...
ventral muscles. It remains unclear when the transition to the adult motor circuit synaptic wiring completes. Regardless, it is difficult to explain how such a circuit can produce the symmetric ventral/dorsal bending that constitutes the undulatory motor behavior.

We have undertaken anatomical and functional analyses of the L1 motor circuit. I will present studies that lead to the exclusion of multiple potential mechanisms, and the potential involvement of additional, uncharacterized cells in facilitating ventral muscle contraction in the L1 stage animals.

References

962A The VER-4/VEGF receptor-related protein regulates GLR-1 glutamate receptors and related behaviors. E.S. Luth, C. Riccio, K. Markoja, P. Juo Developmental, Molecular, and Chemical Biology, Tufts University School of Medicine, Boston, MA.

Glutamate synapses are highly dynamic, and their restructuring provides the cellular basis of learning and memory. Failure to properly form or maintain glutamate synapses can lead to a variety of neurological disorders, and controlling their abundance and activity is critical for maximizing functional recovery after stroke. However, the molecular mechanisms that regulate the formation and function of these connections in vivo during normal nervous system development are not well understood. Expression of channelrhodopsin in the glutamatergic sensory neuron ASH enabled us to combine optogenetic control of a simple glutamate-dependent behavior, the nose-touch response, with a focused RNAi screen to identify novel genes important for glutamate synapse function. RNAi knockdown of ver-4, a VEGF receptor homolog, resulted in a strong defect in ASH-stimulated glutamate-dependent locomotor reversals, but did not affect overall glutamate-independent locomotion based on thrashing assays. Analysis of worms with loss of function mutations in ver-4 confirmed these RNAi results. The intensity and density of the presynaptic vesicle marker RAB-3::mCherry, expressed specifically in ASH, was unaltered in ver-4 mutants. In contrast, we observed widespread alterations in the size and intensity of GLR-1::GFP puncta in the nerve ring and ventral nerve cord of ver-4 mutants, suggesting that VER-4 globally affects GLR-1 levels. Confocal imaging of GLR-1 tagged with both mCherry and pH-sensitive superreceptor pHluorin (SEP::mCherry::GLR-1) indicated that ver-4 mutants exhibit a preferential loss of cell surface GLR-1. Consistent with this data, ver-4 mutants suppressed the increase in glutamate-dependent spontaneous reversals observed in animals expressing a constitutively-active form of GLR-1. Mutants lacking the VER-4 ligand PVF-1 exhibited defects in the nose-touch response suggesting that ligand binding may be required for VER-4-mediated promotion of glutamate signaling. Together, our data identify VER-4 and its ligand as novel regulators of GLR-1 trafficking and signaling. Future experiments will identify the cellular and subcellular site of action of VER-4 and the mechanism by which PVF-1 and VER-4 regulate GLR-1 trafficking.

References:

963B The C. elegans sarcoplasmic reticulum calcium-ATPase regulates nicotinic acetylcholine receptor ACR-16 expression. Ashley A. Martin, Janet E. Richmond Dept Biology, Univ Illinois Chicago, Chicago, IL.

At the C. elegans body wall neuromuscular junctions (NMJs) there are two cholinergic ionotropic receptor types, one that is heteromeric and activated by levamisole (LACR) and one that is homomeric, alpha-7-like, and activated by nicotine (NACHR). Conserved components of a novel synaptic cleft scaffold have been implicated in the clustering of LACRs, but the expression of the colocalized NACHR appears completely normal when these proteins are absent. This suggests that other, unidentified proteins regulate NACHR expression.

The only receptor subunit known to be required for the C. elegans NACHR is ACR-16, which can form functional homopentameric receptors. A forward genetic screen was performed to isolate candidate genes involved in ACR-16 expression. The screen utilized a single-copy integrant of ACR-16::GFP to isolate mutants that decrease the synaptic level of ACR-16::GFP. From this screen a mutant was identified, and whole genome sequencing, using the Variant Density Mapping approach, revealed the sarcoplasmic reticulum calcium-ATPase sca-1 as a likely candidate gene for this mutation. A sca-1 reference mutant strain phenocopies the ACR-16::GFP expression defects. The expression and localization of LACRs and inhibitory GABA receptors seem unaffected in a sca-1 mutant background based on wild-type levels of LACR::RFP and GABAR::GFP fluorescence. Behavioral assays show a more severe uncoordinated phenotype in a sca-1;LACR mutant background, consistent with an effect on ACR-16 receptors. The affects of sca-1 on ACR-16 receptor function are not caused by defects in NMJ patterning, as there is no change in the number of synapses and muscle structure is wild type in these mutants. Electrophysiological recordings show a significant reduction in the evoked response of sca-1 mutants suggesting a functional affect on the receptors. Responses to pressure-ejected nicotine in sca-1 mutants are wild type implying that receptors are present on the muscle membrane but mislocalized. The reintroduction of SCA-1 driven by a muscle specific promoter is able to rescue the reduction in levels of ACR-16::GFP and the increased uncoordination seen in sca-1;LACR double mutants. Due to the role of SCA-1 in calcium level maintenance, calcium imaging was performed. In sca-1 mutants there is a significant increase in baseline levels of calcium, however, stimulated calcium levels are significantly reduced as compared to the control.
 Preliminary calcium imaging in acr-16 mutants suggests there is a similar phenotype to what is seen in sca-1 mutants. This suggests a role for calcium homeostasis mediated through ACR-16 receptors. Further evaluation of this mechanism is on going.

**964C Single cell transcriptome analysis of ASJ thermosensory neuron regulating cold tolerance.** Ayana Maruo, Natsume Takagaki, Akane Ohta, Atsushi Kuhara. Graduate school of Natural Science, Konan University, Kobe-Shi, Hyogo, JP.

ASJ sensory neuron that has been known as a light and pheromone sensing neuron senses temperature and regulates cold tolerance through insulin signaling (Ohta, Ujiiwasa et al., *Nature commun.*, 2014). Cold tolerance in *C. elegans* is a phenomenon that 20°C-cultivated animals cannot survive at 2°C, whereas 15°C-cultivated animals can survive at 2°C. Our previous analyses suggest that cold tolerance is regulated by tissues network containing neurons, intestine and sperm, in which a feedback regulation from sperm to ASJ thermosensory neuron is essential (Sonoda, et al., *Cell Reports*, 2016). However, we have not known thermo-sensing molecules on ASJ and how ASJ is controlled by sperm signaling remain poorly understood.

To identify new genes involved in temperature sensation and temperature tolerance, we conducted RNA-sequencing analysis comparing mRNA expression levels between ASJ and ASE gustatory neuron by using single cell RNA-sequencing analysis (Sugi & Ohtani, *BBRC* 2014). mRNAs were extracted from animals that were cultivated at constant temperature (15°C, 20°C, or 25°C) or cultivated under temperature-shifted conditions (15°C to 25°C or 25°C to 15°C). By comparing gene expression levels between ASJ and ASE in 20°C cultivated-animals, we found the expression levels of ~600 genes such as neuropeptides, neuropeptide receptors, insulin were altered. We measured cold tolerance phenotypes of about fifty mutants defective in those genes, and found that mutants defective in *avr-15* encoding glutamate-gated chloride channel and *clh-1* encoding CLC-type chloride channels showed abnormal cold tolerance.

**965A Neural mechanisms underlying behavioral switching in thermotaxis of *C. elegans*.** H. J. Matsuyama, Y. Tsukada, I. Mori. Neuroscience Institute and Group of Molecular Neurobiology, Graduate School of Science, Nagoya University, Nagoya, JP.

Animals switch behavioral strategy based on environments and experiences. However, neural mechanisms underlying the behavioral switching are poorly understood. *C. elegans* thermotaxis includes behavioral switching and is modified by feeding states (Hedgecock & Russell 1975; Mohri et al. 2005; Kodama et al., 2006; Nishio et al. 2012). Well-fed animals migrate to their cultivation temperature (“navigation”) and move isothermally around the cultivation temperature (“IT”: isothermal tracking) on a thermal gradient without food, whereas starved animals appear to avoid the cultivation temperature and disperse to explore the thermal gradient. We call the former behavioral strategy of well-fed animals as “exploitation mode”, and the later strategy of starved animals as “exploration mode”. How such behavioral switching is regulated by the nervous system remains unclear.

Previous studies showed that the interaction between AFD thermosensory neurons and their major postsynaptic partner AIY is likely to be critical for the behavioral switching. To investigate the neural mechanisms underlying exploitation-exploration switching, we first examined whether the feeding states influence the temperature-evoked Ca2+ dynamics of AFD and AIY for 20°C-cultivated animals. In Ca2+ imaging, we used two types of thermal stimuli oscillating around 17°C and 20°C, where navigation and IT are observed for 20°C-cultivated well-fed animals, respectively.

We found here, that in well-fed animals, the calcium signals of AFD and AIY are synchronized with oscillating thermal stimuli near 17°C (“phase-locked interaction”), whereas “phase-lagged interaction” was observed near 20°C: AII increased intracellular calcium signals at Ca2+ rises or Ca2+ falls of AFD. By contrast, starved animals showed the phase-lagged interaction near 17°C. Our behavioral analysis revealed that starved animals exhibited higher frequency of IT around 17°C than well-fed animals. We hypothesize that phase-locked and phase-lagged interaction between AFD and AIY cause navigation and IT, respectively. To test the hypothesis, we are planning to control the neural activities of AFD and AIY by optogenetics for freely moving animals. In addition, to investigate the molecular basis of the phase shift of AFD-AII interaction, we are currently conducting behavioral analysis and Ca2+ imaging with temporal thermal stimuli for *ins-1* and *aho-3* mutants that exhibit defects in food-associated behavior in thermotaxis. Our study should shed light on revealing a fundamental mechanism by which transitions of circuit states build behavioral switching of animals.

**966B Neuromuscular junction patch-clamp recordings at a primarily undergraduate institution.** K.E. McCluskey, J.P. Merchant, G.G. Ernstrom. 1) Department of Biology; 2) Program in Neuroscience; 3) Program in Molecular Biology and Biochemistry; 4) Middlebury College, Middlebury, VT.

Whole-cell, tight-seal patch-clamp recordings of postsynaptic currents at the neuromuscular junction are the gold standard for analyzing quantal size, frequency, and content. Quantal analysis in *C. elegans* is a technically demanding technique that requires both the proper equipment and skilled dissection. Here we describe how we set up a patch-clamp electrophysiological rig that is accessible to undergraduate researchers. We are currently using this methodology to analyze candidate V-ATPase proton pump mutants that our lab is screening from the Million Mutation Project. We have adapted the methods described by Richmond and Jorgensen (1999): briefly, we immobilize live animals swimming in physiological saline with veterinarian glue and use a glass cutting tool to make an incision along the dorsal side of each animal. Viscera are then aspirated, and the flap of exposed ventral skin, muscle, and nerves is pinned down with spots of glue. Here, we will present analyses of our different attempted dissection methods, our throughput, and our first recordings. A summary of our equipment and space needs will also be provided. Finally, we will describe our plans to integrate this type of experiment into our teaching curriculum. We find that, while technically challenging, *C. elegans* patch-clamping can provide a valuable addition to teaching neurobiology and doing original research with undergraduates.
### 967C Activity-dependent gene expression in the C. elegans motor circuit.

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Locomotion in C. elegans requires balanced cholinergic excitatory and GABAergic inhibitory activity. A gain-of-function mutation in the acr-2 gene (acr-2(gf)), causes increased excitation and decreased inhibition in the motor circuit (E/I imbalance). This results in a spontaneous whole-body shrinking or convulsion phenotype. acr-2 encodes an acetylcholine receptor subunit that is expressed and functions in the cholinergic motoneurons to regulate locomotion. The acr-2(gf) mutation is a valine-to-methionine transition in the second transmembrane domain of these receptor subunits, and interestingly, similar mutations in human acetylcholine receptors are associated with genetic epilepsy. Previous studies from our lab found that neuromodulators can function in a homeostatic response to dampen circuit activity. Here, using cell-specific gene expression profiling, we have identified 234 genes that are differentially expressed in acr-2(gf) neurons compared to wild type. Most of these genes were up-regulated compared to wild type (73%), including the neuropeptide flp-18, which was previously shown to be up-regulated in the motor neurons of acr-2(gf) animals. GO-term enrichment analyses found that terms associated with neuromodulator signaling and G-protein coupled receptor signaling as significantly enriched in this list. In addition, several transcription factors and ligand-gated ion channels were also identified. Studies are now ongoing to validate changes in gene expression identified by RNA-seq and investigate roles for these genes in C. elegans locomotion.

### 968A Investigating how the DAF-7/TGF-beta signaling pathway regulates abundance of the C. elegans glutamate receptor GLR-1.

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Transforming growth factor-beta (TGF-beta) family signaling pathways have roles in both neuronal development and the regulation of synaptic function. We previously identified a role for the C. elegans DAF-7/TGF-beta signaling pathway in the regulation of the AMPA-type glutamate receptor GLR-1. We found that the abundance of GLR-1 increases in animals with loss-of-function mutations in multiple DAF-7/TGF-beta pathway genes. Additionally, DAF-7/TGF-beta pathway mutants exhibit changes in spontaneous locomotion that are dependent on endogenous GLR-1 and consistent with increased glutamatergic signaling. In order to understand the mechanism through which the DAF-7/TGF-beta pathway regulates GLR-1 we sought to identify genes that may be involved in this regulation. Using published data sets we identified a set of 289 genes that are both expressed in GLR-1-expressing neurons and regulated by the DAF-7/TGF-beta signaling pathway. The genes in this data set represent candidate genes that may play a role in regulating GLR-1 in response to DAF-7/TGF-beta pathway signaling. To test whether these candidate genes are involved in the DAF-7/TGF-beta pathway-dependent regulation of GLR-1 we obtained mutants in individual candidate genes and generated double mutants by crossing them with daf-7 mutants. The rate of spontaneous reversals was measured in these double mutants in order to identify mutants with changes in glutamatergic signaling. We have thus been able to identify candidate genes that may be involved in the DAF-7/TGF-beta pathway-dependent regulation of GLR-1. These candidates are currently undergoing further testing to determine whether they play any role in regulation of GLR-1.

### 969B Sentryn and SAD Kinase link Dense Core Vesicle axonal transport and synaptic capture.

Logan Morrison¹, Stacey Edwards¹, Laura Manning², Natalia Stec¹, Janet Richmond², Kenneth Miller¹  
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Dense core vesicles (DCVs) release neuropeptides from the synaptic regions of axons to modulate animal behaviors. Release is preceded by axonal transport of DCVs, followed by their capture in the synaptic region. During transport, motors move DCVs along microtubules bi-directionally, with a bias toward forward transport. Upon capture, DCVs must be protected from the actions of both the forward and reverse motors, known as KIF1A and dynine. The mechanisms that ensure biased forward transport and that protect DCVs from motors after capture are unknown. Here, we use a forward genetic screen to discover two new components of the DCV transport and capture mechanism: SAD Kinase (SAD-1) and a novel protein we named Sentryn (STRN-1). Sentryn is highly expressed in neurons and is conserved in all animals. The name, based on “sentry”, reflects its role in standing guard over captured vesicles and protecting them from counter-productive motor activity during transport. A human Sentryn cDNA rescues the worm DCV phenotypes. Our genetic studies show that Sentryn acts cell autonomously with SAD Kinase to optimize the forward transport and synaptic capture of DCVs. Time lapse and high resolution quantitative imaging of single neuron subregions show that Sentryn and SAD Kinase do this by directly or indirectly regulating the interactions of both the KIF1A and Dynine motors with DCVs. These results provide the first evidence for a mechanistic link between DCV transport and synaptic capture by identifying two conserved proteins that function in both processes.

### 970C Synaptic Vesicle cluster rearrangements in adults driven by a signal transduction pathway used for learning.

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Synapses are modified in adults by multiple mechanisms as behaviors are established, or learned, and then altered. We discovered a new mechanism for rapidly altering the synaptic landscape in adults: rearrangement of synaptic vesicle (SV) clusters. With the aim of testing the stability of SV clusters in unconstrained adults, we quantified the sizes of individual SV clusters in the same animal before and after a behavioral-state change that relies on those clusters. Technical challenges we overcame have prevented similar approaches from being used in any live animal. Remarkably, just 4 hrs after a behavioral state change, ~16% of clusters (1 in 6) in a cholinergic motor neuron increased their sizes by >3.5-fold or diminished by more than the inverse of this. The changes resulted from a rearrangement of existing clusters because they occurred without axonal growth or any net change in total SV content or cluster numbers. Furthermore, we found that hyper-activating a pathway important for learning, the Gas pathway, doubled the number of major rearrangements in adults and caused new clusters to appear in a region that previously had none. We hypothesize that the Gas pathway may normally respond to signals produced during behavior-state changes to alter the levels of SV capture proteins at behaviorally-relevant clusters. This would change the
clusters’ capacities for holding SVs and allow a motor-driven shift of SVs between clusters. These results are the first to suggest a connection between behavioral state changes, SV cluster rearrangements, and a learning pathway in a living adult animal.


Since absent of voltage-gated Na⁺ channels in C. elegans, it has long been assumed that nematode neuron relied on passive propagation rather than classical action potentials. A recent study, however, reports that RMD motor neuron exhibits Ca²⁺-dependent action potential or plateau potentials in C. elegans. Thus, identification of channels that responsible for active propagation of membrane potentials are essential to understand neural mechanisms of information processing. A pair of head chemosensory neurons ASEL and ASER act as main sensor for salt taste cues. With genetic encoding Ca²⁺ indicator, neural activation shown as Ca²⁺ influx into ASEL and ASER are induced by increased and decreased of NaCl concentration, respectively. Although, ASER neuron is isopotential and lacks classical Na⁺ action potential, it is still unclear that electrical neural properties of each ASE neurons in response to NaCl. In this study, we analyze electrical and Ca²⁺ signal propagations of ASE neurons by using a combination of genetics and electrophysiology, and Ca²⁺ imaging techniques. Resting membrane potentials of ASE neurons were about -60 mV and their input resistance over 1.5 GΩ. ASE neurons respond to depolarizing current injection in non-linear and regenerative though excitatory membrane response were increased linearly depend on current intensity by reaching around -40 mV. In NaCl stimulation, ASEL neuron was depolarized by up-steps of NaCl and ASER by down-step of NaCl. Amplitude of membrane depolarization of wild-type ASE neurons evoked by NaCl steps were around 40 mV, indicating that changes in NaCl concentration induce nonlinear potentials. Depolarization of ASEL was diminished by L-type voltage-gated Ca²⁺ channel (VGCC) blocker nemadipine-A or genetic inactivation of L-type VGCC EGL-19. On the other hands, down-step NaCl-induced depolarization of ASER was insensitive to nemadipine-A and egl-19 mutation. In ASEL, Ca²⁺ influx was observed in cilia of ASEL in response to step-up NaCl in egl-19 mutants, but whole cell depolarization was not detected in cell body. In these results suggest that EGL-19 play a role in active signal propagation from the dendritic tip to the soma in ASEL but not in ASER.

972B Neural circuit basis for the behavioral switch in C. elegans chemotaxis to alkaline pH. T. Murayama, I. Maruyama. Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan.

Monitoring of environmental and tissue pH is crucial for the survival of animals. The nematode C. elegans is an excellent model organism for the analysis of neural circuits that regulate behavioral responses to environmental changes, because of its simple nervous system. We are interested in neural circuits responsible for C. elegans chemotactic behavior to environmental alkalinity. The animal is attracted to mildly alkaline pH, and avoids strongly alkaline pH higher than pH 10. Previous genetic dissection and Ca²⁺ imaging demonstrated that ASEL and ASH are major sensory neurons responsible for attraction to mildly alkaline pH and repulsion from strongly alkaline pH, respectively. ASEL was activated by alkaline pH ranging from pH8 to pH 11. ASH was activated by alkaline pH higher than 10. Moreover, ASEL was transiently activated by pH up-step, whereas ASH showed long-lasting activation during stimulation. These results suggest that ASH and ASEL activities compete each other and ASH activity overrides that of ASEL upon stimulation with strongly alkaline pH. The long-lasting ASH activity may override the transient activation of ASEL. Since there is no chemical or electric connection between ASEL and ASH, signals from these 2 alkaline pH sensors must be integrated by downstream circuits. We have now analyzed interneurons responsible for the behavioral switch, and will discuss how the behavioral switch occurs.

973C Regulation of female reproductive behavior states in C. elegans. L.M. Nassar, M. Scheetz, A. Bode, K. Collins. 1) Neuroscience Program, University of Miami, Coral Gables, FL; 2) Department of Biology, University of Miami, Coral Gables, FL.

We are interested in understanding how females make reproductive behavior decisions using Caenorhabditis elegans as a model system. Specifically, we are identifying the neural signaling mechanisms that drive two mutually exclusive vulval motor behaviors: mating with males or the release of progeny during egg laying. We hypothesize that circuit activity that drives egg-laying behavior is inhibited during mating while mating behavior is inhibited when females have sufficient sperm to fertilize oocytes.

To investigate this hypothesis, we are using calcium imaging to record changes in cell activity in the egg-laying behavior circuit during mating and using histamine-gated Cl⁻ channels to test the functional importance of cell activity during female mating behavior. We find that fertile hermaphrodites and sperm-deficient fog-2 mutant females show low vulval muscle activity before mating that resembles the egg-laying inactive state. Consistent with this observation, fog-2 females display low activity in the presynaptic HSN motor neurons and the cholinergic VC motor neurons whose activity coincides with egg release. Vulval muscle activity increases before spicule insertion and silencing of muscle activity with histamine delays mating, suggesting vulval muscle twitching contractions facilitate male spicule insertion. We recently showed that the uv1 neuroendocrine cells are mechanically activated by passage of eggs through the vulva, and we find they are similarly activated upon male spicule insertion during mating. Based on this data, we predict that male prodding of the vulva mechanically activates the VC motor neurons that slow female locomotion and allow mating. During male spicule insertion, the vulval muscles and uv1 neuroendocrine cells are mechanically deformed, with the activated uv1 cells releasing tyramine and neuropeptides that silence the HSN motor neurons to prevent female escape and egg laying that would interrupt mating. Sperm and seminal fluid deposited into the uterus activates the uterine and vulval muscles, allowing for ejection of the male spicules and inactivation of the uv1 cells, signaling mating is complete. Together, our comparative analysis of C. elegans reproductive behaviors will show how altered activity in the same neural circuit can be used to drive distinct reproductive behavior states.
974A  **BKIP-1, an auxiliary subunit critical to SLO-1 function, inhibits SLO-2 potassium channel in vivo.**  L. Niu¹, P. Liu¹, R. Mailler², Z. W. Wang¹, B. Chen¹ ¹) Department of Neuroscience, UConn Health, Farmington, CT; 2) Department of Computer Science, University of Tulsa, OK.

Auxiliary subunits are often needed to tailor K⁺ channel functional properties and expression levels to specific cellular requirements. A number of auxiliary subunits have been identified for mammalian Slo1, a high-conductance K⁺ channel gated by membrane voltage and cytosolic Ca²⁺. Experiments with heterologous expression systems show that some putative Slo1 auxiliary subunits can also regulate other K⁺ channels of the Slo family. However, it remains to be proved that a single auxiliary subunit may regulate more than one Slo channel within the same cell in native tissues. *C. elegans* has two channels of the Slo family: SLO-1 and SLO-2. The latter is also a high-conductance K⁺ channel gated by membrane voltage and cytosolic Ca²⁺. In addition, it is sensitive to cytosolic Cl⁻, which acts synergistically with cytosolic Ca²⁺ in activating the channel. BKIP-1 was initially identified as an auxiliary subunit of SLO-1. It facilitates SLO-1 surface expression, regulates SLO-1 biophysical properties in a Ca²⁺-dependent manner, and is indispensable for SLO-1 physiological function in both neurons and muscle cells (Chen et al., *J Neurosci* 2010). A recent study shows that BKIP-1 genetically interacts with both SLO-1 and SLO-2 to control terminal differentiation of AWC olfactory neurons (Alqadah et al., *PLoS Genet* 2016). However, it remains to be determined how BKIP-1 regulates SLO-2 function. In this study, we recorded SLO-2 whole-cell currents and single-channel activities of motor neurons and muscle cells in dissected worms and compared them between strains with and without BKIP-1. Surprisingly, we found that the BKIP-1 strain displayed smaller SLO-2 whole-cell currents and single-channel open probability (Pₒ) compared with the control strain. In addition, we found that BKIP-1 reduced SLO-2 Cl⁻ sensitivity and activation rate without altering SLO-2 Ca²⁺ sensitivity, and that BKIP-1 shortened the mean open time of SLO-2 single channel events. In contrast, BKIP-1 did not alter whole-cell currents mediated by SHK-1, a Shaker/Kv₁-type K⁺ channel, suggesting that BKIP-1 does not regulate K⁺ channels indiscriminately. These results suggest that BKIP-1 may serve as an auxiliary subunit to inhibit SLO-2. To our knowledge, BKIP-1 is the first example showing that a single auxiliary subunit may exert opposite effects on two evolutionarily related channels in the same cells.

975B  **Natural variations of cold acclimation and analysis of KQT potassium channel in cold acclimation.**  Misaki Okahata¹, Akane Ohta¹, Yohei Minakuchi², Atsushi Toyoda¹, Atsushi Kuhara¹ ¹) Graduate school of Natural Science & Institute for Integrative Neurobiology, Konan University, Kobe, Japan; 2) National Institute of Genetics, Japan.

Temperature is one of the most critical environmental factors in the earth. Organisms have mechanisms to acclimate to environmental temperature changes. We performed two approaches to ravel the cold acclimation mechanisms in animals. 1. Genetic analysis of natural variants showing various cold acclimation

We are studying about temperature acclimation by using *C. elegans* isolated from twelve various areas. 15 degree-cultivated N2 from Bristol can survive at 2 degree, while 25 degree-cultivated N2 can not survive at 2 degree. We previously reported that only 3 hours after cultivation temperature-shift from 25 to 15 degree, cold acclimation was newly established (Ohta, Ujisawa et al., *Nature commun*, 2014). We measured cold acclimation phenotypes in twelve natural variants. AB1 isolated from Australia can rapidly acclimate to new temperature, whereas CB4856 isolated from Hawaii can slowly acclimate to new temperature. In order to identify the responsible gene polymorphism involved in temperature acclimation, we used next generation sequencer and SNP analysis. As a results, the gene responsible for cold acclimation of AB1 was mapped between 1.1~5.1cM. 2. KQT-type potassium channel involves in cold acclimation

We used DNA micro array analysis to identify new gene involved in cold acclimation. 79 genes were changed their expression level when 23 degree cultivated N2 transferred to 17 degree for four hours. We analyzed cold acclimation phenotype in 15 mutants. As a result, kqt-2 mutant defective in KQT-type potassium channel showed abnormal cold acclimation. The expression of KQT-2 was observed in ASK and ADL head sensory neurons, intestine, and vulval muscle. In order to identify the tissue where kqt-2 regulates cold acclimation, cell specific rescue experiments were performed. The abnormal cold acclimation phenotype of kqt-2 mutant was rescued by expressing kqt-2DNA in sensoryneuron but not intestine. *C. elegans* has three kinds of KQT-type potassium channels. Among them, kqt-3 mutant showed abnormal cold acclimation, which was opposite phenotype to that of kqt-2.

976C  **Structural and molecular analysis of a novel model of dendritic spines in C. elegans.**  D. Oliver¹, A. Philbrook¹, K. Nguyen², A. Thackery¹, D. Hall², M. Doitsidou¹, C. Bénard¹, M.M. Francis¹ ¹) University of Massachusetts Medical School, Worcester, MA; 2) Albert Einstein College of Medicine, Bronx, NY; ; 3) The University of Edinburg, Edinburg, UK.

Dendrites are branched neuronal projections that receive and integrate synaptic inputs. Although dendritic structure can be quite varied, a common feature is the presence of thorn-like protrusions, called dendritic spines that are associated with sites of synaptic input. Whereas molecular mechanisms underlying axonal development have been largely elucidated, the molecular processes driving dendritic development, in particular spine outgrowth and stabilization, remain less understood. We recently discovered that excitatory synapses onto GABAergic DD neurons are associated with finger-like protrusions from the GABAergic dendrite. Using these synapses as a model, we aim to elucidate the molecular and cellular basis of the formation and/or maintenance of these dendritic spine-like structures. Using light microscopy, we have obtained evidence that these structures appose presynaptic markers, are decorated with clusters of neurotransmitter receptors, and are highly concentrated in F-actin. Using electron microscopy, we have confirmed the presence of these structures in the wild type and are characterizing them at the ultrastructural level. In addition, we are carrying out a forward genetic screen to elucidate molecular pathways involved in the formation, maintenance and plasticity of these spine-like protrusions. We have isolated several mutants with defects in the DD dendritic protrusions. Using whole genome sequencing and bioinformatic analysis, we have identified candidate genes underlying these defects and are now investigating their specific functions at these spiny synapses.
We are using GCaMP calcium imaging to record activity in cells of the egg laying circuit at the late L4 larval stage and in adults providing a model system to understand the origin and function of cell activity during development.

Type C motor neurons (VC), as well as the tyraminergic uv1 neuroendocrine cells and the vulval muscles which contact to lay muscle cells or GABAergic motor neurons. Photostimulation of cholinergic neurons in wild type animals produces robust Ca\(^{2+}\) transients in both cell types. Compared to motor neurons, calcium responses in muscles are prolonged and larger in amplitude, likely reflecting differences in both synaptic and cellular physiology across these cell types. In order to dissect synaptic mechanisms required for transmission onto each partner, we have been investigating strains carrying mutations in genes required for neurotransmitter release, specific neurotransmitter receptor subunits, and synaptic organizers. By investigating Ca\(^{2+}\) responses in these mutants, we aim to elucidate mechanisms underlying functional connectivity between cholinergic motor neurons and their dyadic synaptic partners.

**977A** Dissection of molecular mechanisms that govern functional connectivity across multiple postsynaptic partners.  
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NEUROBIOLOGY, UNIVERSITY OF MASSACHUSETTS MEDICAL SCHOOL, WORCESTER, MA.

Neurons often make synaptic outputs onto multiple partners. The strength of these connections can vary greatly across partners, but we have limited understanding of how synaptic weights with specific partners are established and regulated. We have been addressing these questions using the motor circuit of *Caenorhabditis elegans* as a model. Cholinergic motor neurons form excitatory dyadic synapses onto body wall muscle cells and GABAergic motor neurons. We have developed a system for investigating functional connectivity onto each of these cell types. Specifically, we have generated transgenic strains that express Chrimson in the cholinergic motor neurons together with the genetically encoded calcium sensor GCaMP6s in either muscle cells or GABAergic motor neurons. Photostimulation of cholinergic neurons in wild type animals produces robust Ca\(^{2+}\) transients in both cell types. Compared to motor neurons, calcium responses in muscles are prolonged and larger in amplitude, likely reflecting differences in both synaptic and cellular physiology across these cell types. In order to dissect synaptic mechanisms required for transmission onto each partner, we have been investigating strains carrying mutations in genes required for neurotransmitter release, specific neurotransmitter receptor subunits, and synaptic organizers. By investigating Ca\(^{2+}\) responses in these mutants, we aim to elucidate mechanisms underlying functional connectivity between cholinergic motor neurons and their dyadic synaptic partners.

**978B** The *C. elegans* RIM-Piccolo homolog CLA-1 is required for normal synaptic transmission.  
Greg Mullen\(^1\), Dennis Frisby\(^1\), Ellie Mathews\(^1\), John McManus\(^1\), Kiely Grundahl\(^1\), Angie Duke\(^1\), Jacob Manjarrez\(^1\), Jim Rand\(^1,2\)  
1) Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) Oklahoma Center for Neuroscience, Oklahoma City, OK.

Members of the vertebrate Piccolo-Bassoon-RIM family of proteins are characterized by a specific set of structural features, including N-terminal zinc fingers, several coiled-coil regions and (except for bassoon) C-terminal PDZ and C2 domains. The proteins are localized to the cytomatrix of the active zone in presynaptic terminals; Piccolo, Bassoon, RIM1 and RIM2 have all been shown to play roles in neurotransmitter release. We have now characterized a large gene locus in *C. elegans* that gives rise to two very long transcripts (26.6 kb and 25.4 kb) encoding proteins with similarities to Piccolo and Bassoon, and two shorter transcripts (4.1 kb and 2.9 kb), one of which encodes a RIM-like protein. This locus, formerly known as either *rip-1* or *tag-80*, is now called *cla-1* (clarinet-1).

The large transcripts (*cla-1A* and *cla-1B*) and the smaller transcripts (*cla-1C* and *cla-1D*) are controlled by two different promoters. With both promoters, we observed reporter expression almost exclusively in neurons, and most or all neurons in wild-type adults appear to express at least one of the reporters. Immunostaining and translational reporters both indicated that CLA-1 proteins are localized to discrete puncta in synaptic regions.

The most prominent feature of the two large CLA-1 proteins is an N-terminal set of 30 tandem 94-amino acid repeats; this repeat structure represents almost one third of the total protein. The repeats are very acidic, rich in serine and threonine, and contain six casein kinase II phosphorylation consensus sites per repeat. Computer modeling of these repeats predicts a dual-repeat compact module structure. We suggest that these repeats are protein interaction domains, and fulfill a comparable function to the N-terminal zinc fingers present in the vertebrate proteins.

The overall *cla-1* gene and CLA-1 protein structures are well conserved in most or all nematode species examined. However, both the number (2-30) and length (57-158 amino acids) of the individual tandem repeats vary considerably among different nematodes, even within the same genus.

*cla-1* mutants are viable and superficially wild-type in appearance, development and behavior. However, they have mild deficits in synaptic transmission, and they enhance the behavioral and synaptic phenotypes of RIM-deficient *unc-10* mutants. The nervous systems of *cla-1* mutants appear grossly normal, and representative presynaptic proteins are properly localized. We suggest that CLA-1A and Piccolo provide a comparable example of two proteins with similar C-terminal domain structures and different N-terminal domain organizations performing conserved functions.

Supported by NIH grant GM059642

**979C** Development of patterned activity in the *C. elegans* egg-laying behavior circuit.  
B. Rav\(^1,2\), K. Collins\(^1,2\)  
1) Neuroscience Program, University of Miami Miller School of Medicine, Miami, FL; 2) Department of Biology, University of Miami, Coral Gables, FL.

Coordinated patterns of activity are required to drive efficient behaviors in mature neural circuits. We are interested in understanding how activity patterns emerge during development that allow for proper execution of behaviors in adults. The *C. elegans* egg laying circuit is a simple, well-characterized neural circuit which develops during the L4 stage and drives a two-state behavior in adult animals with a ~20 minute inactive state and ~2 minute active state where eggs are laid. The circuit comprises two types of motor neurons: the serotonergic Hermaphrodite Specific Neurons (HSNs) and the cholinergic Ventral Type C motor neurons (VC), as well as the tyraminergic uv1 neuroendocrine cells and the vulval muscles which contact to lay eggs. During the L4 larval stage, the cells of the circuit undergo morphological maturation and establish synaptic connections, providing a model system to understand the origin and function of cell activity during development.

We are using GCaMP calcium imaging to record activity in cells of the egg laying circuit at the late L4 larval stage and in adults.
after development. We observe little or no activity in the VC neurons or the uv1 cells in late L4 animals. However, we find the HSN neurons show rhythmic Ca²⁺ transients at the late L4 larval stage even before eggs are produced. HSN L4 activity is rhythmic, occurring every 55 seconds and lacks the characteristic burst-firing pattern of adults during egg-laying active states where Ca²⁺ transients occur every 20s. The vulval muscles show an increase in Ca²⁺ transient strength and frequency as animals develop into adults. During egg laying, vulval muscle Ca²⁺ transients are coordinated with transients occurring in both the anterior and posterior muscle cells. We find this coordinated muscle activity appears at the late L4 stage and is independent of HSN presynaptic input. During the course of development, the ability of the HSN neurons to robustly drive vulval muscle Ca²⁺ activity is coincident with the accumulation of eggs within the uterus. The ‘burst-firing’ activity in the HSN neurons is missing after animal sterilization with Fluoruridine (FUDR) or after acute electrical silencing of the vulval muscles. Together, our data suggests the vulval muscles integrate signals of uterine stretch in response to egg accumulation and promote HSN Ca²⁺ activity that induces the egg-laying active state in adults. Because HSN activity in L4 animals is dispensable for egg-laying circuit development, we are investigating whether HSN activity drives changes in L4-specific behaviors including the reduction of locomotion and the cessation of defecation that accompanies lethargus. Together, these results will help us to understand how mature patterns of cell activity develop and drive robust, stable behaviors.

980A  Molecular basis of antidromic rectification of gap junctions between AVA interneurons and motor neurons in C. elegans escape circuit.  Y. Shui, P. Liu, H. Zhan, B. Chen, Z. W. Wang  Department of Neuroscience, UConn Health, Farmington, CT.

C. elegans AVA command interneurons play important roles in escape behavior, and contact A-type cholinergic motor neurons (A-MNs) through both electrical and chemical synapses. Our recent study shows that the gap junctions (GJs) between AVA and A-MNs only allow antidromic currents (from A-MNs into AVA), and that the function of these GJs depends on UNC-7 innexin in AVA and UNC-9 innexin in A-MNs (Liu et al., Nat Commun 2017). However, molecular basis of the antidromic rectification is unknown. To address this question, we began by expressing UNC-7 and UNC-9 in Xenopus oocytes, and analyzing biophysical properties of homotypic and heterotypic GJs formed by them. While UNC-9 has only one isoform, UNC-7 has at least three different isoforms (UNC-7a, UNC-7b and UNC-7c), which differ in the length of the amino terminal. UNC-7c has a short amino terminal and stability.

Mechanism on how such functional asymmetry arise is largely unknown. Roughly two thirds of L-R homologous pairs. By evaluating new data from reconstruction of serial section electron micrographs (wormwiring.org), we found that most L-R neurons display laterally similar connectivity and connection strength. However, we found that two amphid neurons known to be asymmetric in gene expression and function, ASE and AWC, displayed lateralized connectivity (15 ASEL->AWC synapses vs. 2 ASER->AWC synapses). To corroborate and evaluate this chemical synapse pattern in a large number of L-R homologous pairs, we began by expressing UNC-7 and UNC-9 in Xenopus oocytes, and analyzing biophysical properties of homotypic and heterotypic GJs formed by them. While UNC-9 has only one isoform, UNC-7 has at least three different isoforms (UNC-7a, UNC-7b and UNC-7c), which differ in the length of the amino terminal. UNC-7c has a short amino terminal and stability.

Expression of this specific UNC-7 isoform in AVA interneurons in an unc-7 mutant significantly restored the antidromic junctional currents between AVA and A-MNs whereas the other two UNC-7 isoforms had either no effect or a much weaker effect. Taken together, our results suggest that 1) the amino terminal domain of UNC-7 plays important roles in GJ gating; 2) GJs between AVA and UNC-9 oocytes, and analyzing biophysical properties of homotypic and heterotypic GJs formed by them. While UNC-9 has only one isoform, UNC-7 has at least three different isoforms (UNC-7a, UNC-7b and UNC-7c), which differ in the length of the amino terminal. UNC-7c has a short amino terminal and stability.

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981B  Sentryn is a novel active zone protein that acts with SAD Kinase to promote Synaptic Vesicle cluster Formation and stability.  Stacey Edwards1, Natalia Stec1, Logan Morrison1, Laura Manning2, Janet Richmond2, Kenneth Miller1 1) Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) University of Illinois at Chicago, Chicago, IL.

The motor KIF1A transports synaptic vesicles (SVs) toward microtubule plus ends in axons until they are captured into clusters in the synaptic region. This outward transport is opposed by the minus-end motor dynein, which causes SVs to reverse direction multiple times en route to the clusters. The Core Synapse Stability (CSS) system contributes to the both the dominance of outward transport and the final capture of SVs into clusters. This occurs in part by inhibiting dynein and/or over-riding dynein by regulating KIF1A. Here, we show that the novel conserved protein STRN-1 (Sentryn) is a CSS protein that guards against counter-productive motor activity acting on SVs. Like other CSS proteins, Sentryn can also inhibit the dynein-mediated transport of early endosomes and lysosomes as well as SVs. This activity is normally blocked by JIP3 to ensure organelle clearance from axons. Human Sentryn rescues worm sentryn mutants. Sentryn acts with the CSS protein SAD Kinase to promote the axonal transport of SVs and prevent SVs from escaping capture to clusters. These functions of Sentryn and SAD Kinase occur in parallel and together mediate the function of the CSS protein SYD-2 (Liprin-a). Like other CSS proteins, Sentryn is enriched at active zones, around which SVs are clustered. Sentryn’s localization at active zones is dependent on Liprin-a, but not on SAD Kinase. These results reveal the first new active zone protein in 12 years and advance our molecular understanding of the SV transport and capture processes.

982C  A lateralized circuit of sensory neurons in C. elegans.  Leo T.-H. Tang, Steven J. Cook, Scott W. Emmons, Hannes E. Bülow  Department of Genetics, Albert Einstein College of Medicine, Bronx, NY.

Lateral specialization of the central nervous system is a well-established feature across species, yet the underlying mechanism on how such functional asymmetry arise is largely unknown. Roughly two thirds of C. elegans neurons are present as L-R homologous pairs. By evaluating new data from reconstruction of serial section electron micrographs (wormwiring.org), we found that most L-R neurons display laterally similar connectivity and connection strength. However, we found that two amphid neurons known to be asymmetric in gene expression and function, ASE and AWC, displayed lateralized connectivity (15 ASEL->AWC synapses vs. 2 ASER->AWC synapses). To corroborate and evaluate this chemical synapse pattern in a large number of L-R homologous pairs, we began by expressing UNC-7 and UNC-9 in Xenopus oocytes, and analyzing biophysical properties of homotypic and heterotypic GJs formed by them. While UNC-9 has only one isoform, UNC-7 has at least three different isoforms (UNC-7a, UNC-7b and UNC-7c), which differ in the length of the amino terminal. UNC-7c has a short amino terminal and stability.

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sample of live animals, we generated a reporter strain labelling ASE->AWC connections using in vivo Biotin Labelling of Intercellular Contact (iBLINC). We observe that roughly 85% of animals contain more ASE->AWC synaptic puncta than ASER->AWC. Time-course imaging revealed this connection is present throughout larval development and symmetric at early larval stages (median, left: 3, right: 2) but becomes lateralized by a larger increase of ASE->AWC synapses from late larval to adult stages (median 5) as compared to the increase in that of ASER (median 2.5). We next evaluated the impact of ASEI/ASER cell-fate on connectivity in sly-6 mutant (two ASERs) and sly-6 misexpression (two ASEIs) backgrounds. While we found modest changes in the number of synaptic puncta, a presynaptic cell-fate change was insufficient to symmetricize this connection. We are currently investigating the impact of other cell-fate determinants, laterally expressed genes, and environmental cues on this connection. In conclusion we have added to the rich literature of ASE and AWC asymmetries by describing a lateralized synaptic connection and in part its genetic determinants. We hope that our studies will yield insight into how genes and/or the environment determine the development and plasticity of functional lateralized circuits.

983A Ankyrin and beta-spectrin regulate the unconventional “potassium” channel UNC-58. Philippe Tardy, Marine Mercier, Olga Andrini, Maelle Jospin, Thomas Boulin Institut NeuroMyoGène, Lyon, FR.

Two-pore domain potassium channels (K2P) control neuronal excitability and play a central role in the establishment and maintenance of the resting membrane potential of almost all animal cells. In C. elegans, 47 genes code for K2P channel subunits but only four have been studied so far. We have found that heterologous expression of the uncharacterized K2P UNC-58 in Xenopus oocytes causes a strong depolarisation of the resting membrane potential, which is not consistent with the activity of a potassium-selective channel. In fact, ionic replacement experiments demonstrate that UNC-58 is highly permeable to sodium, which has never been reported in a two-pore domain potassium channel.

unc-58 is expressed in body wall muscles, and A, B and D-type motoneurons. unc-58 null mutants are loopy on solid media and have a significantly decreased thrashing rate in liquid. Conversely, the dominant mutant unc-58(e665) displays a distinctive phenotype, whereby worms flip rapidly around their body axis, but are unable to move forward or backward. To identify factors that directly control the number, activity or distribution of K2P channels in vivo, we have performed a forward genetic screen for suppressors of unc-58(e665). Among 133 mutants, we have found six alleles of unc-44/ankyrin and unc-70/beta-spectrin.

In C. elegans, null mutations in unc-70 and neuronal unc-44 isoforms cause axonal fragility and strong axon guidance defects, respectively. Strikingly, these phenotypes are not observed in mutants from our screen. Using zif-1/2F1-mediated tissue-specific depletion, we show that unc-58(e665) paralysis is due to the activity of the channel in A and B motoneurons, but not in body wall muscles or D-type neurons. Therefore, UNC-44 and UNC-70 must be required for UNC-58 function in these neurons.

In addition to its role in motoneurons, we find that unc-44/ankyrin regulates UNC-58 trafficking to the cell surface in body wall muscles. In vertebrates, ankyrin binding to phosphatidylinositol 3-phosphate (PtdIns(3)P) is required for microtubule-dependent cargo transport. In an unc-44 allele from our screen, a serine to leucine mutation near the PtdIns(3)P binding pocket is sufficient to disrupt UNC-58 localisation, and to relocate the channel to an intracellular compartment. This phenotype is specific for UNC-58, since two other muscle K2P channels, TWK-18 and SUP-9, are unaffected. Surprisingly, our beta-spectrin mutants do not alter the distribution of UNC-58, suggesting that ankyrin functions independently of beta-spectrin in muscle cells.

Taken together, our data highlight new roles for unc-58, unc-44/ankyrin and unc-70/beta-spectrin in the control of neuromuscular function in C. elegans.

984B Analysis of syndecan function at the C. elegans neuromuscular junction. C. VACHON1, L. TANG2, H.E BÜLOW2, J-L. BESSEREAU1 1) Institut NeuroMyogène, Université Claude Bernard Lyon 1-UMR5310 INSERM U1217, VILLEURBANNE CEDEX, Rhône, FR ; 2) Department of Genetics, Albert Einstein College of Medicine, Bronx, USA.

The extracellular matrix (ECM) plays an essential role in the development and function of organs and tissues. Syndecan is an ECM component that belongs to the heparan sulfate proteoglycan (HSPG) family. It is composed of 3 polysaccharidic chains linked to a core transmembrane protein. In C. elegans, syndecan is coded by a single gene sdn-1. It is required for normal vulva development and axonal guidance (Minniti et al., 2004) (Rhiner et al., 2005). Sugar chains are extensively modified including sulfation, acetylation and epimerisation of individual sugar residues. Recent reports suggest that some modifications are cell specific (Attreed et al., 2016) supporting the hypothesis of an HS code.

HSPG are present at neuromuscular junctions (NMJ) but their synaptic functions remain uncharacterized. In C. elegans body-wall muscle cell receive excitatory and inhibitory innervation from cholinergic and GABAergic motoneurons, respectively. Cepunctin / MADD-4 is an ECM protein secreted by motoneurons in the synaptic cleft. Specific combinations of Cepunctin isoforms trigger the clustering of acetylcholine or GABA receptors at synaptic sites. We generated a BFP knock-in allele to detect SDN-1 and observed that SDN-1 is present at NMJs and seems to be enriched at cholinergic neuro-muscular synapses. Using single-chain antibodies to label specific HS modifications in vivo suggests that some modifications could be prevalent at cholinergic junctions. Preliminary data indicate that sdn-1 disruption affects the localization of acetylcholine receptors. Ongoing experiments and future plans will be presented at the meeting.

985C Electrical synapses as modulators of neural activity. L. Voelker^{1,2}, J. Bai^{1,2} 1) Fred Hutch Cancer Research Center, Seattle, WA; 2) University of Washington, Seattle, WA.

An animal must constantly adjust its behavior in order to respond to changing environments and fluctuations in internal states. Animals achieve this by altering neural activity levels through changes known as neural plasticity. While much research has focused on understanding changes that occur at chemical synapses, it is becoming clear that electrical synapses also have important roles to play. Electrical synapses are specialized sites of cytoplasmic communication between neurons and are known to coordinate local electrical activity and to pass the small molecules associated with neural plasticity. In C. elegans, electrical synapses are known to coordinate inputs from multiple different sensory modalities to influence behavior. I have shown that a single electrical synapse gene, LIN-19, is responsible for modulating several different behaviors. LIN-19 mutants are hyper-reactive in their responses to the aversive chemical quinine and fail to chemotax towards lysine. In addition, LIN-19 mutants travel further than wild-type worms in a 3D burrowing assay even in the absence of an attractant. These phenotypes are also observed in mutants lacking the guanylyl cyclase GCY-27, indicating that a decrease of cGMP levels has a similar effect on behavior as lack of particular electrical synapses. This suggests that electrical synapses may modulate neural activity and thus behavior by passing small molecules between cells.

986A Analysis of activity-dependent synaptogenesis in the nematode Caenorhabditis elegans. A. Weinreb, M. Jospin, JL. Bessereau Institut Neuromyogène, Université Claude Bernard Lyon 1 - CNRS UMR5310 - INSERM U1217, VILLEURBANNE CEDEX, FR.

In various systems, activity of neurons or muscle leads to various forms of plasticity, thus shaping network connectivity and regulating synaptic strength. In C. elegans, activity was demonstrated in a few systems to play a role in synapse formation and function. We chose to analyze the development of the 3 cholinergic SAB motor neurons that are innervating the head muscles. In this system, electrical silencing of the muscle cells during development was demonstrated to regulate SAB morphology (Zhao and Nonet, 2000). Using fluorescently-tagged acetylcholine receptors (AChR), we observed SAB overgrowth and ectopic synapse formation in unc-13 and unc-18 mutant worms in which neuromuscular transmission was disrupted. We could confirm that this effect is not due to the loss of movement because there is no SAB overgrowth in the unc-54 myosin mutants that are paralyzed. To silence the electrical activity of muscle cells, we specifically expressed in muscles the Drosophila HisClI histamine-gated chloride channel and the TWK-18 temperature-dependent potassium channel. In both conditions, inhibition of muscle cell activity causes SAB overgrowth, suggesting that a retrograde factor(s) controls SAB development. We could further pinpoint a critical developmental window at the L1 stage during which SAB development is plastic. In addition, we demonstrated that chronic – but not acute – increase of synaptic transmission through acetylcholinesterase inhibition leads to a decrease in the number of synaptic AChRs, suggesting an activity-dependent regulation of AChR number during development. Through a transcriptomic approach, we expect to find genes involved in the overgrowth of the SAB and the regulation of AChR number. We are using RNA-Seq to detect genes differentially expressed upon electrical manipulation of the muscle cells. In parallel, we are using the tools that we developed to better define the conditions leading to SAB overgrowth and AChR downregulation, as well as testing a number of candidate genes.

References:

987B Possible synaptic remodeling of IL2 neurons for behavioral plasticity in C. elegans. Hyunsoo Yim, Sangwon Son, Junho Lee School of Biological Science, College of Natural Science, Seoul National University, Seoul, KR.

Many organisms undergo remodeling of neural circuits among developmental stages, and it is fundamental for them to adopt stage-specific behaviors. Nictation, a dauer-specific dispersal behavior of the C. elegans, is regulated by IL2 neurons which were known as cholinergic sensory neurons. As dauer is an alternative developmental stage and the larvae show quite different behavioral pattern, it is strongly expected that the neural circuit of the dauer stage may have undergone synaptic remodeling. In this study we examined the possibility that IL2 neurons make a novel synapse with body wall muscle. We tried to confirm the neuromuscular junctions between IL2 and BWM by the co-localization of pre- and pos-synaptic markers, and by iBLINC [1]. Further studies include the investigation of signaling pathways responsible for the IL2 NMJ remodeling in dauer larvae by candidate screening, and the necessity of IL2 NMJ in the nictation behavior. Our study will contribute to the understanding of the developmental plasticity of the synapse remodeling and the behavior.


988C Characterizing odor receptors in the AWC neuron. J.J. Young\textsuperscript{1}, M. Harwood\textsuperscript{1}, S. Maher\textsuperscript{1}, L. Resch\textsuperscript{1}, C. Dalton\textsuperscript{1}, S. Nathan\textsuperscript{1}, A Cox-Harris\textsuperscript{1}, R. Morton\textsuperscript{1}, B. Mosqueda\textsuperscript{1}, E. Jerome\textsuperscript{1}, V. Thakker\textsuperscript{1}, W. Mankins\textsuperscript{1}, L. Rost\textsuperscript{1}, L. Daffeh\textsuperscript{1}, K. Tirumalasetty\textsuperscript{1}, A. Quiogue\textsuperscript{1}, I. DiBianca\textsuperscript{1}, C. Ghaffari\textsuperscript{1}, Y.-P. Hsieh\textsuperscript{2}, N. L'Etoile\textsuperscript{3} 1) Dept Biol, Mills Col, Oakland, CA; 2) Institute of Molecular Biology, Academia Sinica, Taiwan; 3) Departments of Cell and Tissue Biology and Medicine, University of California San Francisco.

The nematode Caenorhabditis elegans uses a small number of sensory neurons to respond to a wide variety of odors. We
seek to understand how information is processed in this system. The identities of the odor receptors, the odorants they respond to, and their patterns of expression are largely unknown. Our current goal is to identify and characterize odor receptors in the AWC neuron. We are using translational GFP reporters to localize candidate odor receptors, and are generating odor receptor knockouts using the CRISPR technique. Knockout nematodes will be tested in chemotaxis assays for odor responses. This research program is also being incorporated into undergraduate laboratory courses, engaging undergraduates in authentic research.

989A Mechanisms of receptor guanylyl cyclase-mediated thermosensory signal transduction. Yanxun Yu, Piali Sengupta Biology Department, Brandeis University, Waltham, MA.

The C. elegans genome encodes multiple members of the receptor guanylate cyclase (rGC) family of signaling proteins. Many of these proteins have been implicated in sensing environmental stimuli including salt, gas, and pH. We previously showed that three rGCs (GCY-8, GCY-18, GCY-23) that are expressed specifically in the AFD thermosensory neurons are both necessary and sufficient to mediate thermosensation. Specifically, while each of these rGCs appears to act partly redundantly to mediate thermosensation in AFD, GCY-18 or GCY-23, but not GCY-8, is sufficient to confer thermosensation when ectopically expressed in a subset of non-thermosensory neurons and non-neuronal cells. We are using mutational analyses to identify residues and domains that may mediate thermosensation, and/or define thermosensory response thresholds, of these AFD-specific rGC proteins.

Similar to other rGCs, thermosensory rGCs are single-pass transmembrane proteins, consisting of a divergent extracellular domain (ECD), a transmembrane domain (TMD), and a more conserved intracellular domain (ICD). The ICD can be further divided into an N-terminal kinase homology domain (KHD), a short hinge domain, and a cyclase domain (CD) that mediates catalytic activity. To determine the roles of different rGC domains in mediating thermosensation, we misexpressed mutant proteins in the ASE chemosensory neurons and assessed their ability to confer temperature responses. We find that the ECDs of GCY-18 and GCY-23 are dispensable for conferring temperature responses, but may contribute to determining their response threshold. We also find that replacing the CD of GCY-8 with that of GCY-23 is sufficient to confer responses similar to those conferred by the full-length GCY-23 protein. Conversely, replacement of the GCY-23 CD with the GCY-8 CD abolishes the thermosensory properties of GCY-23. We will also report the roles of the KHD and hinge domains on thermosensation. We are currently further examining the function of the thermosensory rGC CDs, characterizing trans-acting factors that may modulate the functions of these rGCs, and determining whether the roles of the domains identified via misexpression in ASE are similar in the endogenous AFD context.

990B NCS-2 mediates activity-dependent asynchronous cholinergic release. Keming Zhou1,2, Salvatore Cherra1, Alexandr Goncharov1,2, Yi Shi Jin1,2 1) Division of Biological Sciences, Section of Neurobiology, University of California, San Diego, La Jolla, CA 92039, USA; 2) Howard Hughes Medical Institute, University of California, San Diego, La Jolla, CA 92039, USA.

An important principle of brain function is activity balance between excitation and inhibition (E-I balance). Excitation propagates neural firing, and inhibition shapes and refines spatiotemporal patterns of neural activity. Impaired E-I balance leads to dysfunction of neural circuits and is widely linked to neurological and neuropsychiatric disorders, such as epilepsy, autism, schizophrenia and Alzheimer's disease. However, the underlying mechanisms and molecular signaling pathways leading to disruption of E-I balance remain unclear. In Caenorhabditis elegans, sinusoidal locomotion is achieved by balanced cholinergic excitation and GABAergic inhibition. Here, we investigated regulations of excitation and inhibition balance in locomotor circuit using genetic mutants and optogenetic stimulation. We discover that activation of excitatory cholinergic neurons for a short-duration elicits an enhancement of presynaptic strength. In contrast, sustained activation of the excitatory neurons results in disruption of E-I balance, and dampens the excitability of downstream inhibitory GABAergic neurons cell-non-autonomously. The function of NCS-2 depends on its properties of Ca²⁺ binding and membrane association. These results reveal a previously uncharacterized synaptic mechanism and molecular pathway in regulating excitation-inhibition balance, and shed light to the understanding of neurological disorders associated with excitation-inhibition imbalance.

991C Unraveling the fuction of APL-1, the C. elegans ortholog of human APP. A.G. Alexander1,2, A. Nabila1, F. Granovsky1, J. Yang1,2, C. Li1 1) Department of Biology, City College of New York, New York, NY; 2) Department of Biology, The Graduate Center CUNY, NEW YORK, NY.

Mutations in the amyloid precursor protein (APP) are implicated in Alzheimer’s disease (AD). Postmortem observations reveal that one cleavage product of APP, Ab, aggregates to form senile plaques in the brains of AD patients. While APP is a contributing factor in the pathogenesis of AD, it is also required for normal brain development and viability. Similarly, when apl-1 is absent in C. elegans, as in apl-1(yn10) homozygous mutants, larval lethality is observed: C. elegans become trapped in their cuticle as they transition from L1 to L2 and consequently, die. Interestingly, apl-1(yn5) mutants, which express only the extracellular fragment of APL-1, are viable. Furthermore, pan-neuronal expression of APL-1 is sufficient for viability. When apl-1 expression is knocked-down by RNAi feeding in wild-type animals, no phenotypes are seen, presumably because there is residual apl-1 activity. By contrast, when apl-1 is knocked down in apl-
1(y5n) mutants, the F1 apl-1(y5n) progeny are unable to survive. We mutagenized apl-1(y5n) animals with EMS and screened for viable F2 progeny on apl-1 RNAi bacteria. We screened 60,000 haploid genomes and isolated 7 mutants. We are currently mapping these mutants and conducting assays to determine their phenotypes in a wild-type background.

Preliminary data suggest that APL-1 is targeted to mitochondria. We will verify this by driving APL-1 expression under its endogenous promoter and labeling APL-1 with a fluorescent tag. We will be labeling neuronal mitochondria with a complementary tag and looking for colocalization, as well as changes in mitochondrial dynamics. Collectively, these approaches are aimed at determining the role of the extracellular domain of APL-1 and its interacting partners. These experiments will provide insights into the role of APP in normal human development and the pathogenesis of AD.

992A  The Coordination of Reproductive Success and Stress Resilience by TCER-1/TCERG1.  FRANCIS AMRIT1, CARTER MASON1, MAINPAL RANA2, JUDITH YANOWITZ2, ARJUMAND GAHAZI1  1) Departments of Pediatrics, Developmental Biology and Cell Biology, University of Pittsburgh School of Medicine; 2) Department of Obstetrics and Gynaecology, Magee Women's Research Institute (MWRI), University of Pittsburgh.

Reproduction and stress-resistance capacity are life-history traits that govern the evolutionary success of all organisms. However, molecular mechanisms by which these processes are coordinated in metazoans are poorly described. Here, we report a role for TCER-1, the worm homolog of human transcription elongation and splicing factor, TCERG1, in enhancing reproductive capacity in the face of pathogen attack. In C. elegans, eliminating germline stem cells (GSCs) increases lifespan and elevates stress resistance. Previously, we demonstrated that TCER-1/TCERG1 specifically promotes the longevity of germline-less C. elegans but is critical for reproductive health in normal, fertile animals. Many genes that promote the longevity of GSC-less adults also enhance their stress tolerance. Surprisingly, we have discovered that TCER-1/TCERG1 inhibited stress tolerance in adult worms. tcer-1 mutants exhibited elevated resistance against multiple biotic and abiotic stressors, including thermal stress, oxidative stress, ER stress and infection by the opportunistic pathogen Pseudomonas aeruginosa. TCER-1/TCERG1 impairs stress resistance by inhibiting PKM-1, a conserved innate immunity-promoting kinase. PKM-1-target genes are up-regulated in tcer-1 mutants and the immunoresistance of tcer-1 mutants is dependent upon PMK-1. Overall, our data suggest that TCER-1/TCERG1, promotes reproductive fitness and represses stress resilience under normal conditions. Under stressful conditions, TCER-1/TCERG1 is repressed resulting enhanced stress resilience and reduced reproductive capacity. Unlike most pro-longevity genes, TCER-1/TCERG1 appears to have distinct regulatory effects on lifespan, stress resistance, and fertility, suggesting that the protein may function as a molecular rheostat for coordinating major life-history traits. The phenotypic uncoupling observed in tcer-1 mutants provides a unique platform to dissect the pathway that governs resource allocation between procreation, stress response and somatic maintenance in metazoans.

993B  KIN-4/MAST kinase binds DAF-18/PTEN through its PDZ domain and contributes to the longevity of daf-2/insulin/IGF-1 receptor mutants.  S.A. An1, E. Choi1, J.S. Yang1, K. Seo1, W. Hwang1, H. Son1, H.J. Nam1, N. Nguyen1, J.Y. Yoo1, S. Kim1,2, S.J. Lee1  1) Department of Life Sciences; 2) I-BIO, POSTECH, Pohang, South Korea.

PDZ domain-containing proteins (PDZ proteins) comprise a large family of scaffold proteins that participate in organizing components in various signal transduction pathways. However, it remains largely unknown regarding the role of PDZ proteins in C. elegans insulin/IGF-1 signaling (IIS) pathway, which regulates lifespan, stress resistance and dauer formation. To address this issue, we first performed an RNAi-based lifespan screen targeting 49 out of 70 genes that encode putative PDZ proteins using wild-type (N2) and long-lived daf-2/insulin/IGF-1 receptor mutant animals. We found that knockdown of kin-4, a PDZ-containing microtubule-associated serine-threonine (MAST) kinase, largely suppressed the long lifespan of daf-2(e1370) mutants. We confirmed this lifespan result by using kin-4(tm1049) deletion mutants. kin-4(tm1049) mutations also significantly decreased the enhanced oxidative stress resistance caused by daf-2(e1370) mutations. We then showed that overexpression of kin-4 fused with GFP, which was detected mainly in the pharynx and the intestine, was able to increase lifespan. These data suggest that kin-4 is necessary and sufficient for longevity. Next, we performed an extensive yeast two-hybrid screen to identify proteins that interacted with the PDZ domain of KIN-4. Among the 41 candidates, we identified DAF-18/PTEN phosphatase, which acts downstream of DAF-2 and is required for the longevity of daf-2 mutants. We confirmed the physical interaction between KIN-4 and DAF-18 by using in vitro pull down and mammalian cell culture-based co-immunoprecipitation assays. We showed that the PDZ domain of KIN-4 bound to a canonical PDZ-binding motif located at the C-terminal end of DAF-18. Importantly, disruption of the interaction between KIN-4 and DAF-18 partially but significantly suppressed the long lifespan of daf-2(e1370) mutants. Overall, our data suggest that KIN-4 contributes to longevity conferred by reduced IIS via binding DAF-18 through its PDZ domain. We are currently examining which longevity transcription factors among DAF-16/FOXO, HSF-1/heat shock factor 1 and SKN-1/NRF2 mediate the longevity signals transduced from the interaction between KIN-4 and DAF-18. Our study will help understand how PDZ scaffold proteins regulate organismal lifespan by participating in IIS pathway.

994C  A compact and modular lifespan machine for aging studies in Caenorhabditis elegans.  T. Anupom1, M. Rahman2, S. Gupta2, S. Vanapalli2, J. Blawzdziewicz2, N. Szewczyk4, M. Driscoll5  1) Department of Electrical and Computer Engineering, Texas Tech University, Lubbock, TX; 2) Department of Chemical Engineering, Texas Tech University, Lubbock, TX; 3) Department of Mechanical Engineering, Texas Tech University, Lubbock, TX; 4) MRC/Arthritis Research UK Centre for Musculoskeletal Ageing Research, University of Nottingham, Nottingham, United Kingdom; 5) Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ.

The lifespan of an organism is the ultimate indicator of the molecular mechanisms active during aging of an organism. Short lifespan, well-established genetics, and conserved signaling pathways make the nematode Caenorhabditis elegans an attractive model for aging research. Large-scale automated screens for longevity genes in the nematode C. elegans often use bulk liquid culture combined with genetic or drug-induced blocking of progeny, introducing physiological stress on the animals. Common
small-scale pilot screens adopt agar-based methods, which necessitate the tedious task of repetitively picking and transferring animals. In both approaches, it is challenging to add or remove reagents (e.g., food or drugs) at multiple time points during the lifespan, impairing detailed aging investigations.

We report a compact and modular lifespan machine based on microfluidics that addresses the limitations of current methods. Our lifespan machine is comprised of three modules, (i) a microfluidic lifespan chip, (ii) a fluid exchange module and (iii) an imaging module. The microfluidic device houses crawling animals and an on-chip filter that allows the selective removal of progeny. The fluid exchange module controls the flow rate, duration and frequency of fluid injection for washing out progeny and for regularly feeding worms. A microcontroller is integrated into the system that is commanded by a simple GUI, allowing user-defined control of the fluid exchange module. The imaging module consists of a camera sensor and on-board Wi-Fi that captures, transmits and stores images at user-defined intervals. We designed custom software that analyzes the locomotory motion and conducts lifespan analysis. The entire system has a footprint of no more than 20×20 cm² and has been designed such that it does not require any physical interaction, once the lifespan experiment is initiated.

To reduce physiological stress on the animals, we have optimized the device geometry and feeding protocols such that C. elegans gait, body size, and lifespan are consistent with aging assays on agar. We find that the lifespan curves of wild-type and genetic mutants are consistent with the literature reports. In summary, the compactness and modularity of our approach allows building several of these systems, enabling highly parallelized cross-sectional and longitudinal aging experiments.


Reactive oxygen species production is a physiological process necessary for normal cell function, but in excessive amounts, it can lead to oxidative stress, involved in the etiopathogenesis of various diseases. Superoxide radical are highly reactive oxygen species which can be neutralized through catalytic action of superoxide dismutase (SOD). Therefore, three new SOD mimetics were synthesized (I- C24H27Cl2CuN7O8, II- C21H26Cl2CuN4O12, III- C14H23ClCuN5O6) in order to be able to prevent or treat oxidative stress-related diseases. The nematode C. elegans is structurally simple but its genome and its metabolic and biosynthetic pathways are highly conserved in mammals. Thus, in our study to investigate toxicological and antioxidant effects of SOD mimetics in vivo. Wild type (N2) young adult worms were exposed to the three SOD mimetics dissolved in 0.5% dimethyl sulfoxide (final concentration) at various concentrations for 1 hour in M9 buffer without food. After washing, worms were subjected to toxicological and antioxidant assays. To investigate possible toxicological effects, behavior parameters (pharynx pumping rate, defecation cycle length and thrashes) and reproduction (brood size) were analyzed using a microscope. Antioxidant activity was evaluated through survival after juglone (5-hydroxy-1,4-naphthoquinone) exposure, an in vivo superoxide radical generator. Worms were exposed to 200 µM juglone for 1 hour, and then scored as dead or alive using a microscope. C. elegans treated with the higher concentrations used of SOD mimetics were not significantly different from control in pharynx pumping rate (~245/minute), defecation cycle length (~52 seconds), number of thrashes (~204/minute) and brood size (~205/worm), showing that the compounds did not exert toxic effects. Antioxidant activity of the mimetics was demonstrated by the increased survival in treated worms exposed to juglone. Compound I increased survival at 0.50µM (~36%), II at 0.25µM (27%) with maximum effect at 1µM (55%) and III at 0.25µM (18%) with maximum effect at 2µM (32%) compared to control. Thus, the three SOD mimetics showed high antioxidant activity in low concentrations with no toxicity and deserve more studies, including their effectiveness in oxidative stress-related diseases models.

996B Fine scale electrophysiological analysis of pharyngeal aging and a transition-state model of activity. S.A. Banse1, B.W. Blue1, K.J. Robinson2, J.H. Willis1, J.C. Weeks2, S.R. Lockery2, P.C. Phillips1 1) Institute of Ecology and Evolution, University of Oregon, Eugene, OR; 2) Institute of Neuroscience, University of Oregon, Eugene, OR.

Because age-associated loss of neuromuscular function severely impacts quality of life, research models capable of identifying the etiology of lost function are particularly valuable. The C. elegans pharynx is a neuromuscular model whose visually observed age-dependent decline in contraction frequency is used as an experimental health marker. Although informative about overall health state, contraction frequency alone provides little information about the underlying cause for lost function. Fine scale analysis of electropharyngeograms recorded from aging animals provides an alternative assay more amenable to mapping age-related changes to the underlying neuromuscular circuit. We find that decreased pump frequency is associated with bursts of activity punctuated by increased frequency of pauses. Interestingly, although inter-pump interval during an activity bout changes with age, pause duration remains relatively unchanged. This suggests a decreased frequency or efficacy of cholinergic signaling during an activity bout. This reduced efficacy in neuronal modulation of pumping contrasts with M3 related glutamatergic signaling efficacy which holds longer into adulthood. This difference is visible both in the duration of pumps, as well as in average waveform shapes associated with the beginning and end of a pump. We present an activity-state based model to explain the observed decreased pump frequency. We argue that this new approach is an information dense measure that holds promise in mapping age-dependent changes to the functional subunits of pharyngeal activity.


In a world of uncertainty, an organism’s ability to mount a response to stress is fundamental to its interaction with the environment. Experimental exploration of that interaction benefits greatly from tight environmental control, as well as the ability to minimize the behavioral response of fleeing stress. We present a microfluidic platform that enables tight environmental
control, and high-throughput image acquisition while confining single animals to individual arenas that allow a broad range of behavioral activities. The platform is easily scalable, with two 50 arena arrays per chip, and an imaging capacity of 600 animals per imaging scanner. We present validation of the stress platform using osmotic stress, oxidative stress, and starvation, although the system should be adaptable to many stress paradigms.

998A Study and characterization of dynamic and stochastic expression of HSP-16.2 on C. elegans using a high-throughput microfluidic platform. Nadia Vertti-Quintero1, Xavier Casadevall i Solvàs1, Simon Berger1, Oliver Dressler1, Stavros Stavrakis1, Jan Gruber2, Rudiyanto Gunawan1, Andrew deMello1 1) Inst. Chemical- and Bioengineering, ETH Zurich, Zurich, CH; 2) Center for Life Sciences, Yale-NUS College, Singapore.

Heat shock proteins (HSPs) are essential in the maintenance of cellular proteostasis. Their expression and regulation have been shown to vary even among isogenic C. elegans cohorts, with individuals expressing higher levels of HSPs experiencing higher survival rates, which might reflect an underlying ability of each individual to survive upon exposure to heat challenge[1]. Interestingly, though, only worms that express highly at a specific time after heat shock are significantly longer-lived. This suggests that, rather than the level of expression itself, it is the dynamics of expression which correlate with lifespan. Indeed, the expression of HSPs at the whole organism level follows a dynamical behavior in time. We hypothesize that HSPs expression dynamics correlate with worm survival capability, a relationship that we aim to characterize and study.

Towards this end, we use the transgenic C. elegans strain TJ375 [hsp-16.2p::GFP(gpIs1)] and screen the expression of HSP after heat shock. We have built a high-throughput continuous-flow microfluidic platform that enables C. elegans in vivo population screening and sorting based on the quantification of GFP at the whole organism level. Each worm is read in a serial manner while in motion through a straight channel. The quantity of fluorescent proteins within each worm is screened, by means of a laser light sheet which excites the proteins of interest and light is collected by a PMT. Information signals can be processed in real-time for sorting downstream into three possible bins. The sorting mechanism is achieved by hydraulic on-chip valves, which deliver a maximum sorting throughput of 600 worms/min. Worms are then collected in individual vials with no significant loss in viability.

To study how HSPs are dynamically regulated in certain subpopulations and to understand the significance of these dynamics in both, stochastic distribution and longevity, we obtain quantitative assessments of the expression of native HSP-16.2 produced by isogenic cohorts at different ages, as well as at different times after exposure to heat shock. Using this data, we use the LASSO technique to infer the existence of singular subpopulations (with dynamics-specific gene expression profiles), which we will then validate with the appropriate sorting of subpopulations of interest and the measuring of their HSPs expression dynamics separately (through subsequent rounds of screening). We will also corroborate the existence of these specific expression dynamics by imaging single individuals immobilized in a microfluidic trap along 48 h after heat shock. Finally, the characterization of the effect of HSPs expression dynamics (i.e. level of transcriptional drift and time profile of expression) on the lifespan of worms will be assessed.


999B Involvement of a transthyretin-like gene in dietary restriction-mediated longevity in C. elegans. Dena Block1, Katelyn Chen1, Dustin Cox1, Kwame Twumasi-Boateng2, Michael Shapira1 1) Department of Integrative Biology, University of California at Berkeley, Berkeley, CA, USA; 2) BC Cancer Agency, Victoria, British Columbia, Canada.

The transthyretin-like (ttr) gene family of C. elegans consists of 59 genes that are related to vertebrate transthyretin, a protein that carries lipophilic molecules such as retinol and thyroid hormone through the blood and cerebrospinal fluid. Although levels of various ttrs increase during stress stimuli and aging, the functions of ttrs remain largely uncharacterized. We found that RNAi against several ttrs could extend lifespan and increase levels of DAF-16. Focusing on one, we found that ttr-1 disruption increased DAF-16 protein levels and extended lifespan in a daf-16-dependent manner. In contrast, ttr-1 overexpression reduced lifespan. A ttr-1::gfp fusion protein was localized to the periphery of the spermatheca, the vulva, and the intestine. Genetic analysis of ttr-1 mutants in conjunction with known regulators of lifespan revealed that ttr-1 disruption compromised lifespan extension following daf-2 knock-down (although DAF-16 nuclear localization was similar to that in wildtype animals), and abolished lifespan extension in eat-2 mutants, which exhibit slow pharyngeal pumping and represent a genetic model for dietary restriction. These results suggest that ttr-1 may play a role in signaling nutrient availability. Its expression in the intestine and somatic gonad may further suggest a role in communication between the soma and the germline.

1000C Quantitative and functional analyses of the organs and cells of post adult reproductive diapause animals. N. Burnaevskiy, M. Kaebrelein, A. Mendenhall  University of Washington, Seattle, WA.

C. elegans have had a fantastical impact on our understanding of the aging process. In addition to ability to extend lifespan upon many pro-longevity treatments, C. elegans will also live a normal, wild-type lifespan after prolonged (weeks) periods in diapausued juvenile states (L1 arrest, dauer). The mechanisms that preserve the soma of these animals during diapaus are therefore attractive targets for understanding and modulating the aging process. Remarkably, it was found that upon starvation even adult animals are able to enter into diapause state, known as adult reproductive diapause (ARD) (Giana Angelo and Marc R. Van Gilst, 2009). Like diapauses in juvenile animals, upon refeeding and exit of the adult reproductive diapause, their lifespan is fully preserved. After prolonged periods in the ARD state, animals show dramatic morphological improvements upon refeeding, and their post-diapause lifespan is indistinguishable from the animals that never experienced starvation. However, it
is unclear whether post-diapause animals follow the exact same physiological trajectory as their counterparts that were never starved. To gain a better understanding of the post-diapause animals we applied quantitative measures of physiology including detailed electrophysiological analysis of pharynx function, 3-dimensional mitochondrial structure examination and measurement of gene expression capacity. Gene expression capacity, the ability to express genes into functional proteins, is a fundamental cellular property that is highly variable between the intestine cells of individual animals, and may affect many aspects of cellular physiology. Our in-depth quantitative analysis provides some mechanistic insights into the cellular processes underlying longevity and healthspan of post-diapause animals.

1001A  Role of pharmacologically induced-TFEB in aging and age-related neurodegeneration.  Manish Chamoli¹, Shankar J Chinta¹, Minna Schmidt¹, Azar Shahmirzadi¹, Gordon J. Lithgow¹, Julie K Andersen¹ ¹) Buck Institute for Research on Aging, Novato, CA; 2) Touro University California, Vallejo, CA.

An important characteristic feature of aging and many age-related neurodegenerative diseases is a loss of protein homeostasis (proteostasis), which is accompanied by increased accumulation of damaged, misfolded and aggregated proteins. One of the vital components of proteostasis, autophagy, mediates the breakdown and recycling of proteins and other cellular components within the lysosome. Our central hypothesis is that age-related dysfunction in autophagy establishes a prodomal process resulting in decreased protein homeostasis which causes neurodegeneration. Age-related failure in autophagy could result from down regulation of transcription factor EB (TFEB), the master regulator of lysosomal biogenesis. This in turn would impact the cell’s ability to process damaged proteins. Recent studies from our labs reported that up-regulation of TFEB by either rapamycin or trehalose prevented neurodegeneration. Encouraged by these results, we conducted a small molecule screen to identify novel candidates that could induce TFEB and its target genes to levels far exceeding than already existing inducers (e.g. rapamycin and trehalose). Since then, we have discovered new class of compounds that gratuitously induce TFEB and its targets and are protective in in vivo proteotoxic models of neurodegenerative diseases. I will be discussing findings related to this study and potential implication of TFEB in preventing aging and age-related neurodegenerative diseases.

1002B  The adiponectin receptor PAQR-1 couples ER stress resistance with lipid homeostasis during ageing in Caenorhabditis elegans.  N. Charmpilas¹,², E. Kyriakakis¹,³, N. Tavamarakis¹,² ¹) Biology, University of Crete, Institute of Molecular Biology and Biotechnology, Heraklion, Crete, Greece; 2) Department of Biology, University of Crete, Greece.

In addition to serving fat and energy storage functions, the adipose tissue is a highly active metabolic and endocrine organ which exerts its systemic effects through secretion of adipose derived hormones (adipokines). The adipokine adiponectin affects organismal homeostasis through binding to its cognate receptors. Impairment of this signaling axis has been associated with human disease, particularly with type II diabetes and obesity. Manipulation of hormonal signaling pathways may influence lifespan by regulating survival under stress conditions. We examined the involvement of adiponectin signaling in the ER unfolded protein response (UPRER) and the regulation of lifespan in C. elegans. We find that animals with lesions in PAQR-1, one of the three adiponectin receptor homologues in C. elegans, are resistant to ER stressors. Resistance depends on the XBP-1 and PERK UPRER branches. paqr-1(tm3262) mutant animals exhibit elevated HSP-4/Bip baseline levels, and robustly induce chaperone expression and canonical UPRER upon ER stress, in a manner reminiscent of homresis. PAQR-1 deficiency differentially affects lipid content in ER-stressed animals in an age-dependent manner. Our findings indicate that ER stress triggers lipid macroautophagy (i.e lipophagy) and that PAQR-1-mediated signaling negatively regulates the expression of the ATGL-1 lipase. The selective ability of paqr-1 mutant animals to degrade lysids through lipophagy and ATGL-1-mediated lipolysis may underlie their enhanced survival when challenged with ER stressors. Elucidation of the molecular circuitry that links adiponectin signaling with lipid turnover may contribute to the development of effective therapeutic strategies for the treatment of obesity.

1003C  Role of Advance Glycation End products (AGEs) in modulating Caenorhabditis elegans feeding behavior.  Jyotiska Chaudhuri¹, Sanjib Guha¹, Neelanjan Bose¹, Tal Ronnen-Oron¹, Manish Chamoli¹, Brian Hodge¹, Shota Sawano², Austin Lim³, David Hall³, Mark Lucanic³, Gordon Lithgow³, Richmond Sarporg³, Pankaj Kapahi¹ ¹) The Buck Institute for Research on Aging, Novato, CA: 94945; 2) Department of Chemistry, Latimer Hall, University of California, Berkeley, CA: 94720.

Advanced Glycation End products (AGEs) have been implicated in a plethora of diseases including diabetic complications. In an earlier study, we have established a Caenorhabditis elegans mutant that accumulate a-dicarbonyls, the precursor of AGEs, as a model to study diabetes related neuronal damage. Recent RNAseq analyses of this mutant suggest significant differential expression of genes regulating processes such as fat metabolism, neurotransmission etc. that could potentially be mediated by AGEs. Preliminary data on this mutant also suggests that they exhibit enhanced rate of feeding. Supplementation of synthetic AGEs to the diet exacerbates this feeding phenotype in wild type animals early in life, resulting in pan-neuronal damage, accumulation of fat and shortening of lifespan. Therefore, we hypothesize that regulation of food intake could be modulated by alteration in AGE-mediated neurotransmission that could affect the feeding behavior related genes. This is a mechanism which is still poorly understood that could potentially impact diabetes related obesity and other complications.

1004A  Analysis of inter-individual transcriptional variability of stress response genes in C. elegans by high-throughput qRT-PCR in single worms.  Laetitia Chauve¹, Catalina Vallejo²,³,⁴,⁵,⁶, Janna Hastings¹, John Marion²,⁷, Olivia Casanueva¹ ¹) Epigenetics Department, Babraham Institute, CAMBRIDGE, GB; 2) EMBL,European Bioinformatics Institute,
Neurodegeneration. Here we hypothesized that neuronal MT stability regulates neuronal aging and organismal longevity. Caenorhabditis elegans 1006C

Our data suggested that neuronal MT regulation might be involved in stress response to modulate longevity. Examining the localization of synaptic vesicles. We found MT-regulating genes and MT drugs could modulate the age-accelerated age-dependent mobility decline. We further tested the effect of aging on MT-based intracellular transport by

Our data suggested that stabilizing neuronal MT enhance health span by improving mobility, and destabilization of MT

microtubule (MT) regulation is involved on several levels in neuronal function and maintenance of neuronal structure, and also appears to be a general downstream indicator and effector in age-dependent

organism’s overall metabolism and affect homeostasis and longevity. MTs are essential cytoskeleton involved in cell division, shaping the cell and intracellular transport. Microtubule (MT) regulation is involved on several levels in neuronal function and maintenance of neuronal structure, and also appears to be a general downstream indicator and effector in age-dependent neurodegeneration. Here we hypothesized that neuronal MT stability regulates neuronal aging and organismal longevity. Consistent with previous reports, we found that MT-regulating genes played a role in maintaining neuronal integrity during aging. We then tested the lifespan of genetic mutants of MT-regulating genes in C. elegans and found that loss of MT stabilizing genes shortened lifespan, while loss of MT destabilizing genes enhanced lifespan. Decline in mobility is often associated with aging. Our data suggested that stabilizing neuronal MT enhance health span by improving mobility, and destabilization of MT accelerated age-dependent mobility decline. We further tested the effect of aging on MT-based intracellular transport by examining the localization of synaptic vesicles. We found MT-regulating genes and MT drugs could modulate the age-associated changes in vesicle localization. Finally we tested the genetic interaction between MT genes and aging regulators. Our data suggested that neuronal MT regulation might be involved in stress response to modulate longevity.

1005B Neuronal Microtubule Stability Modulates Longevity in C. elegans. Aiping Xu, Lizhen Chen Barshop Institute for Longevity and Aging Studies, Department of Cell Systems and Anatomy, UT Health San Antonio, San Antonio, TX. Aging impacts the function of the nervous system and is the major risk factor for neurodegenerative diseases and is a fundamental problem in basic neuroscience and in human health. On the other hand, the nervous system corporate the organism’s overall metabolism and affect homeostasis and longevity. MTs are essential cytoskeleton involved in cell division, shaping the cell and intracellular transport. Microtubule (MT) regulation is involved on several levels in neuronal function and maintenance of neuronal structure, and also appears to be a general downstream indicator and effector in age-dependent neurodegeneration. Here we hypothesized that neuronal MT stability regulates neuronal aging and organismal longevity. Consistent with previous reports, we found that MT-regulating genes played a role in maintaining neuronal integrity during aging. We then tested the lifespan of genetic mutants of MT-regulating genes in C. elegans and found that loss of MT stabilizing genes shortened lifespan, while loss of MT destabilizing genes enhanced lifespan. Decline in mobility is often associated with aging. Our data suggested that stabilizing neuronal MT enhance health span by improving mobility, and destabilization of MT accelerated age-dependent mobility decline. We further tested the effect of aging on MT-based intracellular transport by examining the localization of synaptic vesicles. We found MT-regulating genes and MT drugs could modulate the age-associated changes in vesicle localization. Finally we tested the genetic interaction between MT genes and aging regulators. Our data suggested that neuronal MT regulation might be involved in stress response to modulate longevity.

1006C The adapted endoplasmic reticulum (ER)-associated protein quality control (ERQC) is critical for the long-lived Caenorhabditis elegans rpn-10(ok1865) proteasomal mutant. M.N. Chinchankar1,2,3, S.K. Maddux1,2,3, K.A. Rodriguez4,5, A.L. Fisher1,2,3,5 1) Barshop Institute for Longevity and Aging Studies, Department of Cell Systems and Anatomy, UT Health Science Center at San Antonio (UTHSCSA), San Antonio, TX; 2) Center for Healthy Aging, School of Medicine, UTHSCSA, San Antonio, TX; 3) Division of Geriatrics, Gerontology, and Palliative Medicine, Department of Medicine, UTHSCSA, San Antonio, TX; 4) Department of Cell Systems and Anatomy, UTHSCSA, San Antonio, TX; 5) San Antonio GRECC, South Texas VA Healthcare System, San Antonio, TX. Protein degradation mechanisms, such as the ubiquitin-proteasome system (UPS) and autophagy, are integral to proteostasis for optimal maintenance and preservation of the proteome. Their reduced efficiency during aging results in the accumulation of misfolded and aggregated proteins which potentiate several age-related proteotoxic disorders. Paradoxically, our lab reported that the Caenorhabditis elegans proteasomal mutant rpn-10(ok1865) mutant exhibits enhanced proteostasis and extended longevity. The RPN-10/PSMD4 subunit is a 19S regulatory particle (RP) ubiquitin receptor of the 26S proteasome that targets polyubiquitinated soluble substrates to the 20S core particle (CP) for degradation. The proteasome dysfunction of the rpn-10 mutant, characterized by reduced but not inhibited ubiquitin fusion degradation (UFD), confers remarkable resistance against many proteomic challenges including aberrant metastable and aggregation-prone proteins. We ascertained that the compensatory upregulation of autophagy and SKN-1/Nrf-mediated responses partially contribute to the robust rpn-10 mutant phenotype. To elucidate its complete suite of protective mechanisms, we analysed our RNA-sequencing data which revealed that several endoplasmic reticulum (ER) protein quality control (ERQC) genes were transcriptionally upregulated in the rpn-10 mutant adult nematodes. From our genome-wide RNA interference (RNAi) screen for enhancers and suppressors of UFD, we identified the ER membrane factor JAMP-1, which is known to recruit proteasomes for ER-associated degradation (ERAD), as a suppressor of the UFD deficit in the rpn-10 mutant. Based on these results, we hypothesized that the rpn-10 mutant might exhibit enhanced ER proteostasis or ERAD activity. I thus found that the rpn-10 mutant cytoplasmic proteostasis and longevity benefits are highly contingent on certain ER components such as the ER master chaperone hsp-3/-4 (BiP/grp78) and ERAD ATPase cdc-48.2 (p97/VCP/CDC48). Additionally, the attenuated expression of the aggregation-prone mutant a-1 antitrypsin (ATZ) reporter proves that the ER proteostasis is also ameliorated in the rpn-10 mutant. Moreover, its high ER stress resistance
but lower ER stress induction than the wild-type indicates optimized ER homeostasis in the rpn-10 mutant. Therefore, I postulate that the rpn-10 mutant possesses adapted ERQC which critically links graded proteasomal function with improved proteostasis and increased lifespan.

1007A Analysis of nutritional supplement effects on Caenorhabditis elegans lifespan. Austin Chriske1, Brandon Metzger2, Kirsten Crossgrove1  1) Biological Sciences, University of Wisconsin-Whitewater, Whitewater, WI; 2) Standard Process Inc, Palmyra, WI.

The molecular basis for the regulation of aging has been extensively characterized in Caenorhabditis elegans. We are using C. elegans to test the effects of nutritional supplements on lifespan. We have observed lifespan extension with purified grape seed extract and the commercial supplement OPC Synergy® (Standard Process Inc.) using manual lifespan assays. We are currently using C. elegans mutants to try to isolate the genetic pathways involved in lifespan extension by OPC Synergy. We have also assembled a lifespan machine with six scanners (Stroustrup et al. 2013. Nature Methods, 10: 665-673) and will discuss how it compares to manual assays, both in terms of results and ease of use. Our goal is to use the higher statistical power of lifespan machine experiments to perform high throughput screening of nutritional supplements for efficacy and pathway analysis.

1008B Electrophysiological characterization of pumping in C. elegans models of frontotemporal dementia. Heather Currey1, Brian C. Kraemer1,2, Nicole F. Liachko1,2  1) Research and Development, Veterans Affairs Puget Sound Health Care System, Seattle, WA; 2) University of Washington, Seattle, WA.

Frontotemporal dementia (FTD) is the second most common pre-senile dementia, affecting more than 50,000 people in the United States alone. This devastating neurodegenerative disease is characterized by the dysfunction and death of neurons in the frontal and temporal lobes of the brain, accompanied by profound changes in behavior and cognition leading to death. Pathologically, this disease is divided into two major groups. Approximately 40% of patients accumulate aggregates of the protein tau in disease affected neurons, while another 50% of patients accumulate aggregates of the protein TDP-43. These proteins appear functionally unrelated, as tau is a microtubule binding protein promoting assembly and stability of microtubules while TDP-43 is involved in many aspects of RNA metabolism from transcription to translation. Therefore, these two pathological variants of FTD may represent two different diseases that impact the same brain region but have different etiologies and cellular consequences and may require different therapeutic strategies for treatment. To study the cellular, molecular, and genetic underpinnings of tau and TDP-43 mediated neurotoxicity in a tractable model system, we have developed C. elegans models expressing these proteins pan-neuronally. Both tau and TDP-43 transgenic animals display early, progressive motor dysfunction, decreased lifespan, and age-dependent degeneration of specific types of neurons. To explore whether neurotoxic tau and TDP-43 affect C. elegans pharyngeal pumping, we utilized a non-invasive microfluidics-based device to record electrophysiological signals from pharyngeal muscles and neurons (electropharyngeogram, EPG). We found pump rate and pump duration have an inverse relationship within tau worms. Worms expressing human tau show a significant increase in pump frequency as compared to N2 worms. We also observed a decrease in pump duration with the same worms. Our initial data indicate that this is true in TDP-43 model worms as well. This phenotype may indicate increased neuronal hyperexcitability prior to neuronal injury and death. Future work will include EPG analysis of potential suppressors of hyperexcitability, as well as EPG analysis of loss of function genetic mutants in C. elegans homologs of tau and TDP-43.

1009C A transient arrest-survival state evolved to enhance fitness during pathogen exposure. H.M. Dalton1,2, S.P. Curran1,2,3  1) Dornsife College of Letters, Arts, and Sciences, Department of Biological Sciences, Molecular and Computational Biology, University of Southern California, Los Angeles, CA; 2) Leonard Davis School of Gerontology; 3) Norris Comprehensive Cancer Center.

Strategies to ensure fitness often slow organism development to delay reproduction and enhance survival. Across multiple organisms, reducing biosynthetic capacity results in developmental arrest early in life, but increased health- and lifespan post-developmentally. Here we demonstrate that these seemingly opposing responses are not an example of antagonistic pleiotropy; rather, we find that the nature of this developmental arrest is not sickness, but a regulated survival program responding to reduced cellular performance and mimicking a perceived reaction to toxin-producing pathogens, or otherwise, that can target eukaryotic protein synthesis. Early impairment of protein synthesis by targeting ribosome biogenesis (psr-11/RPS11) or translation initiation (egl-45/EIF3A) via RNAi, or ribosome progression via cycloheximide treatment, resulted in a specific arrest at larval stage 2 of C. elegans development, and C. elegans can survive in this state for periods that exceed their normal maximum lifespan. This arrest state is benign, as animals that recover can resume reproduction and live a normal lifespan. To protect animals in this pre-reproductive period, the arrest state affords resistance to thermal, oxidative, and heavy metal stress exposure. Although reduced protein synthesis results in cell-autonomous responses, reducing biosynthetic capacity via RNAi alone in the hypodermis was sufficient to initiate the organismal arrest state cell non-autonomously. We further show that loss of protein synthesis results in a reduction of pharyngeal pumping that is dependent upon AMPK-mediated signaling, which likely contributes to the increased longevity when protein synthesis is inhibited post-developmentally. Taken together, these data define the existence of a transient arrest-survival state initiated in response to a loss of protein biosynthesis and provide an evolutionary foundation for the conserved enhancement of healthy aging observed in post-developmental animals with reduced biosynthetic capacity.

1010A Coupling of mitochondrial metabolism and mRNA turnover modulates cellular homeostasis during ageing in C. elegans. I. Daskalaki1,2, M. Markaki1, N. Tavernarakis1,3  1) Institute of Molecular Biology and Biotechnology, Foundation
Mitochondrial metabolism is crucial for the modulation and maintenance of cellular homeostasis. Mitochondrial number and functionality largely determine intracellular ATP, Ca\(^2+\) and ROS levels, which in turn regulate cellular redox state, and may initiate signaling cascades involved in cell death, ageing and disease. Various stressors affect mitochondrial function and trigger retrograde and anterograde responses that enable cells to adapt their metabolism under stress conditions. Cellular metabolic state, upon stress and during ageing, is also determined by the fraction of mRNAs undergoing translation, storage or degradation. Recent studies have shown that tight control of mRNA turnover mechanisms is needed for normal cell physiology, highlighting their global regulatory capacity. The significance of these mechanisms is further supported by their evolutionary conservation from lower to higher eukaryotes. Emerging findings indicate that mitochondrial metabolism is altered in response to cytoplasmic stress, prompted us to examine the functional crosstalk between the two pathways. We are currently investigating this interplay in vivo by combining state of the art optical imaging methodologies with the power of genetic analysis in C. elegans. We find that reduced expression of key mRNA metabolism components causes alterations in mitochondrial morphology and function. Downregulation of the decapping protein DCAP-2 perturbs mitochondrial content and network integrity. In addition, mitochondrial impairment caused by pharmacological treatment, either with paraquat or CCCP, alters the expression of mRNA metabolism factors. Our results indicate that the two mechanisms are coupled and regulate energy metabolism, stress resistance and longevity. Elucidation of this complex association is important towards understanding how metabolic alterations trigger (re)adjustments in mitochondrial physiology that ultimately influence healthspan and ageing.

1011B Age-dependent protein aggregation initiates amyloid-β aggregation. N. Groh\(^1\), A. Bühler\(^2\), C. Huang\(^1\), K. W. Li\(^3\), P. Nierop\(^3\), A. Smit\(^3\), M. Faendrich\(^4\), F. Baumann\(^2\), D. C. David\(^1\) 1) German Center for Neurodegenerative Diseases (DZNE), Tübingen, DE; 2) Hertie Institute for Clinical Brain Research, Tübingen, DE; 3) VU University Amsterdam, Amsterdam, NL; 4) Ulm University, Ulm, DE.

Aging is the greatest known risk factor for neurodegenerative diseases associated with pathological protein aggregation such as Alzheimer’s disease. Identifying age-related factors that are relevant for disease initiation would be crucial for designing any early-stage therapy aimed at preventing neurodegeneration. Recently it has become clear that part of the proteome aggregates during normal aging in the absence of disease. Importantly, these age-dependent aggregation-prone proteins are significantly over-represented as minor protein components in disease-associated aggregates. This co-localization raises the possibility that age-dependent protein aggregation directly contributes to pathological aggregation. We reveal for the first time that highly insoluble proteins from aged C. elegans and aged mouse brains initiate amyloid-β (Aβ) aggregation in vitro. By analyzing insoluble extracts from different stages of adulthood we demonstrate that seeds triggering Aβ aggregation are formed during middle-age in C. elegans. Mass spectrometry analysis shows several late-aggregating proteins also identified in pathologic aggregates highlighting these as strong candidates for cross-seeding. In particular, we find that overexpression of the late-aggregating protein PAR-5 (C. elegans homolog of 14-3-3) accelerates Aβ proteotoxicity in C. elegans. Overall, this proof-of-concept study reveals a novel mechanism involved in the generation of disease-related aggregates and may provide a direct link between the molecular mechanisms of aging and pathogenesis.

1012C Characterization of a CRISPR/Cas9 Mediated C. elegans HSF-1 Model Reveals a Complex Oxidative Response and Novel Oocyte Expression. A. Deonarine\(^3\), M. Noble\(^1\), J. Winskas\(^2\), H. Wang\(^2\), L. Bowie\(^2\), A. Pyat\(^2\), S. Westerheide\(^1\) 1) Department of Cell Biology, Microbiology, and Molecular Biology, University of South Florida, Tampa, FL; 2) Department of Chemical and Biomedical Engineering, University of South Florida, Tampa, FL.

The heat shock response (HSR) is a major cytoprotective pathway induced by various proteotoxic stresses, such as oxidative stress and thermal stress, or pathophysiological conditions, such as neurodegeneration and cancer. These adverse cellular conditions result in the upregulation of mitochondrial chaperones which stabilize dysfunctional proteins, thus promoting cell survival. The major regulator of the HSR is Heat Shock Factor-1 (HSF-1), which is required for robust induction of the HSR, but also serves regulatory roles including growth and development. Previous C. elegans models with tagged HSF-1 relied on rescuing a mutant variant of HSF-1 in an exogenous locus, but it has been noted that mutant variants of HSF-1 can still retain some regulatory function in C. elegans and other organisms. This may lead to non-representational data of HSF-1 regulation collected in existing C. elegans models. To circumvent this and more completely understand HSF-1 activity at the whole-organism level, we have generated a novel, endogenously GFP tagged-model of HSF-1 in C. elegans utilizing CRISPR/Cas9 guided transgenesis. We show that our HSF-1 model produces a protein product that behaves similarly to previous models, though we observe robust HSF-1 expression in the oocytes of C. elegans, which previously not been reported, and a complex response to oxidative damage. Lastly, we also employ a novel approach at modulating focused HSF-1 activation utilizing plasmonic heating via an optical fiber. This model has a unique position as the only endogenously-tagged HSF-1 whole-organism model currently available, and represents a novel, efficient, experimental platform to study HSF-1 function within and outside of the HSR.

1013A An unbiased forward longevity screening reveals ppp-1 as a new regulator of longevity. M.J. Derisbourg\(^7\), L.E. Wester\(^1,2\), R. Baddi\(^1\), M.S. Denzel\(^1,2\) 1) Max Planck Institute for Biology of Ageing, Cologne, DE; 2) CECAD - Cluster of Excellence University of Cologne, Cologne, DE.

Ageing is a physiological process regulated in a multifactorial way. Besides being a major economic and social issue, the ageing process is also a main risk factor for many neurodegenerative disorders, cancer, and metabolic syndrome. Understanding how ageing can be modulated is therefore a main scientific interest. In the past decades, genes and molecular pathways that modulate ageing have been uncovered in the nematode Caenorhabditis elegans using whole genome RNAi screens and many of these genes are conserved in higher vertebrates. Although RNAi-based approaches were very successful,
we now know that chemical mutagenesis screens can reveal interesting gene variants that cannot be generated by RNAi-mediated gene knockdown. Therefore, we interrogated the capacity of the genome to regulate the ageing process by performing a new unbiased forward genetic screen using mutagenesis. This approach targets the entire genome, generates loss- and gain-of-function mutations, and affects genes expressed in any tissue of the worm. We generated over 100 long-lived mutants and causative mutations were detected by next generation sequencing. These aspects take this approach beyond previous longevity screens in the worm. Using this approach, we identified novel candidate longevity genes. Among them, we identified ppp-1, the C. elegans orthologue of the gamma subunit of translation initiation factor eIF2B, as a new regulator of longevity. Thus, this project breaks new ground and will provide a new perspective on lifespan-extending mutations.

**1014B Deconstructing longevity pathways: Drug cocktails for healthy lifespan extension in C. elegans.** T. Dessale1,3, J. Gruber1,2 1) Department of Biochemistry, National University of Singapore, Singapore; 2) Yale-NUS College, Science Division, Singapore; 3) Department of Biochemistry, University of Gonder, Ethiopia.

Many genes, drugs and environmental interventions have been reported to extend lifespan. However, compared to lifespan effects seen with genetic perturbations, the effect size of pharmacological interventions, are typically smaller. Furthermore, simultaneous genetic alteration of two or more pathways has been shown to extend lifespan synergistically. However, it is not clear whether combination of pro-longevity drugs would also have synergistic lifespan extension. Here, we first identified a set of well characterized and evolutionarily conserved ageing pathways. We then identified drugs which targets these pathways and extends lifespan in one or more model organisms. We then systematically explored the efficacy in terms of lifespan extension of all pairs of drugs, identifying two synergistic interactions. Finally, we explored selected triple drug combinations based on the two initial synergistic pairs. Our best triple combination results in doubling of median lifespan with similarly improved healthspan and without any evidence for fitness costs or developmental tradeoffs. To the best of our knowledge this is the largest reported lifespan effect for any pharmacological intervention in C. elegans. To explore the nature of the synergistic mechanism of lifespan extension we employed two approaches. First, we followed a paradigm-driven approach by determined lifespan efficacy of single and combined drugs in different mutants. Second, we used a data driven approach based on transcriptome profile for single drugs and drug combinations. Interestingly, we showed that both the paradigm approach and data driven approach agrees to explain the reason behind drug synergy. Moreover, we showed that TFG-beta signaling pathway is commonly enriched in all synergistic drug combinations but not in non-synergistic combinations. This may indicates that TGF-beta is required for the synergistic lifespan extension. Interestingly, drug combinations fail to show synergistic lifespan extension in TGF-beta downstream mutants. Hence, we believe that tools might be developed to predict synergistic interactions based on transcriptome profiles without the need of time-consuming combinatorial lifespan studies. Furthermore, the synergistic effect of our best combination is quantitatively preserved in Drosophila Melanogaster, suggesting evolutionary conservation of this synergy.

**1015C Longevity in the uncommon context of increased protein production and turnover: insight into the mode-of-action of the lifespan-extending compound royalactin.** G. Detienne1, W. De Haes1,2, B. Braeckman3, L. Schoofs1, L. Temmerman3 1) Functional Genomics and Proteomics, Department of Biology, KU Leuven University, Belgium; 2) Molecular and Functional Neurobiology, Department of Biology, KU Leuven University, Belgium; 3) Aging Physiology and Molecular Evolution, Department of Biology, Ghent University, Belgium.

Via an integrative approach using proteomic, biochemical and phenotypic techniques, we have gained molecular understanding of the physiological changes elicited by the longevity-promoting compound royalactin. While global metabolic heat output remains largely unaltered upon royalactin treatment, translation-associated molecular effectors including eukaryotic elongation factor 2 (EEF-2) and p70 S6 kinase (RSKS-1) are highly upregulated and crucial for the lifespan-extending effect of royalactin in Caenorhabditis elegans. Global protein synthesis measurements and lifespan experiments using cycloheximide-treated nematodes, indeed point towards augmented in vivo protein translation in royalactin-fed animals. This is a rather paradoxical outcome, since longevity is more commonly associated with reduced protein translation and turnover. Yet, here we identify and characterize a novel lifespan-extending intervention, where enhanced translation seems to be beneficial for the aging organism. Additionally, we discovered that royalactin enhances activity of the ubiquitin proteasome system (UPS) in multiple tissues, indicative of an increase in protein degradation as well. This points towards an alternative strategy to maintain protein homeostasis and support healthy ageing, focused more on actively removing damaged proteins and rebuilding cellular components (rather than preventing cellular damage and protein aggregation). The PLZF transcription factor EOR-1 seems to drive this process.

Royalactin is a royal jelly glycoprotein essential for queen differentiation in honeybees. Royalactin plays a central role in this process by switching on the epidermal growth factor (EGF) receptor signaling pathway, which ultimately leads to epigenetic changes and a long-lived queen phenotype. We previously provided the first evidence for its longevity-promoting actions in a non-insect species by studying royalactin-fed C. elegans (Exp. Geront. 2014). We demonstrated that royalactin requires both EGF (LIN-3) and its receptor (LET-23) for extension of nematode lifespan by ~25%. Royalactin also increases body size, maintains fecundity and enhances multiple forms of stress tolerance (Worm 2016) and locomotion in adult nematodes, suggesting a positive effect on healthspan as well. Yet, the mechanism by which royalactin exerts these pro-longevity effects remained fundamentally unknown.

In short, we present new insights into the molecular mechanisms of a rather unique lifespan-extending compound, which paradoxically increases global protein translation and turnover, and displays no trade-off regarding reproductive
capacity, body size and longevity. Further work is now being carried out to unravel which mRNAs are exactly targeted by rosyactin and the role of chaperones in this process, and assess whether its effects are also present in other organisms.

1016A Dissecting NHR-49’s role in stress response regulation. Kelsie Doering, Grace Goh, Stefan Taubert  Medical Genetics, Centre for Molecular Medicine and Therapeutics, UBC, Vancouver, British Columbia, CA.

Cells and organisms constantly experience harmful environmental stresses ranging from suboptimal temperatures to foreign substances such as nutrient contaminants or drugs. Oxidative stress occurs when reactive oxygen species (ROS), obligate and ubiquitous by-products of aerobic respiration, accumulate within the cell. This accumulation is toxic, and high ROS levels are hypothesized to cause and/or exacerbate diseases such as diabetes, cancers, and neurodegenerative disorders. MDT-15, a subunit of the Mediator complex, is a transcriptional coregulator which controls oxidative stress response gene programs in C. elegans. For example, SKN-1, the master regulator of oxidative stress responses in C. elegans, binds to MDT-15 and both proteins are necessary to activate SKN-1-dependent genes and to survive oxidative stress. Surprisingly, responses to the oxidative stressor tert-butyl hydroperoxide (tBOOH), which causes ROS accumulation, are SKN-1 independent. This suggests that additional, parallel mechanisms must exist that protect against excess ROS. We have discovered that the transcription factor NHR-49, which is required to express genes involved in lipid metabolism, including beta-oxidation and fatty acid desaturation, is also involved in this response pathway to induce tBOOH response genes such as fmo-2. To identify new players acting in this pathway, I am performing a reverse genetic screen using RNAi. As readout, I am using a transcriptional reporter, composed of the GFP reporter fused to the promoter of fmo-2. Targeted RNAi will identify which of the ~900 transcription factors, ~400 kinases and ~500 transcriptional coregulators are required for tBOOH-dependent induction of fmo-2, and thus might map into the NHR-49 pathway. In addition, we have also begun to explore whether mdt-15 and nhr-49 participate in other stress and defence responses, and/or whether they interact genetically with factors known to coordinate environmental adaptation.


Aging is driven by a loss of cellular and organismal homeostasis and is the key risk factor for multiple chronic diseases. Interventions that attenuate or reverse systemic dysfunction seen with age therefore have potential to ameliorate the comorbidities associated with old age and prolong healthy years in the elderly. Although loss of protein and transcriptional homeostasis are well-established causes of aging, the decline of RNA homeostasis and splicing fidelity with age remains poorly understood. A recent study in the lab has demonstrated pre-mRNA slicing fidelity as a biomarker and predictor of life expectancy in C. elegans. Using both in vivo alternative splicing reporters and molecular genetics, we have shown that dietary restriction mediated extension of lifespan require specific components of the splicing machinery. However, these factors act downstream of different longevity pathways. REPO-1 was specifically required for lifespan extension in the electron transport chain mitochondrial mutants isp-1(qm150) and clk-1(qm30) but had no effect on longevity through the TORC-1 pathway. On the other hand, SFA-1 was shown to be required for longevity through the TORC1 pathway. This suggests that in addition to the broad dysfunction of splicing with age, alternate pathways that regulate longevity could be influenced by differential composition of the spliceosome. Lastly, overexpression of these splicing factors is sufficient to extend lifespan of wild type nematodes. Thus, understanding the role of these specific splicing factors in modulating alternate longevity paradigms would not only provide information about the mechanisms of these longevity pathways but will also give us new therapeutic windows to multiple age-related pathologies.

1018C RNA Binding Proteins in stress resistance – a screen in C. elegans. Reza Esmailie1,2,3, Michael Ignarski1,2,3, Tim Krüger1,2,3, Rene Neuhaus1,2,3, Francesca Fabretti1,2,3, Ilian Atanassov5, Christoph Dietrich3,4, Roman-Ulrich Müller1,2,3 1) Department II of Internal Medicine and Center for Molecular Medicine, University of Cologne, Cologne, Germany; 2) Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, University of Cologne, Cologne, Germany; 3) Systems Biology of Ageing Cologne, University of Cologne, Cologne, Germany; 4) Department of Internal Medicine III and Klaus Tschira Institute for Integrative Computational Cardiology, University Hospital Heidelberg, Heidelberg, Germany; 5) Max Planck Institute for Biology of Ageing, Cologne, Germany.

RNA binding proteins (RBPs) play an important role in cell biology, regulating expression, stability and localization of all known RNA species. The importance of these proteins is underlined by the increasing body of evidence linking several hereditary diseases, developmental disorders and cancer with mutations in genes encoding RBPs. In the last decade, the list of known and putative RBPs has been increasing in size and complexity across species. Thanks to the development of techniques that allow crosslinking of RNA to interacting proteins followed by both RNA pulldown and mass spectrometry (RNA interactome capture), or immunoprecipitation and next generation sequencing (CLIP). Little is still known about the molecular function of many RBPs and their global dynamics in stress conditions. In this study we choose C. elegans as a model organism to address this complex biological question. Performing RNA interactome capture we identified 641 putative RBPs out of which 582 are conserved in mouse and human. We screened for RBPs involved in stress resistance pathways through heat stress resistance phenotyping using both mutants and RNA interference. In order to identify targets of the RBPs we established a modified CLIP protocol for C. elegans. We tagged RBPs endogenously using CRISPR/Cas9 technology and we performed immunoprecipitation and next generation sequencing. These results broaden the understanding of RBPs function during stress response in the nematode.
1019A The transcription factor SKN-1 and its downstream phase II metabolizing enzyme UGT-22 confer resistance to albendazole in C. elegans. Pauline Fontaine, Keith Choe Department of Biology, University of Florida, Gainesville, FL.

Parasitic nematodes are a global burden to human health and agriculture. Control of these parasites relies on the use of three major classes of anthelmintic drugs including benzimidazoles (BZs). Albendazole and mebendazole are BZs used to deworm agricultural animals and billions of people per year. Nematodes have now evolved resistance, which is threatening sustainable control in agriculture and human treatment programs. Resistance can arise by mutation of the drug target, ben-1, and drug detoxification has been hypothesized as a second potential mechanism. How nematodes metabolize/detoxify drugs, where drug detoxification occurs, and the role of these processes in anthelmintic drug resistance remain largely unknown. The C. elegans transcription factor SKN-1 promotes resistance to a diverse array of reactive small molecules by regulating >89 genes predicted to promote drug detoxification. Nothing is known about the role of SKN-1 in anthelmintic drug resistance. Using RNAi and skn-1 pathway mutants in motility and fecundity bioassays, we show that SKN-1 confers resistance to albendazole and mebendazole in C. elegans. We also demonstrate that ugt-22, a SKN-1-dependent detoxification gene, mediates resistance to albendazole, but not mebendazole. GFP reporters suggest that UGT-22 is expressed predominantly in the intestine, an important site of detoxification and response to stress. Specifically, UGT-22 localizes to the apical plasma membrane and endoplasmic reticulum (ER) of intestinal cells. Interestingly, SKN-1 did not confer resistance to the two other anthelmintic drug class representatives, ivermectin and levamisole, suggesting specificity of the xenobiotic response. Ongoing work includes examining the role of UGT-22 in albendazole biotransformation in vitro.

1020B In the way to identify targets of the RNA-binding protein TIAR-1 under physiological and stress conditions. D.A. Fuentes-Jimenez, R.E. Navarro Departamento de Biología Celular y Desarrollo, Instituto de Fisiología Celular, UNAM, Circuito Exterior s/n, Ciudad Universitaria, Mexico City 04510.

We are interested in studying how germ cells respond to stress conditions. We have observed that stress conditions like heat shock and starvation slow-down translation, increase germ cell apoptosis and induces the formation of RNA granules in the adult gonad. We have shown that the RNA binding protein TIAR-1 is important to content with stress. TIAR-1 is one of the three C. elegans homologs of the TIA-1/TIAR family of RNA binding proteins in mammals. In mammals, these proteins are required for Fas-induced apoptosis, stress granules assembly and translational repression under normal and stressful conditions. We have observed that TIAR-1 is required to induce germ cell apoptosis under several types of stress. During starvation and heat shock, two main classes of ribonucleoprotein complexes are formed in the gonad: one in the core of the gonad and another in the oocytes. Both types of RNA granules are induced when animals are exposed to puromycin while their formation is blocked when animals are exposed to cycloheximide suggesting that these RNA granules are similar to stress granules. We found that TIAR-1 colocalizes to P granules and to gonad core and oocytes RNA granules under stress conditions however it is required exclusively for the formation of gonad core granules. These data support the hypothesis that RNA granules formed in the C. elegans gonad during stress have different nature and perhaps functions. To understand the role of TIAR-1 during stress in C. elegans, we would like to identify its RNA targets. For this purpose, we will isolate TIAR-1 by pulldown from samples of nematodes under normal and stress conditions, and then purify and sequence RNA. Once we identify the trascripts, we will characterize the contribution of specific RNAs to stress protection and we will investigate how TIAR-1 regulates these putative targets. We believe that identifying the targets of TIAR-1 will be helpful to understand how this protein protects C. elegans from stress and particularly could come to understand its role in RNA granules formation.


The Caenorhabditis Intervention Testing Program (CITP) is a National Institutes on Aging supported consortium that screens promising chemicals across diverse genetic backgrounds for effects on lifespan and healthspan. Such chemicals are desirable, as they are likely to target conserved pathways that modulate aging. Pharmacological interventions that target core aging pathways are expected to be useful in treating multiple human aging related diseases. We utilize the model Caenorhabditis elegans, as its relatively short lifespan and predictable responses to stress allow for high-throughput screening with large sample sizes. To increase the data collection capacity of our measurements we have utilized the Automated Lifespan Machines, developed by the Fontana Lab 1. Here we describe our results using these machines to study not lifespan but healthspan. Specifically, we will present our results from testing chemical treatments for their ability to mitigate age-dependent declines in stress resistance. We first characterized the age dependent declines of multiple strains and species in both thermal and oxidative stress resistance. We then go on to describe the results of two reported pro-longevity chemicals on mitigating these declines. We describe our results for Rapamycin, an mTOR inhibitor that has shown robust pro-longevity effects across multiple species, and Acarbose, an alpha-glucosidase inhibitor. The CITP's novel combination of testing diverse genetic backgrounds, use of computer controlled scanners, and devotion to mulit- replicate assays has allowed the CITP to confidently assess the effects of pro-longevity chemicals for their ability to mitigate age-dependent declines in stress resistance. We suggest that this activity may be at least as pharmacologically important as the chemicals' ability to extend lifespan.

1022A The mitochondrial protein import machinery is a determinant of longevity in C. elegans. E. Lionaki, I. Gkikas, I. Daskalaki, N. Tavernarakis 1) Institute of Molecular Biology and Biotechnology, Heraklion, Crete, Greece; 2) Department of Biology, University of Crete, Greece; 3) Medical School, University of Crete, Greece.
Mitochondrial function is a key modulator of the ageing process. Abnormal mitochondria accumulate during ageing and their efficient elimination ensures longevity and stress response in C. elegans. The precise mechanisms through which mitochondrial biogenesis and function impinge on lifespan are currently under intensive investigation. Mitochondrial biogenesis is coordinated at distinct points: a) at the transcriptional level, through the action of specific transcription factors, b) at the organellar level, where conserved mitochondrial protein import translocases assist targeting of nuclear encoded mitochondrial proteins to their final destination within the organelle, and c) at the quality control level, where the mitochondrial pool is maintained through fusion/fission events and autophagic elimination of damaged mitochondria. We are investigating the cause-and-effect relationships between distinct mitochondrial protein import machineries and lifespan in the nematode. Specifically, we targeted channel-forming subunits of the general mitochondrial import pore (tomm-40) and the translocase of the inner mitochondrial membrane complex (timm-23) that mediate transport across the outer and inner membranes respectively. We find that RNAi depletion of tomm-40 or timm-23 alters mitochondrial morphology, lowers mitochondrial content, ATP and ROS levels, and promotes longevity. Tissue-specific knock-down of tomm-40 reveals the critical role of the intestine in mediating the longevity effects. Finally, lowering mitochondrial content confers benefits in a mitochondrial disease model background.

1023B Investigating the role of lysine demethylase UTX-1 in regulating lifespan of Caenorhabditis elegans. Abigail Guillermo1, Karolina Chocian1, Julien Vandamme2, Lisa Salcini2, Jane Mellor1, Alison Woollard1 1) Department of Biochemistry, University of Oxford, Oxfordshire, GB; 2) Biotech Research and Innovation Centre, University of Copenhagen, Denmark.

The lysine demethylase UTX-1 was recently found to influence the lifespan of Caenorhabditis elegans, with knockdown of utx-1 causing a significant improvement in worm longevity (Jin et al. 2011). In addition, an RNAi screen of chromatin factors identified several putative H3K27 modifiers, including utx-1, as having roles in lifespan regulation. utx-1 encodes an enzyme with homology to H3K27 demethylases, suggesting that utx-1 could mediate lifespan through epigenetic regulation. Importantly however, this catalytic activity of UTX-1 has been shown to be completely dispensable for worm development, raising the possibility that utx-1 has functions unrelated to lysine demethylation (Vandamme et al. 2012). Null mutations of utx-1 cause embryonic lethality, necessitating the use of heterozygous mutants or utx-1 RNAi to induce a hypomorphic phenotype. Transgenic overexpression of utx-1 in utx-1 mutants was expected to rescue the lifespan phenotype back to wildtype. However, our results showed that utx-1 overexpression gave a lifespan extension over and above that of the mutant. Furthermore, overexpression of utx-1 in the wildtype background also showed a significant increase in lifespan, suggesting that lifespan expression is very sensitive to the dosage of utx-1. Significantly, overexpression of catalytically dead UTX-1 was not found to be associated with any lifespan increase, suggesting that the catalytic activity of UTX-1 is important in mediating longevity, in contrast to its dispensability for development. We are examining whether under or overexpression of utx-1 is associated with enhanced stress resistance as well as longevity, and also investigating the tissue-specificity of utx-1 function with respect to lifespan regulation and stress resistance. We are also performing RNA-Sequencing analysis to determine what genes are differentially expressed due to the UTX-1 catalytic activity and assess if these genes are important for UTX-1 mediated lifespan extension.


The heat shock response (HSR) is a cellular stress response that senses protein misfolding and restores protein folding homeostasis, or proteostasis. We identified an HSR regulatory network in Caenorhabditis elegans consisting of highly conserved genes that have important cellular roles in maintaining proteostasis. Unexpectedly, the effects of these genes on the heat shock response are distinctly tissue-specific. We investigated the mechanism for muscle-specific regulation of the HSR by the TRIC/CCT chaperonin and found that it is not driven by an enrichment of TRIC/CCT in muscle, but rather it is influenced by one of its most abundant substrates, actin. Knockdown of actin subunits reduces induction of the HSR in muscle upon TRIC/CCT knockdown. Conversely, overexpression of an actin subunit sensitizes the intestine so that it induces the HSR upon TRIC/CCT knockdown. Similarly, intestine-specific HSR regulation by subunits of the signal recognition particle (SRP) is driven by vitellogenins, some of the most abundant secretory proteins. Together, these data indicate that the specific protein folding requirements from unique cellular proteomes sensitizes each tissue to disruption of distinct subsets of the proteostasis network. These findings are relevant for tissue-specific, HSR-associated human diseases such as cancer and neurodegenerative diseases. Additionally, we characterize organismal phenotypes of actin overexpression including a shortened lifespan, supporting a recent hypothesis that maintenance of the actin cytoskeleton is an important factor for longevity.

1025A Acute cold shock response is mediated by thermosensation and stress response genes. L. Gulyas, J.R. Powell Department of Biology, Gettysburg College, Gettysburg, PA.

Environmental stressors can severely endanger an organism’s capability to survive and reproduce, jeopardizing the continuation of a species. Consequently, organisms must launch effective responses to stress, so they can mitigate damage, recover, and ensure progeny survival. One such environmental stressor is acute cold stress, which has the potential to disrupt typical cellular functioning. Using C. elegans as a model system, we previously characterized a set of extreme phenotypic
changes associated with cold shock at 2°C, including loss of mobility and pigmentation. We have now determined that the neuronal cyclic nucleotide-gated channel subunits TAX-2/4, involved in thermosensation, play a role in the cold shock response; mutants defective in these genes show remarkable survival relative to wild-type controls, displaying none of the characteristic phenotypic changes shown by controls. Additionally, we have discovered that the stress-related genes fshr-1 and skn-1 are involved in the regulation of this process. Intriguingly, fshr-1, although important for the survival of worms following immune challenge, seems to positively regulate the phenotypes typically associated with death following cold shock. Furthermore, the ability to undergo vitellogenesis, in which somatic fats are reallocated to the germline, ostensibly for progeny investment, appears to be a factor governing the loss of pigmentation observed in cold shocked wild-type worms. vit-2 mutants defective in this process show retention of intestinal pigmentation that corresponds to high rates of survival following cold shock. Finally, preliminary data suggest that metabolic pathways also influence recovery from cold shock. Taken together, our data suggest that a series of complex pathway interactions in multiple tissues contributes to the sensation, response, and recovery from acute cold stress.

1026B Investigating DNA damage response pathways after exposure to various heavy metals in C. elegans. Christopher Marrinello, Scarlett Koga, Sahil Parag, Katlin Campbell, Megan McMurray, Jennifer Waldroupe, Toni Rockholt, Shawn George, Marrium Siddiqui, Nabiha Haider, Chezna Lee, Xuan Ou Yang, Julie Hall Lincoln Memorial University, Harrogate, TN.

A major route of exposure to various heavy metals is through contaminated soil and water. Research has shown that these substances play roles in the induction of various diseases such as cancer, neurodegeneration and birth defects. In the cell, proteins such as metallothioneins respond to heavy metal exposure and chelate the metal to prevent cellular damage. However, little is known about the cellular response in regards to DNA damage after heavy metal exposure. To provide a better understanding of this cellular response, the induction of both cell cycle arrest and apoptosis were investigated after exposure to copper, cadmium, iron, lead, nickel and silver in the nematode C. elegans. Growth assays were conducted to determine EC10 and EC50 concentrations which were utilized to determine the DNA damage response pathway, apoptosis and/or cell cycle arrest, being induced upon exposure. Apoptosis in the germline was significantly induced in response to all the heavy metals except silver. Silver does appear to induce cell cycle arrest suggesting a different type of damage compared to the other metals. Apoptosis and cell cycle assays for all metals tested will allow us to better understand the damage being caused by the metal exposure as well as mechanisms induced by the cell in response to exposure.

1027C Crosstalk between mitochondrial quality control mechanisms and SGK-1 in ageing regulation. B. Hernando-Rodriguez, M.M. Perez-Jimenez, A. Pla, M.J. Rodríguez-Palero, M. Artal-Sanz Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide, Sevilla, Sevilla, ES.

Mitochondrial dysfunction triggers the activation of different mitochondrial quality control mechanisms (mtQCM), such as the mitochondrial unfolded protein response (UPRmt), to restore mitochondrial homeostasis, and mitophagy, to degrade damaged mitochondria. Although both mtQCMs have been implicated in the regulation of aging, the underlying molecular mechanisms remains largely elusive. The Serum- and Glucocorticoid-induced Kinase 1 (SGK-1) has been implicated in the regulation of the UPRmt. Long lived sgk-1(ok538) loss of function mutants show a mild induction of the UPRmt. However, under severe mitochondrial stress, like depletion of the mitochondrial prohibitin (PHB) complex that strongly induces the UPRmt, sgk-1(ok538) mutants show reduced UPRmt induction and a further lifespan increase. Remarkably, PHB depletion shortens the lifespan of wild type animals. Given the proposed role of SGK-1 in autophagy inhibition, we set out to investigate the interaction of SGK-1 with mtQCM. In this work, we show that severe mitochondrial stress and mtQCM differentially modulate SGK-1 levels during ageing. While inhibition of either the UPRmt or mitophagy increases SGK-1 protein levels, severe mitochondrial stress reduces SGK-1 levels, triggering autophagy. We further show that under severe mitochondrial stress, only the UPRmt is essential to keep low SGK-1 levels and induce autophagy. Furthermore, both, activation of autophagy and activation of UPRmt are essential for the enhanced longevity of sgk-1(ok538) upon PHB depletion. These data underscores a pivotal role for SGK-1 in the maintenance of mitochondrial homeostasis and underpin the importance of the UPRmt and autophagy for lifespan extension under mitochondrial stress conditions.

1028A A heatstroke model in C. elegans. K. Momma, T. Honma, R. Isaka, S. Sudevan, A. Higashitani Graduate School of Life Sciences, Tohoku University, Sendai, Miyagi, JP.

Heatstroke is a life-threatening condition, the risk of which is increasing with a warming climate, the urban heat island effect, and an aging population. Acute onset of organ failure in heatstroke is triggered by breakdown of muscle fiber (i.e., rhabdomyolysis) of skeletal muscle. To gain insight into heat-induced muscle breakdown, we investigated alterations of Ca^{2+} homeostasis and mitochondrial morphology in vivo in body-wall muscles of C. elegans exposed to elevated temperature. Here we analyzed the maximal moving velocity of adult hermaphrodites exposed to heat shock at 35°C for 2 or 3 h. Compared with that at 20°C, the maximal moving velocity of wild-type N2 was lower after exposure to 35°C for 2 h: half of the population was almost paralyzed with severe moving defect, and the other half showed slow behavior. However, 24 h after temperature shiftdown (TSD) to 20°C, their moving activity was remarkably healed. In contrast, the worms exposed to 35°C for 3 h did not recover, and about 80% died within 4 days. We then monitored muscle cytosolic [Ca^{2+}]i in C. elegans HBR4 strain, which expresses the calcium sensor GCaMP3.35 in striated body-wall muscles. The GCaMP3.35 signal intensity was typically highest at the contracting side of a body bend at 20°C. When worms were exposed to transient heat stress at 35°C, the intensity markedly increased in not only the contracting side but also the expanding side. In worms subjected to heat stress for 2 h, but not those subjected to heat stress for 3 h, the elevated GCaMP3.35 intensities decreased to the normal level by one day after
TSD to 20°C, and motility could be recovered. This suggests that Ca\(^{2+}\) homeostasis was disturbed by heat shock for 3 h, much more than 2 h. In addition, heat stress for 3 h at 35°C led to mitochondrial fragmentation and subsequent dysfunction in the muscle cells. Similar progression of mitochondrial fragmentation is increased by treatment with a calcium ionophore, ionomycin. A ryanodine receptor mutant unc-68, linked to Ca\(^{2+}\) release from sarcoplasmic reticulum (SR), could suppress the mitochondrial dysfunction, muscle degeneration, reduced mobility and lifespan by heat stress. In addition, daf-2 mutant, activated DAF-16/FoxO transcription factor caused thermal resistance to Ca\(^{2+}\) leakage, mitochondrial fragmentation, and dysfunction in the muscle cells. These findings reveal that heat-induced Ca\(^{2+}\) leakage causes mitochondrial damage and consequently induces muscle breakdown.

1029B Dissecting the molecular pathways underpinning lifespan extension following exposure to Montmorency Tart Cherries. Inge Vrinds\(^1\), Amber van den Elzen\(^1\), Terun Desai\(^2\), Michael Roberts\(^2\), Christien Lokman\(^1\), Lindsay Bottoms\(^2\), Samantha Hughes\(^1\). 1) HAN BioCentre, HAN University of Applied Sciences, Nijmegen, Netherlands; 2) University of Hertfordshire, Hertfordshire, England, UK.

Montmorency Tart Cherries, MTC, (Prunus cerasus) possess a high anthocyanin content as well as the highest oxygen radical absorbance capacity of all fruits at common habitual portion sizes. MTC have been shown to contribute to reducing lipids, glucose and fat mass in rats and strikingly, similar effects are observed in humans. However, there is a paucity of research examining the molecular mechanisms by which such MTC effects are induced.

Here, we show that when exposed to MTC, C. elegans display a significant extension to lifespan, and these worms are healthier. In contrast, elevated concentrations of MTC shorten lifespan and reveal a developmental delay. Using RNA interference, it is possible to silence genes involved in fat metabolism and provide insight into the molecular pathways through which MTC acts. We have identified that MTC functions via the PPAR signaling pathway, specifically nhr-49 and daf-22. Using chemical analysis of the MTC concentrate, we will demonstrate which of the constituent anthocyanins in MTC provide the health giving benefits. Our data provides encouraging evidence that MTC may be operating as a calorie restrictive mimetic via metabolic pathways.

It is estimated that 1.75 billion people worldwide have Metabolic Syndrome, a cluster of cardio-metabolic criteria including abdominal obesity and elevated blood pressure, that is often a precursor to type 2 diabetes, cardiovascular disease and obesity. As such, our data is contributing to the use of MTC as a nutritional intervention against Metabolic Syndrome, thus improving healthy aging and longevity of human patients.

1030C Insulin ligands affect morphology and function of mechanosensory neurons. S. Hunter\(^1,2\), B. Taylor\(^3\), M. Driscoll\(^4\), A. Bult-Ito\(^5\), E. Vayndorf\(^2\). 1) Department of Biology and Wildlife, Fairbanks, AK; 2) Institute of Arctic Biology, Fairbanks, AK; 3) College of Natural Science and Mathematics, California State University, Long Beach, Long Beach, CA; 4) Department of Molecular Biology and Biochemistry, Rutgers, The State University of New Jersey, Piscataway, NJ.

Insulin signaling plays a key role in the aging of organisms ranging from yeast to mice, and there is evidence that in humans, single nucleotide polymorphisms (SNPs) of the insulin transcription factor, FOXO, can predict whether an individual will reach the age of 100. In C. elegans, peptides, collectively known as ILPs, are regulators of both organismal and neuronal aging. In humans, healthy neuronal aging is characterized by neuronal sprouting, synaptic deterioration and restructuring with little to no neuronal cell loss. However, the physiological factors that affect the decline of neuronal morphology and structure over time in their native context remain poorly understood. This is particularly true at the single cell level. C. elegans ILPs belong to three major subfamilies based on their sequence and structural similarity. We set out to determine how ILPs, individually or as a group, influence the morphology of aging mechanosensory neurons. To investigate the relationship between ILPs and neuronal morphology and function, we conducted a reverse genetic screen of insulin ligands in animals with fluorescently labeled mechanosensory neurons. RNAi was used to individually knock down expression of insulins 1 through 38 and DAF-28, and structural and functional data were collected for ALM and PLM neurons. ILPs of interest were rescreened for a total of three replicates compared to empty vector controls. Our results will highlight ILPs correlated with specific neuronal aberrations and functional outcomes, and show that neuronal morphology may be influenced by ILPs in a family dependent manner.

1031A Uncovering the roles of tyrosine aminotransferase and F01D4.5 in the oxidative stress response. B. Ipson\(^1\), A. Fisher\(^1,2\). 1) University of Texas Health Science Center at San Antonio, San Antonio, TX; 2) GRECC, South Texas VA Healthcare System, San Antonio, TX.

While our understanding is limited regarding the processes by which oxidative stress mediates cell damage and contributes to aging and disease, emerging data suggests the formation of the abnormal tyrosine isomers meta- and ortho-tyrosine to be one mechanism by which this may occur. Because of this, we hypothesized tyrosine metabolic pathways may have previously unknown protective functions in response to oxidative stress. Using the nematode C. elegans, we performed genetic studies to characterize the role of tyrosine aminotransferase in protecting against the adverse effects of oxidative stress in general and, more specifically, m-tyrosine supplementation. We found tyrosine aminotransferase (tatn-1) to be a target of the anti-oxidative stress response transcription factor SKN-1/Nrf2, and biochemical assays have shown TATN-1 maintains specificity for m-tyrosine metabolism; thus suggesting a mechanism by which cells may eliminate this isomer. Furthermore, tatn-1 mutants were more susceptible to developmental delay when placed under general oxidative stress conditions and to germline defects when supplemented with m-tyrosine. To identify regulators or mediators of these adverse phenotypes, we performed a forward genetic mutagenesis screen in which we identified mutants that are resistant to the fertility defects observed in tatn-1 mutant worms supplemented with m-tyrosine. Genome-wide sequencing of the most robust line revealed a nonsense mutation within the
previously uncharacterized gene \textit{F01D4.5} \textit{TCF20}. This gene is a member of the TCF20 family of transcription factors which are also present in multiple other species including people. Intriguingly, \textit{F01D4.5} RNAi not only protects \textit{tatn-1} mutant worms exposed to chronic oxidative stress but renders wildtype N2 worms resistant to the effects of paraquat as well. Utilizing CRISPR technology, we are currently knocking in a fluorescent label to determine tissue localization of the \textit{F01D4.5} protein and to begin to elucidate its function. Collectively, our data points to the cellular metabolism of the abnormal tyrosine isomer \textit{m}-tyrosine by \textit{TATN-1} as a novel oxidative stress defense mechanism and identifies \textit{F01D4.5} as a potential negative regulator of the oxidative stress response.

\textbf{1032B  Feedback system between sperm and temperature sensing-neuron, and isolation of novel genes in cold tolerance.}  \textit{Toshihiro Iseki}, Satoru Sonoda, Natsume Takagaki, Misaki Okahata, Akane Ohta, Atsushi Kuhara Institute for Integrative Neurobiology, Graduate School of natural science, and School of Science and Engineering, Konan University, Kobe, Japan.

Temperature tolerance in animals is important mechanism for survival and proliferation. To investigate temperature tolerance, we are using cold tolerance. 20°C-cultivated animals did not survive at 2°C. In contrast, 15°C cultivated animals can survive. Previously reported, this cold tolerance is regulated by ASJ temperature sensing-neuron, which releases insulin that is received by intestine and neuron (Ohta, Ujisawa et al., \textit{Nature commun}, 2014).

(1) To identify genes in downstream of insulin-signaling, we performed DNA microarray analysis. We found that expressions of sperm genes were changed in insulin receptor mutants, and sperm mutants showed abnormal cold tolerance. Unexpectedly, genetic epistasis analysis suggested that abnormal cold tolerance of sperm mutant was suppressed by the mutations in ASJ temperature sensing-neuron. Calcium imaging analysis showed that ASJ neuronal activity in response to temperature was decreased in sperm mutant \textit{gsp-4}, and rescued by expressing \textit{gsp-4} gene in sperm. Thus, we propose a novel feedback between sperm and ASJ temperature sensing-neuron in cold tolerance (Sonoda et al., \textit{Cell reports}, 2016). To investigate what molecules are required for the tissue network between sperm and ASJ neuron, we are analyzing genes encoding secretory signaling such as nuclear hormone receptor (\textit{nhr}) and other receptors. So far, some \textit{nhr} mutants showed abnormal cold tolerance.

(2) To isolate more genes involved in cold tolerance. By the DNA micro array analysis in this study, the expression level of \textit{Y38H8A.3} mutant showed defect in cold acclimation. However, another null mutant showed normal phenotype. We then speculated that background mutation excepting \textit{m}-tyrosine by \textit{TATN-1} as a novel oxidative stress defense mechanism and identifies \textit{F01D4.5} as a potential negative regulator of the oxidative stress response.

\textbf{1033C  Simple, Fast, High-Resolution Sorting of \textit{C. elegans}.} \textit{N. Jacobs}$^1$, T. Nonet$^2$, Z. Pincus$^1$ 1) Washington University, St. Louis, MO; 2) Rice University, Houston, TX.

Physical separation of \textit{C. elegans} based on visible phenotypes is a basic and important experimental task. Automation of this task can dramatically speed identification of rare individuals (such as positive hits from mutagenesis screens) or bulk stratification of large populations for biochemical or other analyses. Several fluidic and microfluidic devices have been developed for such automation; however, current microfluidic “worm sorter” designs choose between ease of use and manufacture or precise positioning, immobilization, and isolation of individuals. We have now developed a device that is simple in design and easy to manufacture and operate, while retaining good throughput and accuracy. As it employing only a single-layer microfluidic design with no on-chip valves, the device can be fabricated with a single master mold and no complex alignment steps. In addition to its simplicity, our design, which employs high-speed valves off-chip and away from the \textit{C. elegans} flow path, also enables relatively high-speed sorting compared to other microfluidic designs. Time to sort is approximately 1 second per animal; the total throughput of the system is limited only by the concentration of animals in fluid buffer that is delivered to the device. Overall, we can sort approximately 900 animals per hour. Our device can sort into three different outputs, which allows simultaneous sampling of both extremes of a phenotypic distribution, with over 93% average sorting accuracy. This system is non-invasive and gentle, sorting individuals with no identifiable ill effects.

\textbf{1034A  A model for assessing the impact of complex environmental exposures on neurodegeneration in \textit{Caenorhabditis elegans}.} \textit{M. Johnson}$^1$, V. Kalia$^1$, J. Bradner$^1$, M. Niedzwiecki$^1$, D. Walker$^2$, D. Jones$^2$, G. Miller$^{1,3,4,5}$ 1) Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA; 2) Clinical Biomarkers Laboratory, Division of Pulmonary, Allergy and Critical Care Medicine, School of Medicine, Emory University, Atlanta, GA; 3) Center for Neurodegenerative Diseases, School of Medicine, Emory University, Atlanta, GA; 4) Department of Pharmacology, School of Medicine, Emory University, Atlanta, GA; 5) Department of Neurology, School of Medicine, Emory University, Atlanta, GA.

Neurodegeneration is a complex molecular process that involves a combination of genetic, environmental, and age-related risk factors. Little is known about how environmental exposures interact with genetic factors and aging to induce neurodegeneration. Here, we describe a platform to assess the impact of complex environmental exposures in \textit{Caenorhabditis elegans} (\textit{C.elegans}). Work from our laboratory and others has previously shown that disruption of the vesicular monoamine transporter 2 (VMAT2; \textit{SLC18A2}) confers vulnerability to dopamine toxicity in mice and Parkinson’s disease in humans. The mutant \textit{cat-1} lacks the worm ortholog to VMAT2. We evaluated the effect of \textit{cat-1} deletion on lifespan using the hands-free, high-throughput Lifespan Machine, as developed by Strostrup et al. (2013, 2016). We found that \textit{cat-1} mutants have an altered lifespan as compared to wild-type controls. In order to study the impact of exposures to complex chemical mixtures, we
needed to develop a high-throughput method to measure hundreds of exogenously applied chemical and subsequent metabolic products in the worms. Using three different high-resolution metabolomics platforms, the Agilent 6560 Ion Mobility Q-TOF LC/MS, the Thermo Q-Exact HF LC/MS, and the Thermo Q Exactive GC hybrid quadrupole-Orbitrap GC-MS/MS, we are able to detect and quantify low levels of a wide variety of environmental chemicals (>350) representative of realistic exposures. These include polychlorinated biphenyls, brominated flame-retardants, organochlorine pesticides, phoshoester chemicals, herbicides, insecticides and pesticide synergists. Moreover, the combination of liquid chromatography (LC) and gas chromatography (GC) metabolomic platforms allows us additional measures of polar metabolites from environmental chemicals and endogenous metabolites (>5000). This combined analytical strategy thus provides a means to capture differential toxic effects of chemical metabolic products and the biological response to chemical exposures through metabolome-wide association study (MWAS). The high-resolution metabolomics platform can provide comprehensive coverage on as few as 100-1000 worms. The combination of genetically vulnerable mutants, the automated analysis of lifespan, and the high-resolution metabolomics analysis of endogenous and exogenous chemicals creates an ideal platform for studying gene-environment interactions in models of neurodegeneration and aging in C. elegans.


Understanding healthy aging is of great importance given that the average age in developed societies continues to increase. While much research on the biology of aging has focused on interventions that affect lifespan, it is also important to evaluate healthspan, defined as the period when an organism is generally good health; an increase in lifespan does not always imply a proportionate increase in healthspan.

As part of the 'Ageing with elegans' consortium, we aim to discover processes and interventions that influence healthspan, using C. elegans as a model. We have developed the WorMotel, a microfabricated multi-well device for the longitudinal observation of activity of a large number of individual C. elegans during their lifespans. We have integrated the WorMotel method with a custom integrated robotic plate handler and imaging systems for serially monitoring up to 240 plates containing 57,600 individual animals. Using this system, we are performing a large-scale candidate RNAi screen to identify genes that control healthspan. We use the observed maximal locomotor activity of the worms upon blue light illumination as representing their level of health. We then define healthspan as the total time during which the worm shows at least one-quarter of its highest maximal activity. We select candidates based on the significantly altered healthspan/lifespan (HS/LS) ratio. Candidate genes are grouped in 8 classes, established by the relative changes in lifespan and HS/LS ratios, and prioritized for detailed study accordingly.

We present results for lifespan and healthspan from a subset of genetic knockdown experiments. We find that long-lived populations typically show overall lower HS/LS ratio whereas short-lived groups show higher HS/LS ratio, consistent with the published literature. We discuss exceptions to this pattern. In future work, consortium collaborators will study the role of these genes in other aging-related phenotypes, such as sarcopenia-relevant muscle integrity, maintenance of neuronal functionality, and lipid content, prior to further study in mice models.

Multi-OMICS approach revealed involvement of daf-16/FOXO pathway in neuro-behavioural toxicity of graphene nanomaterials in C. elegans. Youngho Kim1, Carlos P. Roca2, Nivedita Chatterjee3, Mina Kim4, Suhkmann Kim5, Jongki Hong6, Inhee Choi7, Jinhee Choi7 1) University of Seoul, Seoul, Korea; 2) Aarhus University, Silkeborg, Denmark; 3) Pusan National University, Busan, Korea; 4) Kyunghee University, Seoul, Korea.

Graphene nanomaterials have attracted great research interest for their potential applications in electronics, energy, materials and biomedical areas. However, little information is available about their toxicity mechanisms. In this study, the potential hazard of graphene nanomaterials were investigated in the nematode Caenorhabditis elegans, using an integrated systems toxicology approach. To gain an understanding of the underlying molecular mechanisms of graphene toxicity, microarray, metabolomics, and lipidomics assays were performed, followed by an integrative pathway analysis. Results suggested the daf-16/FOXO pathway, drug metabolism, Fat metabolism, and MAPK pathway as potential toxic mechanisms of GO toxicity. To validate the detected pathways, functional genetic studies were performed, using loss-of-function mutants of genes of those pathways. Involvement of daf-16/FOXO pathway in neuro-behavioral toxicity was validated by increased nuclear translocalization of the transcription factor daf-16 by graphene exposure. Hence, we provide insights into the mechanism of toxicity of graphene and suggest the direction for the further examination of graphene toxicity. The comprehensive approach allowed us to integrate all assay results in a consistent understanding of graphene toxicity. Thus, this approach will serve as a proof-of-principle for the general suitability of multi-OMICS approaches for the elucidation of toxicity and stress responses, providing valid hypotheses which allowed to obtain unbiased knowledge.

A screen to identify miRNA predictors of individual lifespan in C. elegans. H. Kinser, Z. Pincus  Department of Genetics, Washington University in St. Louis, St. Louis, MO.

Genetically identical C. elegans individuals reared in highly uniform environments nonetheless exhibit widely different lifespans. We propose that early-life differences in the expression of key regulatory genes direct otherwise identical individuals into different gene expression states, resulting in profound differences in lifespan. To identify such regulators, we screened miRNA::GFP reporters to identify those whose expression correlates with lifespan in wild-type C. elegans individuals. We developed a novel series of high-throughput, automated methods to overcome the technical challenges of this approach, which requires observation of large numbers of individuals to generate statistical significance while maintaining single-animal resolution for longitudinal measures of gene expression and lifespan. Briefly, we deposited adult animals into 384 well plates,
one per well, and acquired fluorescence images of each individual. We maintained the animals in liquid culture and conducted daily whole-plate time-lapse imaging with modified flatbed scanners to measure the lifespans of many individuals in parallel. We performed multiple rounds of screening with 48 different miRNA::GFP reporter strains and identified 8 whose expression is significantly correlated with lifespan. We have thus far validated one of the hits from the screen, miR-63, in a single-animal solid culture system, showing that early-life expression of this miRNA is predictive of individual lifespan in multiple contexts. By continuing to characterize these miRNAs and their downstream targets, we will define gene expression states associated with longevity and the broader pathways and networks involved in determination of individual lifespan.

1038B Environmental stresses induce transgenerationally inheritable survival advantages via germline-to-soma communication in C. elegans. S. Kishimoto, M. Uno, E. Okabe, M. Nono, E. Nishida Kyoto University, Kyoto, JP.

Hormesis is a biological phenomenon, whereby exposure to low levels of toxic agents or conditions increases organism viability. It thus represents a beneficial aspect of adaptive responses to harmful environmental stimuli. Recent studies suggest that ancestral environmental conditions can influence the phenotypes of progeny. However, it is unclear whether beneficial hormesis effects can be transmitted to the offspring. Here we show that hormesis effects induced in the parental generation can be passed on to the descendants in C. elegans. Animals subjected to various stressors during developmental stages exhibit increased resistance to oxidative stress and proteotoxicity. The increased resistance is transmitted to the subsequent generations grown under unstressed conditions. Remarkably, exposure of the male parents to these stressors also increases the stress resistance of the descendants and extends their lifespan. These findings suggest that the memory of hormesis effects can be transmitted to progeny through germline epigenetic alterations. Our analysis also reveal that the insulin/insulin-like growth factor (IGF) signaling effector DAF-16/FOXO and the heat-shock factor HSF-1 in the parental somatic cells mediate the formation of epigenetic memory, which is maintained through the histone H3 lysine 4 trimethylase complex in the germline across generations. The elicitation of memory requires the transcription factor SKN-1/Nrf2 in somatic tissues. We propose that germ-to-soma communication across generations is an essential framework for the transgenerational inheritance of acquired traits, which provides the offspring with survival advantages to deal with environmental perturbation.

1039C Cellular effects of copper chloride and nickel sulfate in Caenorhabditis elegans. Scarlett Koga, Jennifer Waldroupe, Kaitlyn Campbell, Julie Hall Lincoln Memorial University, Harrogate, TN.

Copper and nickel are elemental nutrients that are necessary for human biochemical and physiological functions and also used by healthcare professionals to help patients overcome disease and surgeries. In high concentrations, exposure to these metals can cause adverse health consequences damaging organ functionality. These metals are naturally found in food sources such as crab, almonds, cocoa, black pepper, and many more. Exposure to nickel and copper can also occur through contamination of water and soil due to manufacturing of metals, forest fires, and decaying vegetation. To further understand the cellular effects of copper and nickel exposure, apoptosis and cell cycle arrest were investigated in the nematode C. elegans. EC10, EC30, and EC50 concentrations were determined using a growth response assay for copper. Due to extreme phenotypic abnormalities observed at the EC30 and EC50 nickel concentrations, studies with nickel were performed below the calculated EC10. For copper (III) chloride the determined EC10, EC30, and EC50 were 200µM, 300µM, and 400µM, respectively. For nickel (II) sulfate, the EC10 concentration was calculated to be 800µM and additional concentrations of 200µM, 400µM, and 600µM were used. Apoptosis was assessed in the germline after 24h exposure to copper or nickel. After exposure to 400µM copper, statistically significant increase in germline apoptosis was observed when compared to the untreated. Exposure to nickel sulfate demonstrated similar trends in apoptosis with statistically significant differences at 400µM, 600µM, and 800µM (2.72, 3.6, and 4, respectively) when compared to the untreated (0.89). Due to induced embryonic apoptosis and phenotypic abnormalities observed during exposure to metals, reproduction assays were performed to investigate the effect of metal toxicity on average brood size and embryonic lethality. Trends demonstrated a drastic decrease in reproduction and an increase in embryonic lethality with increased concentrations of copper and nickel. Cell cycle arrest and chromosome number are being assessed to determine other possible pathways effected by these metals. These results indicate that copper and nickel may effect germ cell development and/or embryonic development from parental exposure through apoptosis and other DNA damage pathways.

1040A Functional Characterization of Novel circular RNA Molecules Synthesized from Aging Associated Genes: Studies Employing Transgenic C. elegans Model of Parkinson's Disease. L. Kumar, ., Shamsuzzama, A. Nazir Toxicology, CSIR-Central Drug Research Institute, Lucknow, uttar pradesh, IN.

Neurodegeneration, the process through which some functions of the brain die progressively with degeneration of neurons, is well known to be associated with aging and leads to neurodegenerative diseases (NDs) like Alzheimer’s disease (AD) and Parkinson’s disease (PD). At present, lack of effective treatment or complete cure against NDs warrants looking into possibilities of employing newer strategies in treating such diseases. With the large amount of genetic information available, studies on novel molecules like circRNAs are providing significant insights into various biological processes. circRNAs are a class of non-coding RNAs, which are produced by scrambling of exons at the time of splicing. They are highly enriched in the mammalian brain and are up-regulated during the neuronal differentiation but functionally the role of these circRNAs needs to be explored. To better understand the aspect of age associated circRNAs in NDs we carried out bioinformatics studies, employing GenAge and circbase tools, towards identifying genes which were associated with aging and also biosynthesize circRNAs. Using the approach of RNaseR exonuclease treatment to total RNA followed by amplification using divergent primers, we validated the presence of nine circRNAs, of which five circRNAs and expression of their biosynthesizing genes was found to be up-regulated in early and late stages of PD model of C. elegans. We further carried out the functional genomics studies with RNAi induced gene silencing of rpt-1 gene and studied the associated endpoints of alpha synuclein aggregation in age specific manner in transgenic C. elegans model alpha synuclein. Our studies reveal that novel circRNA molecule cincrpt-1 and its
biosynthesizing gene rpt-1 could form interesting targets for further understanding age associated Parkinson’s disease and towards possibly designing better therapeutic strategies.

1041B A new path to lifespan extension in C. elegans: bacterial colonization, innate immune response, and food avoidance behavior. Sandeep Kumar, Zuzanna Kocsisova, Brian Egan, Daniel L Schneider, Kerry Korndfeld Development Biology, Washington University School of Medicine, Saint Louis, MO.

To identify new genes that modulate the rate of reproductive aging, we performed a forward genetic screen for mutant worms with an extended mated reproductive span, leading to the identification of the am117 mutation (Hughes et al., 2011). We used positional cloning approaches to identify the am117 molecular lesion as a premature stop codon in the phm-2 gene, which was previously identified genetically by not molecularly. phm-2 encodes a protein with homology to human scaffold attachment factor B, a protein proposed to bind DNA and influence transcription in vertebrates. phm-2(II) animals also displayed substantially extended lifespan and increased stress resistance, indicating the affected gene modulates somatic and reproductive aging. The mutant worms display an abnormal pharynx grinder and a scrawny morphology, suggesting that the mutation might lead to caloric restriction. Consistent with this hypothesis, we showed that genetic interactions of phm-2(II) behaved as predicted with eat-2, rsks-1 and pha-4. Multiple alleles of the phm-2 gene were discovered based on a defect in pharyngeal pumping; our molecular analysis of these alleles showed they affect the same open reading frame as am117. These results identify the phm-2 locus, a new allele of phm-2, and novel phm-2 phenotypes.

We observed that phm-2 mutant animals are frequently outside the bacterial lawn, a phenotype called food avoidance. We hypothesized that the pharyngeal grinder defect results in bacterial colonization of the gut, which was confirmed. Furthermore, this bacterial colonization resulted in the activation of the innate immune response based on transcriptional changes and the nuclear localization of HLH-30. Bacterial colonization caused food avoidance and caloric restriction, since the phm-2 mutant phenotypes (extended lifespan, reproductive span and scrawny body morphology) can all be suppressed by culturing the mutant animals on UV-killed E. coli or non-pathogenic bacteria such as Comamonas DA1877 and B. subtilis. To elucidate the basis of the food avoidance behavior, we demonstrated that phm-2 animals required serotonergic signaling to avoid food. We conclude that the pharynx grinder defect leads to bacterial colonization, which activates innate immunity pathways and leads to food avoidance, which leads to caloric restriction. The combination of these factors results in delayed somatic and reproductive aging. The analysis of phm-2 represents a new mechanism of longevity extension in C. elegans - bacterial colonization and behavioral food avoidance - and we discuss implications for the interpretation of other genetic models of caloric restriction in worms. Caloric restriction is the original lifespan extending intervention; Different CR regimes are reported to have different consequences in worms, and our results may clarify these puzzles.

1042C A C. elegans model of Wolfram Syndrome type 2. Emmanouil Kyriakakis1,2, Christina Ploumi1,3, Nektarios Tavernarakis1,3 1) Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology – Hellas; 2) Biozentrum, University of Basel, Basel, Switzerland; 3) Department of Basic Sciences, Faculty of Medicine, University of Crete, Greece.

Wolfram syndrome (WS) is a rare autosomal neurodegenerative disorder, characterized by early juvenile diabetes mellitus, a gradual loss of vision due optic nerve atrophy, deafness and often behavioral and mental changes. The average life expectancy is 30 years due to systemic complications. Treatment is merely symptomatic and supportive. There are two genetics forms of WS; type 1 is caused by mutations in the WFS1 (wolframin) gene, whereas WS type 2 is caused by mutations in the CISD2 gene. The C. elegans cisd-1 gene encodes a homolog of the mammalian CISD2, based on sequence analysis and the presence of the conserved iron sulfur domain. Lesion of cisd-1 recapitulates key features of CISD2 loss associated with WS type 2, including lifespan shortening and neurodegeneration. Intriguingly, dietary restriction (DR) completely reverses the detrimental effects of CISD-1 deficiency on longevity. This effect is at least in part due to autophagy induction. In addition, cellular proteostasis mechanisms are deferentially affected by CISD-1. The mitochondrial unfolded protein response (UPRmito) is significantly suppressed, while the endoplasmic reticulum UPRER is upregulated during ER stress. The heat shock response remains unaffected. These findings suggest an involvement of CISD-1 in organelle-specific proteome homeostasis. The C. elegans WS model will facilitate elucidation of the molecular mechanisms underlying WS pathogenesis and provide a platform for drug discovery.

1043A Resveratrol extends lifespan and delays germline aging through the activation of both SIR-2.1/Sirtuin and MPK-1/ERK. DS Yoon1, DS Cha3, MH Lee1,2 1) Internal Medicine, East Carolina University, Greenville, NC, USA; 2) Lineberger Comprehensive Cancer Center, UNC-Chapel Hill, NC, USA; 3) Woosuk University, Jeonbuk, Republic of Korea.

Chemicals can change the lifespan and also delay the onset of age-associate disease. Resveratrol (RSV) has been emerged as a highly effective longevity chemical. Although several studies showed that RSV extends lifespan through Sirtuin, the findings remain controversial. Using C. elegans, we found that RSV-mediated longevity requires both SIR-2.1/Sirtuin and MPK-1/ERK. Specifically, the longevity largely depends on MPK-1/ERK in the absence of RSV. However, in the presence of RSV, the longevity is regulated by both SIR-2.1/Sirtuin and MPK-1/ERK in an inversely proportional manner. In addition to somatic aging, the number of germ cells is gradually reduced during aging. Intriguingly, RSV delayed age-associated germ cell loss through SIR-2.1 and MPK-1. We also found that RSV can promote the formation of MPK-1-associated tumors in C. elegans germline. Therefore, our results provide insights into the understanding of controversial effects of RSV on the longevity but also propose the effects of RSV on germline aging and tumorigenesis in other organisms.
During aging and stress, nematodes may encounter a variety of xenobiotics in their environment. Organisms such as C. elegans protect themselves against xenobiotics by transforming them to water soluble excretable metabolites. A sensitive and convenient method to study this phenomenon is to use fluorescent or pro-fluorescent model xenobiotics. The aim of this study was to determine if fluorescent 7-hydroxy-4-trifluoromethylcoumarin (HFC) is transformed to a non-fluorescent metabolite by C. elegans. When 6.25 µM HFC was incubated with live and frozen C. elegans in M9 solution, the fluorescence decreased immediately in incubation with the live worms and after six to eight hours after melting and keeping the frozen worms at room temperature. The decrease of fluorescence was enzyme dependent, since heating the worms at 100°C for 20 min prevented the reaction. The magnitude of the decrease of fluorescence depended on time and number of worms. In 10 mM KOH alkaline condition the fluorescence of HFC disappeared in one hour. We could detect 3-(2,4-dihydroxyphenyl)-4,4,4-trifluorobut-2-enoic acid (DiHPFEacid) in the incubates with both alkaline solution and C. elegans. Therefore we conclude that lactone of HFC is hydrolyzed to DiHPFEacid via an enzymatic reaction in C. elegans. The enzyme responsible for this reaction in C. elegans remains unknown, however western blot data suggests the presence of an ortholog of CYP2A6, an enzyme which catalyzes this reaction in higher organisms. Taken together, these results provide a convenient whole animal model system to study fluorescent based xenobiotic metabolism.

**1045C Time-series quantitative proteome and transcriptome analyses revealed evidence of programmed aging in C. elegans.** Shang-Tong Li, Wen-Hong Zhang, Pan Zhang, Han-Qing Zhao, Joshua Mitteldorf, Meng-Qiu Dong  National Institute of Biological Sciences, Beijing, China.

Aging is a subject where the theorists have found no consensus, and where every existing theory is challenged by some experimental finding that contradicts it. Historically, this has been the hallmark of a field ripe for new insights, often with ramifications that ripple through science. Using C. elegans as a model system, we address a fundamental question “what is aging?” Our approach is to characterize the aging process in a systematic and quantitative manner in the hope to tease apart elements of programmed aging (result of group selection) from those of non-programmed aging (owing to declining force of selection on older individuals). To this end, we have quantified abundance changes of mRNAs and proteins in the early phase of aging of wild type, daf-2, and age-1 mutants in a temperature-sensitive (ts) sterile or embryonic lethal background (to eliminate offspring), as well as ts glp-1 and gon-2 mutants.

From our high temporal resolution data (1-2 samples per day, starting from L4 or adult day 1 to adult day 6 or day 10 at 25 °C), we observed a sudden change in both the transcriptome and the proteome between adult day 1 and day 2, followed by much more gradual changes. This pattern was seen in wild type and the long-lived daf-2 and age-1 mutants, and the rate of change of proteins or mRNAs was indeed slower in the daf-2 and age-1 mutants, appearing scaled to the rate of aging. The sudden proteome remodeling between adult day 1 and day 2 was also apparent in the germline-less glp-1 and gon-2 mutants, indicating that reproduction is not required for this to happen. The transcriptome data of glp-1 and gon-2 mutants are being processed and will be interrogated in the same way. In addition, this proteome remodeling phenomenon was distinctly different from the proteome change from L4 to adult day 1, suggesting that it is not simply an extension of the developmental program.

In summary, our time-series quantitative proteome and transcriptome analyses suggest that signs of aging at the molecular level occur much earlier than the signs of aging in morphology or behavior such as mitochondrial fragmentation or reduced locomotion. Also, we think that these results provide evidence for programmed aging, which appears to be turned on shortly after reproduction starts, although it does not absolutely require the presence of a reproductive system for its onset. Our data also provide a set of potential molecular aging markers for chronological aging and another set for physiological aging.

**1046A Functions of CLIC proteins and TGF- beta signaling in heat stress in C. elegans.** Jun Liang  Dept of Science, Borough of Manhattan Community College, New York, NY.

Chloride intracellular channel proteins (CLIC) are multifunctional proteins. Mammalian CLIC family, which has seven members, varies in subcellular localization and cellular functions. Cellular stress molecules induce endogenous CLIC4 to translocate from the cytoplasm into the nucleus. Mammalian Schnurri-2 is required for CLIC4 nuclear translocation in response to Transforming Growth Factor – beta (TGF-beta), but not required for CLIC4 nuclear function. Schnurri-2 is a transcription cofactor in the BMP pathway. However, the physiological functions of CLIC in whole animals level are not well understood, in particular how the genes regulate thermotolerance is largely unknown. To address these issues, we took advantage of viable CLIC mutants in C. elegans and characterized its functions in heat stress and aging. Schnurri-2 is homologous to C. elegans SMA-9 which functions in the DBL-1/TGF-beta pathway. There are two CLIC homologs in C. elegans: EXL-1 and EXC-4. exc-4 mutants develop cysts in the excretory canal, while abnormal phenotypes of exl-1 mutants have not been identified. We analyzed integrated EXL-1::GFP lines in wild type background and observed strong fluorescence in intestinal cells, which is consistent with previous study. EXL-1::GFP indeed is translocated into the nucleus under various heat shock conditions. Supporting functional importance of this, exl-1 loss-of-function mutants are thermo-sensitive, in compare with wild type animals. Meanwhile, we found that EXL-1, not EXC-4, bears a non-classic Nuclear Localization Signal (NLS). This may explain why exl-1 translocates into the nucleus upon heat stress, while exc-4 still remains in the cytoplasm. Furthermore, we generated double mutants of exl-1 and DBL-1/TGF-beta pathway components. To our surprise, the double mutants survived better than any single mutants. Our results are different from the finding in mammalian system, which indicates the need to further investigation. In the future, we will dissect the genetic interactions between exl-1 and the DBL-1 pathway.
1047B  The mitophagy receptor FUNDC-1 contributes to hypoxic injury in C. elegans. Yunki Lim, Stephanie Viteri, Keith Nehrke    School of Medicine and Dentistry, University of Rochester Medical Center, Rochester, NY.

Mitochondria are important for metabolism and cell signaling, while mitochondrial damage and dysfunction can contribute to multiple disease pathologies. Thus, the molecular components and signaling pathways that comprise mitochondrial quality control (MQC), including the mitochondrial unfolded protein response (UPRmt), proteases, anti-oxidants, and mitophagy, are tightly regulated. FUNDC1 (FUN14 domain containing 1) is a hypoxia-induced mitophagy mediator that has been characterized mainly in human cultured cells. FUNDC1 is located on the mitochondrial outer-membrane and directly binds to LC3 and Drp1. However, a FUNDC1 homolog had not been studied in any other system, and how it contributes to MQC and interacts with other signaling pathways during MQC is unknown. We have identified a C. elegans FUNDC1-like protein T06D8.7 and begun to analyze its role in MQC during hypoxia. T06D8.7 is in an operon together with three other genes that are involved in mitochondria or proteasome function. Here, we demonstrate that transcription of T06D8.7 is activated by hypoxia through HIF1 binding sites in both intragenic and operon promoters. We have generated T06D8.7 mutant worms using CRISPR-Cas9 mutagenesis and find that loss-of-function worms are protected against hypoxic injury and death. Atfs-1 codes for a central regulator of UPRmt and atfs-1 loss-of-function suppresses the T06D8.7 hypoxic-protected phenotype. This suggests that disrupting mitophagy elicits a UPRmt and subsequent adaptation to hypoxia. We are currently utilizing fluorescent biosensors to test the physiologic impact of the interaction between these fundamental signaling pathways in MQC.

1048C  Non-Autonomous Regulation of Systemic Mitochondrial Stress Response and Morphology by Neuronal fzo-1/Mitofusin. Liang-Yi Lin, Chih-Ta Lin, Jiun-Min Hsu, Hao-Ching Jiang, Chun-Liang Pan  Institute of Molecular Medicine, National Taiwan University School of Medicine, Taipei, TW.

Tissue-specific stress responses are protective mechanisms against proteotoxic stress and could be regulated in a non-autonomous fashion. Inhibition of mitochondrial respiration or proteostasis triggers systemic mitochondrial unfolded protein response (UPRmt), and recently serotonin and the FLP-2 neuropeptide had been shown to be important for this regulation. Here we report that disrupting mitochondrial dynamics in the neurons, by silencing the mitochondrial fusion gene fzo-1, induced UPRmt and mitochondrial fragmentation in the intestine. Acetylcholine, tyramine, glutamate and neuropeptides were required to mediate non-autonomous UPRmt. Our data suggest that tyramine signals derived from the RIM and RIC neurons target neurons that express the TYRA-3 tyramine receptor. Strikingly, neuropeptides, but not neurotransmitters, are important for non-autonomous regulation of mitochondrial dynamics in non-neural tissues. Consistent with previous studies linking UPRmt and bacterial defense, fzo-1 mutants showed avoidance to bacterial food. We are now exploring the neural mechanisms that link mitochondrial dynamics to non-autonomous UPRmt regulation and pathogen avoidance. (supported by National Health Research Institutes, NHRI-EX106-10529NI and the Ministry of Science and Technology, MOST 104-2320-B-002-058-MY3)

1049A  Screening Pro-Longevity Chemicals for Reproducible and Robust Positive Effects across Diverse Genetic Backgrounds. Mark Lucanic1, Max Guo2, Monica Driscoll3, Gordon Lithgow1, Patrick Phillips4, Caenorhabditis Intervention Testing Program 1) Buck Institute for Research on Aging, Novato, CA; 2) Rutgers University, Dept. of Molecular Biology and Biochemistry, Nelson Biological Laboratories, Piscataway, NJ; 3) Institute of Ecology and Evolution, University of Oregon, Eugene, OR; 4) Division of Aging Biology, National Institute on Aging, Bethesda, MD.

The Caenorhabditis Intervention Testing Program is a multi-institutional effort to screen promising chemicals for pro-longevity effects across diverse genetic backgrounds. Chemicals that act robustly, across diverse genetic backgrounds, are likely to target conserved pathways and will be promising leads to test in higher organisms, including vertebrates. This project makes use of the short-lived and genetically diverse Caenorhabditis genus to rapidly assess the robustness of pro-longevity chemicals. The project further emphasizes reproducibility of results as a primary goal. Towards this end, all experiments are performed in parallel at each testing site. Additionally all research sites perform multiple replicates of each assay, with large cohorts. By utilizing this rigorous approach we expect to facilitate and expedite the identification of promising drug leads for eventual use in treating human age-related diseases. Here we describe lifespan results for the 22 Caenorhabditis strains utilized, as well as our results from the first set chemical assays. While many of these chemicals extended the lifespan of at least one strain, the amyloid dye ThioflavinT was the only chemical to promote longevity across all strains tested. The CTP has demonstrated the ability to identify chemicals that act robustly across diverse genetic backgrounds. Currently we are focused on screening more chemicals for similar robust effects, as well as chemical testing the most promising chemicals for their efficacy in mitigating age-related declines in physiology. In parallel we are investing in and developing automated assays in an effort to increase the throughput of the CITP.


Previous work showed that chemosensation of hermaphrodite produced pheromones ascr#2 and ascr#3 increases C. elegans adult lifespan and stress resistance (Ludewig 2013), whereas male-produced pheromones of then unknown identity shorten hermaphrodite lifespan (Gems 2000, Maures 2014). We show that the male-produced ascaroside ascr#10 underlies male-induced hermaphrodite progeria, and that pheromone-mediated lifespan increase and decrease depend on several different sirtuins (Ludewig, 2013; Ludewig, unpublished). Sirtuins, a family of NAD+ dependent histone deacetylases, have been implied induced hermaphrodite progeria, and that pheromone-mediated lifespan increase and decrease depend on several different sirtuins (Ludewig, 2013; Ludewig, unpublished). Sirtuins, a family of NAD+ dependent histone deacetylases, have been implied.
Mitochondrial ROS production is sirtuin-dependent and requires an intact nicotinamide catabolic pathway, indicating that ascaroside-mediated effects on lifespan depend on conversion of NAD$^+$ into nicotinamide by sirtuins, similar to previously reported effects of sir-2.1 over expression (Schmeisser 2013).

Our research uncovered an endogenous lifespan regulatory system based on antagonism of longevity-promoting and progeria-inducing ascarosides that act by modulating sirtuin-dependent ROS production. Furthermore, our work suggests that the intensely discussed variability of sirtuin dependent lifespan phenotypes in C. elegans may be due to the confounding effects of worm-produced ascarosides accumulating on experimental plates, in conjunction with other environmental stimuli activating skn-1/Nrf.

1051C The Nucleosome Remodelling Factor subunit pyp-1 negatively regulates the heat shock response upon aging in Caenorhabditis elegans. M. Noble1, A. Deonarine1, D. Lugano1, L Bowie1, E Guisbert2, S Westerheide1 1) University of South Florida, Tampa, FL; 2) Florida Institute of Technology.

Title: The Nucleosome Remodelling Factor subunit pyp-1 negatively regulates the heat shock response upon aging in Caenorhabditis elegans

Mark Noble, Andrew Deonarine, Doreen Lugano, Lori-Ann Bowie and Sandy D. Westerheide

The heat shock response is a conserved eukaryotic stress response known to regulate protein homeostasis (proteostasis) and it is regulated by the transcription factor hsf-1. Previous studies have shown tremendous effects of the HSR on life span, health span, and disease models of neurodegenerative diseases such as Alzheimer’s, Parkinson and Huntington’s disease. In C. elegans, proteostasis has been shown to decline with the onset of adulthood in parallel to the decline of the HSR. Therefore, understanding the molecular mechanisms and regulation of this response is vital in revealing its effects on various stresses. Recent studies have shown a link between chromatin remodelers and regulation of the heat shock response. Thus, utilizing RNAi genetic screen, we were able a uncover pyp-1, a pyrophosphatase and subunit of the Nucleosome-remodeling factor (NURF) which showed tremendous effects on the HSR. From a different screen, pyp-1 was also found to be a negative regulator of the heat shock response in C. elegans. We hypothesize that the age-dependent decrease in HSR induction may be due to repressive chromatin changes conferred by chromatin remodeling complexes. As a result, we identified pyp-1 as a novel negative regulator of the heat shock response and proteostasis upon aging in C.elegans.

1052A Regulation of neurogenesis by stress. S. Luo1,2, B. Horvitz1,2 1) HHMI; 2) Dept. Biology, Massachusetts Institute of Technology, Cambridge, MA.

Unlike most neurons, which are derived from ectoderm, the C. elegans pharyngeal I4 neuron is generated from a mesodermal cell lineage. We found that efficient generation of I4 is dependent on HLH-3, the C. elegans homolog of the mammalian proneuronal protein Mash1, and the Mediator CDK-8 kinase complex (DPY-22, LET-19, CDK-8, CIC-1), which is conserved throughout evolution. HLH-3 and the CDK-8 Mediator function synergistically to promote efficient I4 neurogenesis. Recently we discovered that I4 neurogenesis is also modulated by environmental stresses, such as DMSO or ethanol. We found that DMSO and ethanol induce endoplasmic reticulum (ER) stress, as they elicit expression of hsp-4p::gfp, an ER stress reporter, but not of the mitochondria stress reporter hsp-6p::gfp or the cytosolic stress reporter hsp-16.2p::gfp. DMSO and ethanol each enhance I4 loss in hlh-3 but not in Mediator mutants, suggesting that DMSO and ethanol-induced stresses might function by suppressing CDK-8 Mediator function. Interestingly, we found that the p38 MAPK innate immunity pathway is required for DMSO and ethanol to suppress I4 neurogenesis. We hypothesize that DMSO or ethanol activates the p38 MAPK pathway, which suppresses I4 neurogenesis by inhibiting the CDK-8 Mediator function. Since in mammals the p38 MAPK pathway has been implicated in inflammation and neurodegeneration, by identifying the molecular mechanisms by which the p38 MAPK pathway regulates I4 neurogenesis, we hope to generate novel insights into the mechanisms of stress-induced neurogenesis defects and neuropathology.

1053B Role of the protein kinase MBK-1/DYRK1A in lifespan regulation of Caenorhabditis elegans. Hildegard Mack1, Peichuan Zhang2, Bryan Fonslow2, John Yates3 1) Institute for Biomedical Aging Research, University of Innsbruck, Innsbruck, Tyrol, AT; 2) Calico Life Sciences, South San Francisco, CA, USA; 3) Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA, USA.

In Caenorhabditis elegans, reduction of insulin/IGF-1 like signaling and loss of germline stem cells both increase lifespan by activating the conserved transcription factor DAF-16 (FOXO). While the mechanisms that regulate DAF-16 nuclear localization in response to insulin/IGF-1 like signaling are well characterized, the molecular pathways that act in parallel to regulate DAF-16 transcriptional activity, and the pathways that couple DAF-16 activity to germline status, are not fully understood at present. When analyzing DAF-16 posttranslational modifications by mass spectrometry, we observed phosphorylation at Ser326 in wildtype animals. This site corresponds to Ser329 in human FOXO1, which has been reported previously to be subject to inhibitory phosphorylation by the Down Syndrome-associated kinase DYRK1A. prompted us to investigate the role of the C. elegans DYRK1A ortholog MBK-1 in lifespan regulation. Unexpectedly, we found that inactivation of MBK-1 substantially shortened the prolonged lifespan of daf-2 and gfp-1 mutant animals while decreasing wild-type lifespan to a lesser extent. On the other hand, lifespan-reduction by mutation of the MBK-1-related kinase HPK-1 was not preferential for long-lived mutants. Interestingly, mbk-1 loss still allowed for DAF-16 nuclear accumulation but reduced expression of certain DAF-16 target genes in germline-less, but not in daf-2 mutant animals. These findings indicate that mbk-1 and daf-16 functionally interact in the germline- but not in the daf-2 pathway. Together, our data suggest mbk-1 as a novel regulator of C. elegans longevity upon both, germline ablation and DAF-2 inhibition, and provide evidence for mbk-1 regulating daf-16 activity in germline-deficient animals.
Investigating the regulatory pathway of C. elegans metallothionein gene mtl-2. Christopher Marinello, Melissa Henderson, Julie Hall Lincoln Memorial University, Harrogate, TN.

Metallothioneins are small, cysteine-rich, metal-binding proteins that are important in metal detoxification and homeostasis in the cell. MTs are highly conserved and C. elegans has two, mtl-1 and mtl-2. Interestingly, C. elegans metallothionein genes lack MREs, the well-known transcriptional metal regulatory region found in the promoters of most eukaryotic metallothionein genes. In addition, a homolog of the metal-regulatory transcription factor, MTF-1, has not been identified in the C. elegans genome. Genetic screens have identified a negative regulatory pathway for mtl-1 which includes ATF-7, PMK-1 and members of the insulin signaling pathway, PDK-1 and the AKT-1/-2 complex. As determined by qRT-PCR mtl-2 expression is not affected by these genes found to regulate mtl-1. While it has been identified that ELT-2 is required for metallothionein gut expression this alone does not explain the response to cadmium and other cellular stresses. To identify potential regulators of mtl-2 expression two major strategies are being employed: (1) creation of transgenic strains of C. elegans containing GFP under the control of mtl-2 promoter using the CRISPR/CAS9 system and (2) forward genetic screen using RNAi knockdown of genes associated with cellular detoxification and response to stress. GFP expression will be measured after exposure to cadmium and other heavy metals along with the knockdown of candidate genes to determine the regulatory pathway of mtl-2.

Functional decline of the nervous system associated to pathological aging. C.N. Martineau1, 2, B. Baskaner2, R.I. Seinstra2, W.R. Schaffer3, A.E.X. Brown4, P. Laurent1, E.A.A. Nollen2 1) ULB Institute for Neuroscience, ULB, Brussels, BE; 2) European Research Institute for the Biology of Ageing, UMCG, University of Groningen, NL; 3) Laboratory of Molecular Biology, MRC, Cambridge, UK; 4) London Institute of Medical Sciences, MRC, London, UK.

Accumulation of protein aggregates in the brain is a hallmark of several progressive neurodegenerative disorders, including Parkinson’s disease. We developed new Caenorhabditis elegans models to integrate information from live animals on age-related protein aggregation and toxicity. While protein aggregation can be followed over time with fluorescent reporters, the physiological decline associated to proteo-toxicity is assessed by behavioural approaches. Using our models combined to genetic, biochemical and behavioural tools, we aim to identify and characterize modifiers of proteo-toxicity to the brain to better understand age-related cognitive impairment.

One of our models is based on the heterologous expression of human alpha-Synuclein fused to YFP in the 8 dopaminergic neurons of C. elegans. No neuronal loss could be observed in this model, but the distribution of the YFP fluorescence evolves with age and an accumulation of fluorescent foci, suggesting a relocation of alpha-Synuclein-YFP into protein inclusions. The median lifespan of these animals is moderately shortened in comparison to wild-type. Behaviour is the principal output of C. elegans nervous system functions. To better understand the impact of aggregation-prone proteins on the functions of the nervous system and on healthspan of the organism, we performed a comprehensive characterization of the locomotory behaviour of the worm. We generated a dataset describing the behaviour of wild-type worms, disease-model worms, and control worms throughout their lifespan, at the steady state. Individual animals were followed every day to capture individual variability and compare normal and pathological ageing patterns. Our preliminary data indicates that at steady state, the behaviour of animals expressing alpha-Synuclein evolves similarly to the one of control animals over age, suggesting that overall fitness might be conserved at the organism scale. In contrast, data obtained on worm populations showed that the locomotory behaviour in response to different stimuli is altered over age when alpha-Synuclein is expressed in dopaminergic neurons.

The behavioural repertoire of the worm is complex and variations in behaviour from single-animal measurements under stimulation should give new insights on the evolution of neuronal functions and interactions during pathological aging.

Repetitive starvation leads to fat accumulation in C. elegans. Shinya Matsumoto, Rena Yamamoto, Kanae Yamamoto, Misaki Nagao, Nao Sato, Sayuri Yamamoto, Akari Sawanaga Department of Food and Nutrition, Kyoto Women’s University, Kyoto, Kyoto, JP.

Our aim is to define how repeated starvation affects the biology, physiology and molecular biology of organism, by using C. elegans. Adult worms were exposed to two cycles of 6hr starvation-18hr feeding diet pattern, and their fat accumulation was analyzed by Nile Red staining and biochemical quantification. The expression of genes involved in fat synthesis (pod-2, fasn-1, mboa-2, sbp-1) and fat degradation (hosl-1, lipl-4, cpt-1, cpt-2, B03003.3, F53a2.7) in the worms were also determined by real-time RT-PCR.

Both fluorescent analysis and biochemical quantification showed that fat content of worms experienced repeated starvation increased. There was no obvious change in the expression of genes involved in fat synthesis, but those of fat degradation tended to decrease, which is consistent with the increment of fat in worms experienced repeated starvation. The life span, fecundity and mobility of worms experienced repeated starvation did not show differences compared to those of the control worms.

Our results indicate that repeated starvation causes metabolic and nutritional effect on organism. It is often mentioned that repeated, and often unsuccessful, dieting leads to weight regaining of all or more than initial loss, which is referred as rebound weight gain. However, it is difficult to know whether such weight gain is caused by metabolic and/or genetic alteration. Our data in worms may provide a molecular basis of rebound weight gain.

Age-related decline in muscular strength and coordination observed in C. elegans pharynx. Adela Chicas-Cruz, Yoanne Clovis, Terra Hiebert, Kathryn McCormick NemaMetrix, Inc, Eugene, OR.

Aging has been associated with a progressive decline of feeding behavior, but detailed quantification of how this process affects pharyngeal muscle function remains largely unexplored. We profiled the feeding behavior along the lifespan of adult C. elegans worms. Taking advantage of microfluidics combined with whole-body electrophysiology, we found that in older adults,
the pharyngeal activity declines, not only in frequency, but also by in pumping regularity and strength. Our data uncover previously undocumented changes in *C. elegans* feeding behavior associated with age. We show that the duration of pharyngeal contractions (pumps) become more variable as the worm ages. We also show that pumps are interspersed with longer pauses in older worms, as shown by a 35% increase in interpump duration between Day 1 and Day 10. Because of the worms’ larger size, we expected to measure a stronger signal in older worms than in young adults. Surprisingly, a decrease in pump amplitude was observed in older worms, showing that despite being larger in size, 10-day old worms produce weaker pumps, consistent with an overall loss of muscle strength in the pharynx. We then exposed aging worms to trehalose, a stress protectant previously shown to increase longevity in *C. elegans*, and found that trehalose can mitigate the onset of the observed pharyngeal pumping deficiency. In this study, we describe a novel method to reliably quantify the effect of aging on multiple aspects of feeding behavior. We demonstrate that beyond pharyngeal pumping rate, the feeding behavior and muscular activity of *C. elegans* are strongly impaired by aging. However, such defect can be mitigated by exposure to trehalose.

1058A  **Genes involved in translation have increased antisense transcription during heatshock.**  **Marko Melnick,** Patrick Gonzales, Joseph Cabral, Christopher Link  Institute for Behavioral Genetics, Integrative Physiology, CU Boulder, Boulder.

Deletion of TDP-1, the worm ortholog of the ALS-associated RNA binding protein TDP-43, results in the accumulation of double-stranded RNA (dsRNA). Foci of dsRNA can be detected in a TDP-1 deletion strain using the dsRNA-specific monoclonal antibody J2. While screening for conditions that influenced the formation of dsRNA foci we discovered that heat shock robustly induced nuclear dsRNA foci in intestinal and hypodermal cells of wild type worms. To assay this unexpected formation of dsRNA, we performed strand-specific RNA-seq on total and J2-immunoprecipitated RNA from control and heat-shocked wild type worms. We found that the dsRNA pool in heat-shocked wild type worms differed significantly from that observed in the TDP-1 deletion strain: it included extensive antisense transcripts mapping to a limited set of loci in the genome. This antisense transcription correlated with reduced sense transcription of nearby genes. Interestingly, many of these affected genes appear to be involved in translation. For example, we observe increased antisense and decreased sense transcription of eukaryotic translation initiation factor 3 subunit (eIF-3b), which is required for several steps in the initiation of translation of mRNAs.

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1059B  **Endosomal/autophagic regulation of FOXO transcription factors.**  **I. Meras**\(^1\), L. Chotard\(^2\), C., E. Rocheleau\(^1,2\) 1) Anatomy and Cell Biology, McGill University, Montreal, Quebec, CA; 2) Department of Medicine, Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada, H4A 3J1.

FOXO proteins are a conserved family of transcription factors that regulate metabolism, stress responses, and lifespan. Loss of FOXO proteins is associated with cancer, neurodegenerative and metabolic diseases. Insulin/IGF signaling (IIS) pathway negatively regulates FOXO by activating the Akt1/2 kinase, which phosphorylates FOXO. The 14-3-3 proteins bind phospho-FOXO (pFOXO), sequestering it in the cytoplasm. FOXO proteins were first identified as downstream targets of IIS in the nematode *C. elegans* which has a single FOXO, DAF-16, that is most closely related to human FOXO3a. During fed conditions, *C. elegans* IIS is constitutive which keeps DAF-16 in the cytoplasm. Under starvation conditions (and other stresses), there is no IIS, and DAF-16 enters the nucleus to regulate gene expression. We found that in the intestine of fed *C. elegans*, DAF-16 localizes to a subset of RAB-5-positive early endosomes. DAF-16 endosomes are lost in rab-5(RNAi) animals and significantly expanded in tbc-2 mutants that result in increased RAB-5 activity. In starved animals, DAF-16 endosomes are lost, and DAF-16 localizes mainly to the nucleus, whereas re-feeding results in relocalization of DAF-16 onto endosomal membranes. These results suggest that IIS promotes DAF-16 localization to endosomes. Consistent with this hypothesis, loss of daf-18 pten, a negative regulator of IIS, results in an increase in the number of animals with DAF-16 endosomes, while knockdown of the 14-3-3 protein, FTT-2, results in loss of DAF-16 endosomes. Furthermore, we found that FTT-2 14-3-3 protein colocalizes with DAF-16 on endosomes. This suggests that the endosomal pool of DAF-16 is phosphorylated. This is conserved in human cells as we discovered that pFOXO1/3a colocalizes with activated Rab5 on the endosomes in HEK293 cells.

We do not know the ultimate fate of pFOXO in endosomes, but our data shows that LGG-1 engulfs DAF-16 endosomes and knockdown of lgg-1/2 increased the number of DAF-16 endosomes, suggesting that they are degraded by autophagy. We hypothesize that IIS pathway regulates FOXO proteins on endosomes where it can be released if needed or degraded via selective autophagy. Relevance: Here we are proposing a new mechanism of FOXO regulation on signaling endosomes and turnover by selective autophagy. We believe this project will provide new insights into the regulation of FOXO proteins and open new avenues for drug development to regulate FOXO in cancer, neurodegenerative and metabolic diseases.

1060C  **Role of Sphingolipid Enzymes in the Regulation of Stress Response and Health Span.**  **C.I. Meyer**, J. Brown, K. Heasley, A. Kalil, J. Chan  Department of Biology, Juniata College, Huntingdon, PA.

Sphingolipids play an important role in different regulatory processes including those for structural support and cell signaling. In particular, sphingosine is a sphingolipid involved in regulating cell death, whereas sphingosine-1-phosphate (S1P) is a sphingolipid that induces cell proliferation and inhibits apoptosis. Thus, the enzymes that control cellular levels of sphingolipids, such as sphingosine kinase, S1P lyase, and ceramide synthase, are critical to cell survival decisions. Cells face these decisions during stress, which might increase molecules such as reactive oxidative species (ROS). The accumulation of ROS can potentially act as a stressor and cause cellular damage. Cellular response to ROS includes the upregulation of detoxifying proteins such as glutathione S-transferases (GSTs) and superoxide-dismutases (SODs). Recently, it has been shown that...
sphingolipids are important for stress and starvation responses in animals. Our lab has demonstrated that C. elegans mutants lacking sphingosine kinase (sphk-1) have shorter health spans and express poor stress responses. However, it is not known whether sphingolipids directly influence stress response through detoxification protein pathways. To examine this, we further analyzed stress response in a panel of C. elegans sphingolipid metabolism mutants, including sphingosine kinase (sphk-1), ceramide synthase (hyl-1, hyl-2), and S1P lyase amongst others. We will present our analyses of transcriptional reporters of the genes, gst-4, a GST-like protein, and sod-3/SOD in animals under control or stress-induced conditions. Furthermore, we aim to examine how stress responses change as animals age, and whether the dynamics of sphingolipid metabolism might contribute to poor aging. Our data will enhance our understanding of the role of sphingolipid metabolism in the stress response pathways in all eukaryotic cells. Since longevity is correlated to how well a cell can respond to environmental or internal stress, our conclusions will give insight to how bioactive lipid-regulated stress response impacts health span.

1061A A screen for reproductive span extension genes identifies repx-1, a novel regulator of reproductive span.  Rebecca Moore1, Cheng Shi1, Shijing Lou1, Eileen Zhuang1, Coleen Murphy1,2 1) Molecular Biology, Princeton University, Princeton; 2) LSI and Genomics, Princeton University, Princeton.

Female age-related reproductive cessation, which is generally caused by decreasing oocyte quality, is one of the earliest age-related declines observed in humans, but little is known about its genetic regulation. C. elegans experiences a similar decline of oocyte quality with age, but can be maintained through the manipulation of endocrine signaling. In order to better understand the biology of reproductive aging, we performed a small Mo1-mediated insertional mutagenesis screen to identify mutants with extended reproduction span. Five genes were identified, including repx-1 (reproductive span extension-1), a putative tyrosine kinase with homology to FGFR. repx-1 mutants extend reproductive span and slow age-related declines in oocyte and germline morphology, but are not long-lived, uncoupling these aging phenotypes. repx-1 does not act through known reproductive span regulators (i.e. TOF-b and Insulin signaling pathways), but shares downstream biomarkers of oocyte quality. repx-1 acts non-autonomously in the intestine to regulate germline quality. To identify potential interacting partners, we performed a yeast 2-hybrid screen, and found that FAR-2, predicted to be involved in fatty acid and retinoid scavenging, bound to REPX-1. Like repx-1, far-2 mutants also extend reproductive span, and far-2 functions upstream of repx-1 in the body wall muscle and vulval cells of adult hermaphrodites. These results suggest that additional pathways that may modulate late-life reproduction, independent of lifespan regulation, await discovery.


Remarkably the c-Jun-NH2-terminal kinase (JNK) pathway is all evolutionarily conserved across species. In view of the hypothesis that increased stress resistance subdue aging, we investigated the role of ursolic acid (3β-Hydroxy-urs-12-en-28-oic acid; UA) in the pioneering aging model Caenorhabditis elegans with an increase in mean and maximum lifespan by up to 30%. Our genetic study unravelled the underlying pathway (where JNK-1 is acting independently of insulin-IGF-1 signalling (IIS) pathway to modulate longevity. In support of in vivo results in silico docking study of UA with C. elegans JNK-1 ATP-binding site suggested promising binding affinity exhibiting binding energy of -8.11 kcalmol$^{-1}$. UA induced JNK-1 activation in wild-type animals underline the importance of pharmacological interventions in the delineation of molecular targets for aging and associated pathologies.

1063C Movement decline and lifespan trajectories of C. elegans insulin/insulin-like signaling pathway mutants.  B. Newell Stamper1,2, K. Kechris3, D. Kitzenberg4, J. Cypser2, P. Tedesco2, TE Johnson1,2 1) Integrative Physiology, University of Colorado Boulder, Boulder, CO; 2) Institute for Behavioral Genetics, University of Colorado Boulder, Boulder, CO; 3) Biostatistics and Informatics, University of Colorado Denver, Denver, CO; 4) Cell and Developmental Biology, University of Colorado Boulder, Denver, CO.

Aging researchers have identified several pathways that are molecularly conserved across species and that extend lifespan when mutated. The insulin/insulin-like signaling (IIS) pathway is one of the most widely studied of these. It has been assumed that extending lifespan also extends healthspan (the period of life with minimal functional loss). However, data supporting this assumption conflict and recent evidence suggests that life extension may, in and of itself, extend the frail period. In this study, we use Caenorhabditis elegans to further probe the link between lifespan and healthspan. Using movement decline as a measure of health, we assessed healthspan across the entire lifespan of nine IIS pathway mutants. In one series of experiments, we studied healthspan in mass cultures, and in another series we studied individuals longitudinally. We found that long-lived mutants display extended mid-life movement, and do not extend the frailty period. Lastly, we observed that early-adulthood movement was not predictive of late-life movement. Overall, these observations show that extending lifespan does not extend the period of frailty, and that both genotype and a stochastic component modulate aging.

1064A Identification of CLP-1 atypical calpain substrates in C. elegans.  L. Newman, P. Kuwabara  Department of Biochemistry, University of Bristol, Bristol, GB.

The global shift towards an increasingly aged society has led to an acute awareness of age-related degenerative disease. C. elegans has become a widely-accepted model for studying the molecular mechanisms underlying ageing and age-related diseases.

Calpains are regulatory Ca$^{2+}$ activated cysteine proteases present in nearly all eukaryotes$^1$. Dysregulation of Ca$^{2+}$ homeostasis resulting in aberrant calpain activation is implicated in contributing to age-related pathologies, such as Alzheimer’s disease and
sarcopenia. However, it has been difficult to identify substrates targeted by calpain activation, because cleavage specificity is not determined by primary sequence alone\(^2\).

Our present understanding of human calpain activity is based primarily on studies of ‘typical’ calpains, which contain EF hand domains; less is understood about the six ‘atypical’ calpains lacking this domain. The \(C.\) elegans genome only encodes atypical calpain genes, which were previously shown to be differentially expressed in a variety of tissues\(^3\). Overexpression of CLP-1 in body wall muscle was also shown to cause a low penetrance paralysis phenotype in adults accompanied by muscle degeneration. The level of paralysis was increased when CLP-1 was overexpressed in \(egl-19(gf)\) mutants due to elevated levels of intracellular calcium.

We have now performed a proteomic screen to identify CLP-1 interacting partners by co-immunoprecipitation followed by mass spectrometry. Our study has identified a subset of proteins known to be required for muscle integrity and normal locomotion, which are being validated by quantitative Western analysis. Other potential substrates identified that had not been previously associated with the maintenance of muscle integrity are being analysed by RNAi. We are also carrying out domain truncations of CLP-1 to understand how its intracellular localisation in muscle is specified and what impact this has on substrate binding. From these experiments we aim to identify the physiological and pathological roles of atypical calpains, and in doing so, understand how atypical calpains participate in age-related degenerative conditions in higher organisms.


\textbf{1065B} \textbf{The role of SKN-1 and DAF-16 in protein homeostasis and polyglutamine aggregation in the \textit{Caenorhabditis elegans} intestine.} 1) I.T.B.R. Paula\(^1\), V.S. Pereira\(^1\), R. Guerra-Sá\(^1\), R.P. Oliveira\(^2\)
\(\text{\(^1\) Núcleo de Pesquisas em Ciências Biológicas, UFOP, Ouro Preto, MG, Brasil; \text{\(^2\) Biologia Celular e Genética, UFRN, Natal, Rio Grande do Norte, Brasil.}\)

DAF-16 and SKN-1 are both transcription factors that play highly conserved roles in regulating stress resistance and longevity genes. Besides that, they also contribute to protein homeostasis, activating genes related to protein folding and degradation. Here, we attempt to further characterize SKN-1 functions on protein homeostasis. Analysis of gene expression at the L4 stage showed that fumarilacetoacetase and proteasome subunits genes were significantly reduced in \(skn-1\text{(RNAi)}\). Also, the proteasomal activity was significantly decreased in \(skn-1\text{(RNAi)}\) and \(daf-16\text;skn-1\text{(RNAi)}\) animals but not in \(daf-16\text{RNAi}\) animals. Since SKN-1 is predominantly expressed during larval and adult stages in the intestine, we examined the aggregation pattern in transgenic animals expressing 44, 64 and 82 glutamine repeats (PolyQ) in the intestinal cells fused with the Yellow Fluorescent Protein (YFP). As expected for polyQ-containing proteins, we observed an increase in the number of aggregates in Q44 and Q64 animals on \(control\text{(RNAi)}\) as they aged. However, the average number of aggregates in Q82 animals was reduced at the 8th day. Surprisingly, knockdown of \(skn-1\) resulted in a significant reduction in the number of aggregates in Q44, Q64 and Q82 animals. We next explored three possible scenarios to explain why there is a reduction in the number aggregates at the 8th day and on \(skn-1\text{(RNAi)}\) condition. First, we measured the sizes of individual aggregates and found no correlation with the number of aggregates. Second, we observed that all transgenic lines treated with 5-fluoro-2-deoxyuridine (FUdR) showed reduced number of polyQ aggregates at all stages, suggesting a trade off mechanism between reproduction and proteostasis input. Lastly, we observed that the proteasomal activity of 8-day-old Q82 animals on \(skn-1\text{(RNAi)}\) or \(daf-16\text{RNAi}\) is increased compared to \(control\text{(RNAi)}\). The inactivation of the proteasomal and lysosomal activity by MG-132 and chloroquin, respectively, resulted in a significant increase in the number of aggregates at the 8-day-old Q82 animals on \(skn-1\text{(RNAi)}\). Moreover, knockdown of both \(skn-1\) and \(daf-16\) increased the aggregation in the intestine of 8-day-old Q82 animals. Our findings suggest that in wild type animals \(skn-1\) plays an important role in proteostasis by upregulating proteasome gene expression and proteasomal activity. Besides that, the presence of toxic polyQ aggregates in the intestine, the major site of detoxification response, appears to trigger a compensatory mechanism of the proteostasis network coordinated by \(SKN-1\) and \(DAF-16\).

\textbf{1066C} \textbf{Larval density during L1 arrest impacts recovery from starvation through changes in DAF-16 nuclear localization.} 1) M. Olmedo\(^1\), M. J. Rodríguez-Palero\(^2\), A. Fernández-Yáñez\(^2\), A. Mata-Cabana\(^1\), L. Cerero\(^1\), M. Merrow\(^3\), M. Artal-Sanz\(^2\)
\(\text{\(^1\) Department of Genetics, University of Sevilla, Spain; \text{\(^2\) CABD, University Pablo de Olavide, Spain; \text{\(^3\) Department of Chronobiology, Ludwig-Maximilians-University Munich, Germany.}\)

The developmental progression of \textit{Caenorhabditis elegans} can be slowed down or interrupted when animals face unfavourable conditions like food scarcity. \textit{C. elegans} larvae arrest as L1s when hatching in the absence of food. Arrested L1s can survive several weeks without food and present increased resistance to stress. Several genetic and environmental factors impact the survival of arrested L1s. These include the Insulin/Insulin-like growth factor signalling (IIS) pathway and larval density during arrest.\(^1,2\) The effect of density is mediated by exposure of unknown compounds secreted after hatching.\(^2\)

When food becomes available, larvae resume postembryonic development but animals that have been starved for long periods of time take longer to reach adulthood\(^3\). This effect of starvation was attributed to a developmental delay after extended starvation. However, using a method we developed to measure developmental timing\(^4\) we have been able to measure the duration of each stage of development after extended starvation. We have observed that developmental speed is resilient to time in starvation. The later entry into adulthood after extended arrest corresponds, instead, with a delay to resume development. That is, extended starvation increases recovery time.

Using the same method, we observe that recovery time is affected by interventions that affect survival to starvation, like
mutations in the IIS pathway and density of animals during arrest. In addition, we show that high density of animals contributes
to the maintenance of DAF-16 nuclear localization during arrest, contributing to longer survival and fast recovery from arrest.
Our results indicate that density and low insulin signalling impact DAF-16 function in a similar manner, possibly allowing
integration of the two signals to control developmental arrest.


1067A Dietary restriction promotes healthspan via a glucagon-like signaling pathway in C. elegans. B. Onken Centenary University, Hackettsown, NJ.
A major goal of aging research is to understand the underlying relationship between nutritional intake, metabolism, and
healthy aging Low-glycemic index diets have been shown to reduce risk of age-related metabolic diseases such as diabetes and
cardiovascular disease, and reduced caloric intake via dietary restriction (DR) increases healthspan across species One
potential approach for supporting healthy aging is via interventions that engage healthspan-promoting metabolism
My previous work demonstrated that interventions that block glycolysis result in striking healthspan gains, while increased
 gluconeogenic activity can similarly promote healthy aging I also showed that DR increases healthspan in a manner that
requires gluconeogenic gene expression These results suggest that molecular pathways that engage gluconeogenic metabolism
and inhibit glycolysis may generally work to improve the quality of aging in mammals, the glucagon signaling pathway works
opposite to the insulin pathway to regulate blood sugar levels: when circulating glucose levels are low, glucagon promotes
glucose production in the liver by stimulating glycogenolysis and gluconeogenesis while inhibiting glycogen synthesis and
glycolysis I reason that glucagon signaling, like DR (and unlike insulin signaling), may have an overall positive impact on
healthspan
To investigate this, I screened for potential glucagon receptors in Caenorhabditis elegans, and found one candidate, pdfr-1,
which is required for the induction of gluconeogenic gene expression under DR and for the long lifespan of dietary-restricted
animals In the mammalian glucagon signaling pathway, the G protein alpha subunit coupled to the glucagon receptor activates
adenylate cyclase, which increases cAMP levels to activate protein kinase A, which in turn inhibits glycolytic activity and
promotes gluconeogenesis Similar to pdfr-1, I found that a C. elegans adenylyl cyclase ortholog, acy-1, is required for
increased lifespan under DR I also found that disruption of kin-2, which encodes the inhibitory subunit of the C. elegans PKA
ortholog kin-1, triggers biomarkers for the DR state and results in dramatic healthspan increases that mirror those seen under
DR My observations reveal a potential C. elegans glucagon pathway that is required for DR healthspan bene ts, and suggest
novel approaches to improving the quality of aging via interventions that promote glucagon pathway signaling

1068B The impact a glucose diet has on physiology, stress responses, behavior and gene expression in C. elegans. Dennis Dumesnil, Saifun Nahar , David Burks, Abhishek Shah, Rajeev Azad, Pamela Padilla Dept Biological Sci, Univ North Texas, Denton, TX.
Hyperglycemia, increased free fatty acids, and insulin resistance are metabolic abnormalities associated with diabetes which
in-turn contribute to microvascular and macrovascular dysfunctions leading to severe health issues including organ dysfunction,
tissue damage, blindness and amputations. Here we are using C. elegans to gain a greater understanding of the impact a
glucose diet has on physiology, stress responses, behavior and gene expression profile. A diet supplemented with sugar
increases lipids levels indicating that glucose diet can be used as an obesity mimetic.

Mitochondria are essential for energy production and have vital roles in calcium signalling and storage, metabolite synthesis
and apoptosis in eukaryotic cells. Neuronal cells depend, perhaps more than any other cell type, on proper mitochondrial
function. Thus, maintenance of neuronal homeostasis necessitates a tight regulation of mitochondrial biogenesis, as well as, the
elimination of damaged or superfluous mitochondria. Mitochondrial impairment has been implicated in several age-related
neurodegenerative diseases. Mitophagy is a selective type of autophagy mediating elimination of damaged mitochondria, and

Mitophagy and neuronal homeostasis in C. elegans. K. Palikaras1, N. Tavernarakis1,2 1) Institute of Molecular Biology and Biotechnology, Heraklion, Crete, GR; 2) Medical School, University of Crete, Greece.

1069C Mitophagy and neuronal homeostasis in C. elegans.
the major degradation pathway, by which cells regulate mitochondrial number in response to metabolic state. However, little is known about the effects of mitophagy deficiency on neuronal physiology. To address this question, we developed two composite, in vivo imaging systems to monitor mitophagy in neurons. We find that neuronal mitophagy is induced in response to oxidative stress. Mitochondrial dysfunction leads to transport of axonal mitochondria towards the neuronal cell body, in a calcium- and an AMPK/AKT-2-dependent manner. Impairment of autophagy increases the number of mitochondria in neurons and abolishes mitochondrial axonal transport upon stress. Additionally, mitophagy deficiency results in enhanced cell death in C. elegans models of neurodegeneration. Our findings indicate that mitophagy contributes critically to the preservation of mitochondrial homeostasis and neuronal health.

1070A Mechanistic insights into SIN-3 regulated autophagy and modulation of Caenorhabditis elegans lifespan. Renu Pandey, Meenakshi Sharma, Daman Saluja Dr. B. R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi, Delhi, IN.

Statement of Purpose: Any change in the environmental steady state, or homeostasis at the cellular and genetic levels has direct implications on fecundity and the lifespan of an organism. Reactive oxygen species (ROS) long reflected to be mere toxic spin-offs of oxidative metabolism have now been established to be essential mediators in tightly controlled signalling cascades. Understanding the part played by ROS and redox dynamics in stress response, development and aging requires concurrent evaluation in an appropriate living system and Caenorhabditis elegans becomes an obvious choice. SIN-3 (Swi-independent) first isolated in a yeast genetic screen as a negative regulator is now established to be a master transcriptional scaffolding agent and a co-repressor manifesting itself via the Histone Deacetylase Complex (HDACs). With many functions being attributed to SIN-3 from yeast to humans and the paucity of work on the model system C. elegans in this regard led us to study this protein in the model system. The objective of our study was to understand the dynamics of aging regulated through the SIN-3 network.

Methods: The methodology employed to gain insights into our study includes- culture of C. elegans strains, gene expression analysis, biochemical analysis of various oxidative stress markers like catalase activity, lipid peroxidation, ATP assay, NADPH redox status and microscopy to visualise autophagic events

Summary: Our results indicate a reduction in the mean lifespan of the mutant sin-3(tm1276) worms as compared to the wild type. Both the mitochondrial and overall ROS levels were found to be elevated in the mutant sin-3(tm1276) strain. The mRNA expression analysis through quantitative real time PCR showed differential regulation of ROS associated genes like sod(s), hif-1a, dct-1 and hcf-1. Staining with DCFDA also exhibited higher ROS in mutant worms as compared to the control. Other hallmarks of excessive ROS like lipid peroxidation and levels of other enzymatic antioxidants were found to be significantly elevated in the mutant strain. Autophagy has long been associated with cell survival and rescue in stress. Our data demonstrates a ROS dependent increase in autophagy. Hence providing extensive insights into the sin-3 mediated regulation of lifespan.


Nematocida parisii is a fungal-related natural intracellular parasite that infects the Caenorhabditis elegans intestine. Using transcriptional analysis our lab identified a response to this parasite distinct from responses to other known bacterial and fungal pathogens of C. elegans, but similar to the response to Orsay virus infection (Bakowski et al, PLoS Pathogens 2014). We have named this response the Intracellular Pathogen Response (IPR) and it involves the transcriptional up-regulation of several components of a predicted Cullin-RING ubiquitin ligase complex (CRL) such as cul-6, skr-3,4,5 as well as several genes of unknown function including pals-5. The IPR appears to be distinct from other stress responses. Using EMS screening we have found that the gene pals-22 is a repressor of the IPR – pals-22 mutants have the IPR constitutively on and show a reduced level of polyQ aggregates, as well as a heat stress phenotype dependent on cul-6 (Reddy et al, in preparation). Thus our model suggests the existence of a new stress pathway repressed by PALS-22, whose activation leads to an increased proteostasis capacity through the activity of ubiquitin ligases containing CUL-6, which target unfolded proteins with ubiquitin for subsequent degradation. GFP-tagged version of these proteins show that the effector proteins CUL-6 and PALS-5 are strongly expressed in the intestine after activation of the IPR, as well as in the pharynx for CUL-6. PALS-22 and the related protein PALS-25 (a suppressor of PALS-22 we also identified through EMS screening) are expressed broadly in the worm but show stronger expression in the phasmid and amphid neurons. This result suggests there may be neuronal involvement in the IPR pathway. To provide insight into proteins of unknown function like PALS-5, PALS-22 and PALS-25, as well as to determine if CUL-6 functions in a true CRL, we have performed co-immunoprecipitation to identify binding partners. Preliminary results show a direct interaction between PALS-25 and PALS-22, which together with their common tissue expression suggests that PALS-22 may repress PALS-25 activity through a direct protein-protein interaction. CUL-6 binding partners included SKR-3 and NEDD-8 indicating that CUL-6 acts in a CRL. The other effector protein PALS-5 was found to interact with SQST-1 suggesting a role for this protein in autophagy. Indeed, our previous results have shown that SQST-1 plays a role in resistance again this pathogen. In future studies we will investigate the functional role of identified binding partners as well as the ubiquitylation profile of worms expressing the IPR to obtain a better understanding of this novel response.

1072C Eugenol, Xenohormesis and Longevity Promotion. A. Pant, R. Pandey Microbial Technology and Nematology, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, IN.

Aging is a quasi-programmed phenomenon which is universal to all organisms. Understanding the molecular mechanisms regulating aging and age-related diseases is most sought after for designing therapies and interventions for managing age-
related mortality and morbidity. Thus, the discovery of age-defying natural molecules might cure age-related diseases in humans. Therefore, in an effort to identify safe and effective therapy of natural origin, the present study was designed to evaluate the stress modulatory and lifespan modulating potential of eugenol employing Caenorhabditis elegans model. The Eugenol pre-exposure was found to promote lifespan and alter the stress level in C. elegans in a dose-dependent manner. Therefore, to further unravel the possible genetic mechanism underlying eugenol mediated longevity and stress resistance, the interaction of eugenol with gene regulating lifespan was deciphered by studying effect of eugenol on gene mutants and gene expression of these genes. The daf-2, daf-16, eat-2, sir-2.1, mev-1, skn-1(zu67) and skn-1(zu135) gene mutants were exposed to eugenol (50 µM) and vehicle control. In addition to that knock down of wdr-23 was achieved by RNAi and employed for studying the interaction of eugenol on wdr-23. The eugenol mediated lifespan extension, antiaging effects, stress tolerance and maintenance of healthy lifespan is regulated by multiple cellular signaling pathways. As eugenol treatment failed to augment lifespan of daf-2, eat-2, sir-2.1, skn-1(zu135), skn-1(zu67) and wdr-23 significantly suggesting the involvement of these genes in eugenol mediated stress modulation and lifespan extension. The eugenol exposure was found to mediate DR like effects and xenobiotic stress response in worms which later on modulated insulin/IGF-1 signalling response regulated by master transcription factor DAF-16, SKN-1/NRF pathway regulated by SKN-1, DR like effects, stress response and autophagy by SIR-2.1/SIRTUIN-1 (NAD-dependent deacetylase), P38 MAPK pathway which is regulated by PMK-1, WDR-23/SKN-1 pathway regulated by wdr-23, heat shock response by HSF-1. Altogether, these present study for the first time reports anti-aging and stress modulatory effects of eugenol. These interesting findings highlight the significance of natural molecules in designing therapeutics for managing aging and age-related diseases.

Keywords: Aging, Eugenol, Anti-aging, Natural molecules, Xenohormesis, Caenorhabditis elegans

1073A DNA damage-induced autophagy and necrotic neurodegeneration during ageing in C. elegans. M.E. Papandreou 1,2, N. Tavernarakis 1,2 1) Institute of Molecular Biology & Biotechnology, FORTH, Heraklion, Crete, Greece; 2) Department of Basic Sciences, Faculty of Medicine, University of Crete Heraklion, Crete, Greece.

Accumulation of DNA damage is a key determinant of ageing and has been implicated in neurodegeneration. Although it is well known that ultraviolet (UV) radiation induces apoptosis, the contribution of necrotic cell death to DNA damage-related pathology remains elusive. To address this question, we developed a nematode model for DNA damage-induced neurodegeneration by using UV-C irradiation to trigger DNA damage in C. elegans neurons. Initial observations using this model show a marked increase of cytoplasmic calcium concentration upon UV irradiation. To examine whether this acute cytoplasmic calcium elevation triggers necrosis in neurons, we exposed DNA repair-defective mutants to UV light. These mutant animals are hypersensitive to UV irradiation and exhibit widespread necrotic cell death in somatic tissues upon exposure, while neurons are particularly affected. Runaway autophagy has previously been implicated in necrotic neurodegeneration. In this context, we investigated the contribution of autophagy in DNA damage-induced cellular pathology and nuclear dynamics. Notably, we found that DNA damage induces autophagic flux and alters nuclear dynamics both in nematodes and mouse cells. We are currently dissecting the interplay between DNA damage-induced autophagy, nuclear membrane alterations and necrotic cell death, aiming to identify evolutionarily conserved molecular mechanisms interfacing these processes.

1074B Homeostatic stress responses regulate selfish mitochondrial genome dynamics. M. Patel, B. Gitschlag, C. Kirby Biological Sciences, Vanderbilt University, Nashville, TN.

Age-dependent disorders are a hallmark of diseases associated with mutations in the mitochondrial genome (mtDNA). Hundreds to thousands of wildtype mtDNA copies are normally present in each cell. However, a fraction of these mtDNA can acquire mutations and coexist with wildtype mtDNA in a state of heteroplasmity. Mutant mtDNA that persist despite having potentially deleterious consequences can be viewed as selfish genetic elements. Mechanisms that allow selfish mtDNA to achieve and sustain high levels are poorly understood. We establish a large 3.1kb deletion bearing mtDNA variant uaDf5 as a bona fide selfish genome in C. elegans. Next, using droplet digital PCR to quantify mtDNA copy number, we show that uaDf5 mutant mtDNA replicates in addition to, not at the expense of, wildtype mtDNA. These data suggest existence of homeostatic copy number control for wildtype mtDNA that is exploited by uaDf5 to hitchhike to high frequency. We also observe activation of the mitochondrial unfolded protein response (UPRmt) in animals with uaDf5. Loss of UPRmt results in a decrease in uaDf5 frequency whereas constitutive activation of UPRmt increases uaDf5 levels. These data suggest that UPRmt allows uaDf5 levels to increase. Interestingly, the decreased uaDf5 levels in absence of UPRmt recover in parkin mutants lacking mitophagy, suggesting that UPRmt protects uaDf5 from mitophagy. We propose that cells activate two homeostatic responses, mtDNA copy number control and UPRmt, in uaDf5 heteroplasmic animals. Inadvertently, these homeostatic responses allow uaDf5 levels to be higher than they would be otherwise. In conclusion, our data suggest that homeostatic stress response mechanisms play an important role in regulating selfish mitochondrial genome dynamics.

1075C Cold-induced suspended animation. T. Pekec 1,2, R. Ciosk 1 1) Friedrich Miescher Institute, Basel, CH; 2) University of Basel, Basel, CH.

Wild-type C. elegans are sensitive to a sudden temperature drop but, following a short adaptation at an intermediate temperature, the animals survive at 4 °C for many days (1, 2). While in cold, the animals stop feeding and moving, suggesting that they enter a hibernation-like, suspended animation state. To investigate molecular pathways involved in hibernation, we have performed an unbiased RNAi screen, searching for genes essential for cold survival. Among others, this screen identified a conserved ribonuclease, REGE-1. One target of REGE-1 is the ets-4 mRNA (2). ETS-4 is a member of the ETS transcription
factor family, which regulates life span (3). We found that the loss of ets-4 rescues the cold sensitivity of rege-1 mutants, as well as other rege-1 phenotypes, suggesting that ETS-4 is a key effector of REGE-1. To identify genes functioning downstream from ETS-4, we perform a forward genetic screen, searching for mutants suppressing the cold sensitivity of rege-1 animals, despite high levels of ETS-4. I present and discuss characterization of candidate mutants at the meeting.


1076A The NEET homolog CISD-1 modulates ageing through mechanisms involving autophagy and components of the intrinsic apoptosis pathway. P. Ploumi1,2, E. Kyriakakis1, N. Tavernarakis1,2 1) Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology – Hellas; 2) Medical School, University of Crete, Greece.

The NEET family of proteins comprises a special type of Iron Sulfur Cluster (ISC) binding proteins implicated in various human pathologies ranging from neurodegeneration to cancer and age-related diseases. Despite the well known structural properties of the mammalian NEETs, the mechanisms by which they influence longevity remain largely enigmatic. The C. elegans gene cis-1 (W02B12.15) encodes a functional homolog of the mammalian CISD1 (CDGSH Iron Sulfur Domain 1) and CISD2 (CDGSH Iron Sulfur Domain 2) proteins, based on sequence analysis and the presence of the conserved domain CDGSH for binding to ISCs. Similar to its mammalian homologs, CISD-1 is an outer mitochondrial membrane protein, ubiquitously expressed in neuronal, intestinal and muscle cells. Downregulation of cis-1 shortens animal lifespan, boosts mitochondrial activity and induces germline apoptosis. Our findings indicate that CISD-1 exerts differential effects on ageing through its involvement in both the autophagic degradation process and the intrinsic apoptosis pathway. We aim to further elucidate the mechanisms through which CISD-1 function in mitochondria modulates ageing, and gain insight into the functional interplay between autophagy and apoptosis that influences longevity.

1077B How a mutation that slows aging can also disproportionately extend end-of-life decrepitude. K. Podshivalova1,2, R.A. Kerr1,2, C. Kenyon1,2 1) Calico Life Sciences LLC, SOUTH SAN FRANCISCO, CA; 2) University of California San Francisco, San Francisco, CA.

The goal of aging research is to extend healthy, active life. For decades, C. elegans daf-2 insulin/IGF-1 receptor mutants have served as a model for extended lifespan and youthfulness. However, a recent report suggested that their longevity is associated with an undesirable phenotype: a disproportionately long period of decrepitude at the end of life. In a human population, such an outcome would be a burden to society, bringing into question the relevance of daf-2 mutants as a model for life extension. We set out to accomplish three goals: to undertake a quantitative large-scale analysis to corroborate the reported disproportionately extended end-of-life decrepitude in a daf-2 mutant; to determine whether this phenotype could be due to behavioral particularities of the specific daf-2 allele that was examined; and, if not, to elucidate the cause of this apparently undesirable phenotype. We found that, following an extended period of movement, daf-2 mutants survive longer in a decrepit state because of a beneficial trait: they are resistant to colonization of the digestive tract by dietary bacteria, a condition that leads to premature death in wild type and prevents their manifestation of decrepitude. If bacterial colonization is prevented, daf-2 mutants lead both chronologically and proportionately healthier lives relative to wild type.

1078C SUMOylation regulates lifespan and mitochondrial homeostasis in C. elegans. Andrea Prinz1,2, Nektarios Tavernarakis1,2 1) Institute of Molecular Biology and Biotechnology; Foundation for Research and Technology-Hellas, Heraklion, Crete, Greece; 2) Department of Biology, University of Crete, Heraklion, Crete, Greece; 3) Medical School, University of Crete, Heraklion, Crete, Greece.

Posttranslational modifications have pivotal roles in numerous cellular processes. SUMOylation, the attachment of SUMO (small ubiquitin-related modifier) to a protein, is implicated in the regulation of DNA damage response, cell division, sub-cellular protein localization and protein-protein interactions, among others. Protein SUMOylation levels increase progressively during ageing. Nevertheless, whether elevated SUMOylation is only an unrelated consequence of the ageing process or it serves a causative, regulatory role in senescent decline is not understood. The C. elegans genome contains a single gene encoding SUMO (smo-1), rendering the nematode a convenient model in which to genetically dissect the role of SUMOylation in organismal physiology and ageing. The insulin/IGF-1 pathway is a well-characterized and conserved signal transduction cascade, which has a major role in determining the lifespan of animals, mainly through the DAF-16/FOXO transcription factor and the stress response-related transcription factor SKN-1/NRF2. Interestingly, these two key transcription factors contain putative SUMOylation sites. Deletion of smo-1 causes embryonic lethality. However, we find that RNAi knockdown of smo-1 initiated at the L4 stage shortens the lifespan of both wild type and long-lived animals. Notably, knockdown of a SUMO protease gene (ulp-1), extends the lifespan of long-lived mutants (clk-1, daf-2, ife-2), but does not modify the longevity of wild type animals. The lifespan-altering effect of SUMOylation is tissue-specific. In addition, we observed that manipulation of SUMOylation levels by either knockdown of smo-1 or ulp-1 influences the activity of DAF-16 and SKN-1, as well as, stress resistance, energy metabolism and mitochondrial homeostasis, in a genetic background- and age-dependent manner. These findings directly implicate SUMOylation in the regulation of key stress responses and longevity.

1079A DAF-16/FOXO and HLH-30/TFE3 cooperate as combinatorial transcription factors to regulate stress responses and aging in C. elegans. Xin-Xuan Lin1,2, Georges Janssens1, Bryan Fonslow3, Iike Sen1,2, Nicholas Stroustrup4, Peter Swoboda5, John Yates5, Gary Ruvkun6,7, Christian Riedel1,2 1) ICMC, Department of Medicine, Karolinska Institute, Stockholm, SE; 2) European Research Institute for the Biology of Ageing, University of Groningen, Groningen, NL; 3) Department of
When an organism experiences dire conditions (e.g. stress or nutrient deprivation), dedicated signaling cascades get activated to convey appropriate cellular and organismal responses that ensure the organism's survival. Central to these responses is the conserved transcription factor DAF-16/FOXO that under low insulin/IGF-like signaling (IIS) but also a broad variety of other distress signals gets activated and translocates into nucleus, to drive expression of stimulus-specific sets of stress resistance and longevity promoting genes. Although DAF-16/FOXO has been extensively studied, it remains somewhat elusive how this transcription factor alone can integrate such multitude of upstream stimuli and eventually relay them into customized transcriptional responses.

Using C. elegans, we show that for many purposes DAF-16/FOXO does not function alone but as a combinatorial transcription factor together with HLH-30/TFEB, a conserved master regulator of autophagy and lysosome biogenesis. Just like DAF-16, also HLH-30 normally resides in the cytoplasm and translocates into the nucleus under a similar (although not identical) panel of dire conditions to regulate transcription. Using proteome and genome-scale techniques, we found that DAF-16 and HLH-30 can form a complex and that they co-occupy many target promoters. Consistent with these observations, they co-regulate many target genes in long-lived IIS mutant or germline-deficient animals, under heat, or under oxidative stress, indicating that HLH-30 plays an important role in DAF-16-dependent transcriptional responses.

Interestingly though, despite DAF-16 and HLH-30 frequently co-translocating into the nucleus, forming a complex, and co-regulating many target genes, we found that their genetic interaction strongly depends on the upstream stimulus, carefully tuning the physiological outcomes for the animal: i.e. they function in the same pathway when promoting longevity under low IIS or germline-deficiency or when promoting resistance to oxidative stress, but they elicit heat stress responses independently. And while DAF-16 is required for dauer formation, HLH-30 even inhibits this process.

Conducting yet more detailed analyses, we eventually explain our observations by a model where DAF-16 and HLH-30 dynamically integrate stimuli convergent on either transcription factor and relay them into the combinatorial regulation of their large panel of target genes.

1080B  HSP25 overexpression extends lifespan through protective aggregation in an HSF1-dependent manner.  K.A. Rodriguez1,2, M. Khan3, R. Buffenstein4, A.L. Fisher1,3,5,6 1) Sam and Ann Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center San Antonio, San Antonio, TX; 2) Cell Systems and Anatomy, University of Texas Health Science Center San Antonio, San Antonio, TX; 3) Department of Medicine, University of Texas Health Science Center San Antonio, San Antonio, TX; 4) Calico, San Francisco, CA; 5) Center for Healthy Aging, University of Texas Health Science Center at San Antonio, San Antonio, TX; 6) GRECC, South Texas VA Healthcare System, San Antonio, TX.

Long-lived animals show resistance to a broad spectrum of environmental stressors. Our previous work indicated that this is attributed in part to altered changes in molecular chaperone levels that influence the transport and disposal of damaged proteins. Particularly striking were the high levels of the small molecular weight chaperone heat-shock chaperone 25 kDa (HSP25) and its putative transcription factor heat shock factor-1 (HSF1). These showed as significant correlation with rodent longevity and were 5-fold higher our longest-lived species compared to the shortest. This suggests that HSP25 may play a key role in age-related maintenance of protein homeostasis in long-lived animals. To examine if this correlation was more causal we constructed a transgenic C. elegans worms which ubiquitously overexpress HSP25 fused to GFP. Worms overexpressing this construct show resistance to heat stress (20% increase in mean survival) and have a 25% extension of lifespan. This lifespan extension depended on the expression of hsf-1 but not daf-16 as determined by RNAi experiments. Generally, an increase in life span and heat resistance corresponds with decreased aggregation. However, when we crossed our longer-lived, heat-resistant worms with worms containing a repeat of 44 glutamines targeted to the worm intestine (Q44::YFP) we surprisingly observed that the HSP25 transgenic worm had a higher percentage of animals showing aggregation and more aggregates per worm than the control-crossed worm. Yet the lifespan of the HSP25 transgenic animals was still longer. This could suggest a role for this protein in promoting controlled aggregation which then removes unstable and/or damaged proteins by packaging them into insoluble aggregates. In theory, these now sequestered, aggregated proteins might be removed by autophagy or other protein degradation pathways which we are currently testing. Thus, the sequestration of these aggregate-prone structures by HSP25 and other shSPs could be a protective mechanism within the cell.

1081C  Echoes of early life exposure: delayed methylmercury toxicity in Caenorhabditis elegans.  M. Rudgalvyte1, J. Peltonen2, R. Nass3, G. Wong1 1) Faculty of Health Sciences, University of Macau, Macau, CN; 2) A. I. Virtanen Institute of Molecular Sciences, Department of Neurobiology, University of Eastern Finland, Kuopio, FI; 3) Indiana University School of Medicine, Indiana University, Indianapolis, Indianapolis, Indiana, USA.

Background. Methylmercury (MeHg) is a well-known environmental pollutant, causing adverse effects even in small concentrations. Exposure to MeHg may lead to physical disorders, impaired development, or/and neurobehavioral alterations. MeHg causes oxidative stress, disrupted Ca2+ homeostasis, mitochondrial dynamics changes, cell cycle alterations and cell death. MeHg may lead to delayed toxicity since the first symptoms of poisoning appear long after initial exposure. Clinical signs of exposure may also appear later in life as the result of additional stress, aging, or disease. Since time may pass until the emergence of the first signs of exposure, delayed toxicity is very challenging to study.

Results. Age synchronized N2 worms were treated with MeHg (25uM) for 30 min at room temperature at L1 stage and allowed to develop until stage L4 at +20°C on NGM/OP-50 plates. Animals were analyzed at L4 and at 12-day old adult stages to determine the delayed effect of MeHg treatment. We observed that acute MeHg treatment delays animal development and
shortens lifespan. Moreover, a basal slowing response assay suggests that treated animals exhibit dopaminergic signaling impairment. Interestingly, early MeHg exposure had no effect on either brood size of P0 generation or development, brood size, and longevity of their progeny (F1). A single exposure to MeHg resulted in mitochondria membrane potential decrease and reduced oxygen consumption rate. Global RNA sequencing was performed and data analysis revealed 624 down-regulated and 35 up-regulated genes. During gene annotation analysis, enriched groups of protein modification and metabolic processes, as well as regulation of cell morphogenesis, phosphorylation, protein kinase activity and poly(A) RNA-binding were uncovered. The members of dpy and lpr gene families were further investigated. Knockdown of single genes from these families resulted in a changed animal tolerance to MeHg exposure. Among downregulated genes, abu-8 and abu-11 were observed suggesting the involvement of the unfolded protein response in MeHg-induced animal toxicity.

**Conclusions.** Our study indicates that a single acute MeHg exposure during the development of *C. elegans* results in adverse effects that are observable later in life, and thus provide a model for delayed toxicity.

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**1082A Contributions of the C. elegans JNK homolog kgb-1 to ER stress resistance.** C. Ruediger, L. Liu, M. Shapira Integrative Biology, University of California Berkeley, Berkeley, CA.

JNK proteins are conserved stress-activated kinases that coordinate stress responses to adverse conditions. The broadly expressed Caenorhabditis elegans JNK homolog KGB-1 provides protection from protein folding stress (ER stress) and heavy metals. Previous results from our lab showed that this contribution, while beneficial during larval development, reverses with age, becoming detrimental to both stress resistance and lifespan in adult worms. To understand the age-dependent switch, we sought to characterize the contributions of KGB-1 to stress resistance. Currently, very little is known about upstream activators or downstream effectors linking KGB-1 and ER stress resistance, or the tissues in which they act. To characterize ER stress-related upstream activation of KGB-1, we analyzed the effects of tunicamycin, an ER stress inducing agent, on larval development of mutants for unfolded protein stress sensors ire-1, peak-1, and atf-6 either disrupted alone or in conjunction with kgb-1 disruption. peak-1, atf-6, and xbp-1, a downstream target of IRE-1 activation, were each found to have an added contribution to stress sensitivity of kgb-1 mutants. Furthermore, a transcriptional reporter for hsp-4/Bip, a chaperone protein downstream target of XBP-1, was also induced normally following tunicamycin exposure in kgb-1 mutants. Known KGB-1 targets AP-1 components fos-1 and jun-1, as well as daf-16/FOXO, were also found to be dispensable for kgb-1-dependent ER stress protection through developmental analyses and analysis of a DAF-16::GFP fusion protein reporter. These results demonstrate that KGB-1’s larval contributions to ER stress resistance are independent of canonical pathways involved in the unfolded protein response. The mechanism underlying KGB-1 contribution to stress remains unknown, but interestingly, tissue-specific expression of KGB-1 either in the intestine, the epidermis, muscle, or neurons was equally capable of providing partial rescue in a kgb-1 mutant background. Importantly, tissue-specific contributions were mediated by cell-autonomous effects, suggesting a role for KGB-1 in integrating stress signaling at the level of the entire organism.

**1083B Sexually antagonistic male signals manipulate germline and soma of C. elegans hermaphrodites.** E. Aprison, I. Ruvinsky Molecular Biosciences, Northwestern University, Evanston, IL.

Males and females pursue different reproductive strategies, which often bring them into conflict – many traits exist that benefit one sex at a cost to another. Decreased female survival following mating dramatically demonstrates one aspect of this phenomenon. Particularly intriguing is the evidence that secreted compounds can shorten lifespan of members of the opposite sex in Drosophila and Caenorhabditid nematodes even without copulation taking place. The purpose of such signals is not clear, however. While it is possible that they could limit subsequent mating with competitors or hasten post-reproductive demise, thus decreasing competition for resources, they are also likely to harm unmated individuals. Why would a system exist that reduces JNK homolog KGB-1 provides protection from protein folding stress (ER stress) and heavy metals. Previous results from our lab showed that this contribution, while beneficial during larval development, reverses with age, becoming detrimental to both stress resistance and lifespan in adult worms. To understand the age-dependent switch, we sought to characterize the contributions of KGB-1 to stress resistance. Currently, very little is known about upstream activators or downstream effectors linking KGB-1 and ER stress resistance, or the tissues in which they act. To characterize ER stress-related upstream activation of KGB-1, we analyzed the effects of tunicamycin, an ER stress inducing agent, on larval development of mutants for unfolded protein stress sensors ire-1, peak-1, and atf-6 either disrupted alone or in conjunction with kgb-1 disruption. peak-1, atf-6, and xbp-1, a downstream target of IRE-1 activation, were each found to have an added contribution to stress sensitivity of kgb-1 mutants. Furthermore, a transcriptional reporter for hsp-4/Bip, a chaperone protein downstream target of XBP-1, was also induced normally following tunicamycin exposure in kgb-1 mutants. Known KGB-1 targets AP-1 components fos-1 and jun-1, as well as daf-16/FOXO, were also found to be dispensable for kgb-1-dependent ER stress protection through developmental analyses and analysis of a DAF-16::GFP fusion protein reporter. These results demonstrate that KGB-1’s larval contributions to ER stress resistance are independent of canonical pathways involved in the unfolded protein response. The mechanism underlying KGB-1 contribution to stress remains unknown, but interestingly, tissue-specific expression of KGB-1 either in the intestine, the epidermis, muscle, or neurons was equally capable of providing partial rescue in a kgb-1 mutant background. Importantly, tissue-specific contributions were mediated by cell-autonomous effects, suggesting a role for KGB-1 in integrating stress signaling at the level of the entire organism.


Defects in the DNA Damage Response (DDR) affect tissues differently suggesting that genome maintenance operates in a tissue-specific manner. A prime example is given by ERCC1/XPF deficiency, which gives rise to pleiotropic symptoms including neurodegeneration, developmental defects, cancer, bone marrow failure and accelerated aging, depending on the type of mutation. ERCC1/XPF is a structure specific endonuclease that is involved in several DNA repair pathways and has a critical role in Nucleotide excision repair (NER). This major DDR pathway is responsible for removing bulky DNA lesions, in particular those formed by UV light. NER consists of two sub-pathways: Global Genome NER (GG-NER) deals with damage anywhere in the genome, whereas Transcription-Coupled NER (TC-NER) deals with damage that blocks transcription. Following damage detection and verification, a DNA stretch containing the damage is excised by endonucleases ERCC1/XPF and XPG. The resulting gap is filled in by DNA synthesis and ligation. Although the function of ERCC1/XPF has been studied in great detail in biochemical and cell biological experiments, it is not entirely clear how deficiency of this complex can lead to such a variety of tissue-specific symptoms.

Previously, we have shown that in *C. elegans*, GG-NER mainly protects germ cells while TC-NER mainly protects somatic cells.
against UV-induced DNA damage. Furthermore, ERCC-1/XPF-1 loss-of-function causes developmental defects and accelerated replicative aging, which is reminiscent of symptoms in human patients. To better understand to what extent DNA repair functions differently in tissues, we expressed fluorescently tagged ERCC-1/XPF-1 in the germline, hypodermis, intestine, neurons and muscles. Next, we set up new live cell imaging methods to monitor the tissue-specific spatio-temporal dynamics of ERCC-1/XPF-1 in response to DNA damage. We show that in oocytes the endonuclease complex quickly but transiently relocalizes to and binds UV-damaged chromosomes in a GG-NER-dependent manner. Intriguingly, we find that re-localization and chromatin binding in response to DNA damage changes upon differentiation of cells. Our results confirm that in vivo the main role of GG-NER is to safeguard the whole genome in the totipotent germline, while the main role of TC-NER is to safeguard active genes to promote cell function in differentiated tissues.

1085A EGF/EGFR signaling confers healthspan benefit in *C. elegans*. S. Salam¹, S. Yu², H. Iwasa³, M. Driscoll¹ 1) Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ; 2) Taiwan Supra Integration and Incubation Center, Taiwan; 3) Tokyo Medical and Dental University.

Aging is associated with major health decline leading to diseases like diabetes, cancer and neurodegenerative diseases. Improving healthspan (the period of healthy maintenance prior to age-associated decline) is one of the major goals of the current medical research and identifying biological factors that signal for robust healthspan is of interest. The EGF growth factor acts through the tyrosine kinase receptor family to influence cell development and function. EGF signaling is also known for mammalian neurometabolic regulation in both normal and pathophysiological aging. However the role of EGF signaling in aging is under-studied. Previous study from our lab documented the exciting facets of EGF signaling pathway in maintaining healthspan in *C. elegans* model (Iwasa et. al, 2010). In *C. elegans*, enhanced EGF pathway activated mutant EGFRI/let-23(gf) maintains swim vigor in advanced age. Enhanced EGF signaling acts via the itr-1/lip3 receptor ER calcium channel to extend physical vigor and lower gut lipofuscin, conferring a proportionately longer period of health and functionality over the life. Deletion of a negative regulator of *C. elegans* EGF signaling, *hpa-2* (high performance in aging), increases EGF signaling such that mutants swim more youthfully at old age, and accumulate less age pigment/lipofuscin, although the lifespan is not markedly extended. This improvement in health over unit lifetime is important and distinctive: whether the heavily studied insulin signaling longevity pathway and other pathways merely extend a period of poor adult health has become a recent controversy, but the EGF pathway modulates a definitive vigor improvement.

Our unpublished recent work has shown that strains expressing different isoforms of EGF ligand/LIN-3 can confer anti-aging activity such as better swim prowess, less accumulation of age pigment and increased median life span in adult animals, suggesting specific EGF ligands confer healthy benefits. We have shown that: 1) transgenic over-expression of activated EGFRI/let-23(gf) only in neurons can restore the healthspan measures mentioned above; 2) genetic mutants that reduce EGF signaling EGFRI/let-23(ry) increase indications of neuronal proteo-stress. Together with on-going experiments, our work informs on how elevated or maintained EGF signaling confers healthspan benefits.

1086B Stress induced nuclear granules in the germline. K.M. Sampuda, J. Evers, L. Boyd  Biology, Middle Tennessee State University, Murfreesboro, TN.

Environmental stress can have negative impacts on general and reproductive health. We are investigating the effects of environmental stress on protein quality control systems in the germline and other tissues. We have found that salt stress, oxidative stress, and starvation induce the relocation of ubiquitin and proteasome into distinct subnuclear regions referred to as stress induced nuclear granules (SINGs). SINGs form quickly; within one hour of stress initiation. The formation of SINGs does not require intertissue signaling since isolated gonads or intestines form SINGs when exposed to stress. Worms with a mutation in the conjugating enzyme, *ubc-18*, do not form SINGs. Additionally, a combined knockdown of *ubc-20* plus *ubc-22* reduces the level of SING formation as does knockdown of the ubiquitin ligase *chn-1*, a CHIP homolog. Early embryos form SINGs when stressed and fail to hatch. Cell division in these embryos is halted. The formation of SINGs can be prevented by pre-exposure to a brief period of heat shock before stress exposure. Heat shock inhibition of SINGs is dependent upon the HSF-1 transcription factor suggesting that expression of chaperones can prevent SING formation. Thus, we hypothesize that SINGs occur as the result of an accumulation of misfolded proteins. In order to further characterize this stress response, we isolated ubiquitinated proteins from stressed and unstressed populations. Analysis of those proteins showed that certain proteasome subunits are ubiquitinated in response to stress. Homologous subunits have also been identified as targets of ubiquitination in human cell lines (Besche et al, Embo J 2014). We plan to determine whether ubiquitination of proteasome subunits is required for SING formation and whether those subunits are targets of the UBCs mentioned above.

1087C Microfluidic platforms for aging studies: longitudinal high-resolution phenotyping and post-reproductive screening. Sahand Saberi Bosari, Daniel Midkiff, Adriana San Miguel  Department of Chemical & Biomolecular Engineering, North Carolina State University, Raleigh, NC.

*C. elegans* has been fundamental to our current understanding of aging and the molecular mechanisms that play a role in determining healthspan and longevity. Aging studies, however, pose technical challenges that are typically solved by labor-intensive picking and transfer of populations, or by the use of reproduction-inhibiting agents. In this work, we are developing microfluidic platforms that allow performing aging studies in the absence of reproduction-inhibiting agents, and with far less manual involvement. Our platforms are focused on two main areas which have not been fully developed due to these limitations. First, we have developed a platform that allows for lifelong longitudinal, high-resolution (sub-cellular) phenotyping of *C. elegans* populations. This platform allows tracking subcellular processes in the same population for hundreds of animals, and we are currently aiming to quantify the morphological changes that neurons and synapses undergo during the aging
process in a variety of conditions. In our second platform, our goal is to perform genetic screens in aged individuals (i.e., post-reproduction). We aim to identify mutations that result in "late-onset" phenotypes, which are only exhibited late in life. Our platform allows monitoring of individual mutagenized animals, cultured in individual microfluidic chambers, while isolating their progeny by interfacing with a deep-well system. Our platforms dedicated to challenging aging studies can result in the identification of genes and morphological changes associated with the aging process under natural conditions.

1088A  How do worms determine their readiness to cope with environmental stress? Jodie Schiffer, William Heath, Julian Stanley, Stephanie Stumbur, Abigail Vogelaar, Hannah Tam, Natalie McGowan, Javier Apfeld  Biology, Northeastern University, Boston, MA.

At any time in their life, worms may encounter harmful environmental conditions such as high concentrations of oxidants or high temperature. We are interested in understanding how worms prepare for these potentially lethal events. We want to know: what establishes how prepared they are?

To determine the mechanisms that control survival under harmful conditions, we are examining the effects of strong loss-of-function and null mutants in a collection of intercellular signaling receptors and transcription factors regulated by these receptors. We measure survival under oxidative stress and at high temperature using a "Lifespan Machine" cluster of flat-bed scanners [1]. This automated technology presents substantial advancements in throughput and sensitivity compared to manual methods. Using this approach, we have identified several signaling receptors and transcription factors that regulate survival under oxidative conditions. Interestingly, we identified both receptors and transcription factors that function to either confer or limit oxidative-stress resistance. This indicates that the combined level of activity of these genes sets the worm’s readiness to cope with oxidative stress. We are currently investigating whether these genes act together or independently to determine the worm’s normal level of resistance to various stressors, and the mechanisms that establish the normal activity levels of each of these genetic determinants of stress resistance.

Reference:

1089B  The histone chaperone lin-53 (Rbbp4/7) in muscle maintenance and aging regulation.  S. Seek, B. Tursun
Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association, Berlin.

The histone-chaperone LIN-53 (Rbbp4/7 in mammals) is part of several complexes such as PRC2, NuRD or Sin3 regulating chromatin modification and structure. Immunostaining reveals that LIN-53 is expressed virtually in all tissues of C. elegans (Lu, 1998; Seek, unpublished). It was previously shown that lin-53 functionally interacts with PRC2 in the germline in order to prevent reprogramming of germ cells (Patel et al. 2012; Seek et al. 2016). However, it is unclear whether it has similar roles and functions in different cell types.

Our ongoing study shows that LIN-53 interacts mainly with the Nucleosome remodeling and deacteylase (NuRD)-complex in C. elegans muscles. Staining of muscle proteins shows that lack of lin-53 leads to a disruption of the muscle structure resulting in motility defects, which is phenocopied by depletion of members of the NuRD-complex. Muscle-specific interaction of LIN-53 with NuRD members was detected using mass-spec and interactions could be confirmed by Co-IPs. In contrast, while lin-53 mutants have a shortened lifespan, NuRD mutants live normal or longer than control animals. This suggests that LIN-53 depletion has combinatorial effects possibly by affecting another chromatin regulating complex. Indeed, our work revealed that removal of the Histone deacetylase complex Sin3 affects lifespan similar to lin-53 depletion.

To identify affected gene expression due to lack of lin-53 we carried out a transcriptome analysis. Muscle-specific genes but also the expression of genes, which are associated with both muscle maintenance and lifespan regulation are affected in lin-53 mutants. Several small heat-shock proteins (sHSPs) were highly upregulated, among them hsp-43 and sip-1. RNAi against hsp-43 in lin-53 mutants suppressed the motility defects but not the short lifespan suggesting that de-repression of hsp-43 contributes to the muscle defects. Besides a number of genes belonging to the oxidative stress pathway (e.g. skn-1, sod-genes) and metabolic enzymes (e.g. tps-1) show decreased expression. We performed a metabolome analysis, which revealed that trehalose levels are significantly decreased in lin-53 mutants. Feeding of trehalose indeed partially rescued the short lifespan of worms lacking lin-53.

Reference:

1090C  Octopaminergic signaling mediates neural regulation of innate immunity in Caenorhabditis elegans.  C. Yuan, Y. Liu, D. Sellegounder, P. Wibisono, J. Sun  Department of Biomedical Sciences, Elson S. Floyd College of Medicine, Washington State University, Spokane, WA.

Upon pathogen infection, the nervous system regulates the immune system to confer coordinated protection to the host. However, the precise mechanisms of such regulation remain unclear. Previously we demonstrated that in Caenorhabditis elegans, the G protein-coupled receptor OCTR-1 functions in the sensory neurons designated as ASH to suppress innate immune responses. In the current study, we show that both endogenous and exogenous neurotransmitter octopamine (OA) inhibit immune responses in C. elegans and identify OA as an endogenous ligand for OCTR-1 in immune regulation. OA is synthesized in two RIC interneurons and non-neuronal gonadal sheath cells. Blocking synaptic transmission of RIC neurons led
to enhanced worm survival against pathogen infection and upregulation of OCTR-1-regulated immune genes, indicating that RICs function in the OCTR-1 neural circuit to negatively regulate innate immunity. Our study uncovered an octopaminergic immuno-inhibitory pathway in *C. elegans*, which could be helpful to the understanding of the complex mammalian neural-immune regulatory circuits and could benefit the development of more effective treatments for innate immune disorders.

**1091A  The JNK homolog KGB-1 integrates stress signaling through cell non-autonomous contributions.** L. Liu, C. Ruediger, M. Shapiro

The JNK homolog KGB-1 is known to provide protection from protein folding stress (ER stress) and heavy metals. However, previous results from our lab showed that this contribution was age-dependent, dominant in developing larvae, but not in adults, where KGB-1 activation instead reduced stress resistance and shortened lifespan. This switch was associated with age-dependent antagonistic regulation of DAF-16 by KGB-1. In parallel, KGB-1 also activated FOS-1-dependent gene expression, but unlike DAF-16 regulation, this program was age-invariant. KGB-1 is broadly expressed, but very little is known about the significance of that. To better understand the involvement of KGB-1 in stress-resistance, we investigated its tissue-specific contributions.

Expression of KGB-1 in either the epidermis, muscle, or neurons provided partial protection to developing larvae from ER stress induced by tunicamycin, whereas intestinal KGB-1 showed only a marginal contribution. While this might be taken to imply additive and distributed cell autonomous contributions to stress resistance, intestinal expression of a GFP reporter for a KGB-1 FOS-1-dependant target revealed that neuronal KGB-1 activation induced intestinal gene expression better than intestinal KGB-1. qRT-PCR measurements of additional KGB-1 targets corroborated this, showing that extra-intestinal KGB-1 (in neurons, but also in muscle) was required for full-blown expression of intestinal targets, indicating dominant cell non-autonomous contributions of KGB-1 that did not require its expression in the target tissue. In contrast, a KGB-1 FOS-1-dependent epidermal target presented cell autonomous regulation by KGB-1, but no significant effects of extra-epidermal KGB-1 activation. Analysis of KGB-1-dependent induction of a DAF-16 target showed similar cell non-autonomous contributions, and further demonstrated that not only gene induction was activated (in larvae) by extra-tissue KGB-1, but also DAF-16-dependent gene repression, in adults. Together, these results demonstrate that KGB-1 integrates stress signals at the level of the entire organism, and that a significant part of its contributions to stress resistance depends on cell non-autonomous functions. The ability to relay both gene induction and repression further suggests (and supported by preliminary results) modulation of a secreted tonic signal.

**1092B  Applying the Q system for spatiotemporal gene expression in adult *C. elegans*.** X. She, M. Hansen

While *C. elegans* has long been on the leading edge of aging research, a spatial-temporal controlled gene expression system is currently lacking to fully understand tissue- and adult-specific functions of genes modulating lifespan and healthspan in this organism. The Shen lab recently adapted the so-called Q-system for inducible gene expression in a subset of *C. elegans* neurons (Wei et al., Nature Methods, 2013). Originally discovered in the fungus Neurospora crassa, this binary system is comprised of the transcription factor QF and its repressor QS. In the presence of quinic acid, the QS repressor is released from binding with QF, allowing QF to activate the expression of transgenes. Temporal control is achieved through quinic-acid supplementation to the media plates, and spatial control by use of tissue-specific promoters that drive the expression of Q and QS. Here, we further developed the Q-system and tested it for its usability in *C. elegans* aging studies. First, we drove the elements of the Q-system with tissue-specific promoters expressing in several major tissue types to show the inducibility of the system in adult animals. Our results indicate that the Q-system can be used to induce expression in such tissues. Second, we tested the effects of quinic acid and Q-system components on *C. elegans* lifespan and stress resistance and detected no apparent effects on longevity or fitness, suggesting that the Q-system can be used for lifespan studies. We will present current progress towards applying the Q-system as a powerful system for spatial- and temporal analysis of gene function in adult *C. elegans*.

**1093C  The putative actin-binding and scaffold protein *filamin*-2 affects *C. elegans* survival in response to different bacterial food sources.** Deniz Silfoglu, Zahabiya Husain, Wolfgang Maier, Martin Regenass, Joy Alcedo

Animal survival can be affected by either food levels or food composition, where the effects can be mediated by the perception and/or metabolism of macro- and micro-nutrients. Indeed, different bacterial food sources have distinct effects on the physiology and survival of the nematode *C. elegans*, presumably by changing certain gene activities within the animal. Recently, we have found that the predicted actin-binding and scaffold protein, *filamin*-2, shortens *C. elegans* lifespan on some bacteria, but not on other bacteria. The bacterial food source may serve not only as a source of nutrients and metabolites, but also as cues that elicit the animal's innate immune responses. However, the effects of *filamin*-2 do not appear to be part of a general innate immune response. We find that *filamin*-2 mutants show increased survival on some pathogenic bacteria, but not on other bacteria that confers a higher degree of pathogenicity in *C. elegans*. Thus, *filamin*-2 likely regulates the animal’s response to specific bacteria-derived cues. At present, we are elucidating the molecular and cellular mechanism(s) through which *filamin*-2 affects survival in a bacteria-specific manner.

**1094A  Coordinating lipid storage with lysosomal lipolysis enhances longevity.** M.J. Silvestrini, B.C. Baggett, S.B. Williams, N.E. Seah, R.H. Houtkooper, L.R. Lapierre

The autophagy-lysosomal pathway is a conserved process that modulates intestinal lipid homeostasis and mediates lifespan...
extension. In long-lived animals, maintaining sufficient intestinal lipid stores is an important feature of lifespan extension. Our recent study (Seah et al. 2016, Autophagy) demonstrated that lipoprotein biogenesis impairs longevity by depleting intestinal lipids that would otherwise be converted to neutral lipids and lipophagic cargo that can generate lipid signaling molecules. We found that long-lived animals have reduced lipoprotein biogenesis as well as enhanced lipid droplet formation and remodeling, which are coordinated with increased lysosomal lipolysis and result in the production of lipid signals for nuclear hormone receptor signaling and lifespan extension. Here, we present global transcriptional and lipid metabolic changes associated with altered lipoprotein biogenesis. Also, we find that specifically coordinating lipid storage with lysosomal lipolysis improves longevity. Our findings provide a rationale to explore novel therapeutic strategies to enhance lifespan via modulation of lipid dynamics.

1095B Lipoprotein aggregation enhances host susceptibility to pathogen infection by suppressing the expression of immune genes. Jogender Singh, Alejandro Aballay Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, 27710, USA.

Protein aggregation is a major contributor to aging and age-related diseases. The unfolded protein response (UPR) is a stress response pathway that is activated upon increased unfolded and/or misfolded proteins in the endoplasmic reticulum (ER), and enhanced ER stress response improves lifespan and immunity. However, the mechanism by which ER stress affects immunity remains poorly understood. Using the nematode Caenorhabditis elegans, we show that mutations in the lipoproteins vitellogenins, which are homologs of human apolipoprotein B-100, resulted in the upregulation of the UPR. Lipoprotein aggregation adversely affected the immune response and the lifespan of the organism. We also show that lipoprotein aggregation inhibited the expression of several immune genes encoding proteins secreted by the intestinal cells in an IRE-1 independent manner. Our studies provide a mechanistic explanation for adverse effects caused by protein aggregation on immunity and highlight the role of an IRE-1-independent pathway that may sense protein aggregates in the suppression of the expression of genes encoding secreted proteins.

1096C Single-animal assessments of health suggest that long lifespan does not necessarily confer short healthspan. Drew Sinha123, William Zhang23, William Pittman123, Zachary Pincus123 1) Dept. of Biomedical Engineering, Washington University in St. Louis, St. Louis, MO; 2) Dept. of Genetics, Washington University School of Medicine in St. Louis, St. Louis, MO; 3) Dept. of Developmental Biology, Washington University School of Medicine in St. Louis, St. Louis, MO.

A fundamental question in the study of aging is whether long life is associated with better health (i.e. high physiologic function). Despite the simplicity of this question, it has been practically difficult to study the relationship between health and lifespan in humans or model organisms. In lieu of looking at animals over time, early work in C. elegans inferred that long-lived animals experienced longer time in good health (i.e. healthspan) by comparing wild-type and mutant animals of the same chronological age. However, recent reports suggest that while long-lived mutants spend a longer time in good health (longer absolute healthspan), these individuals spend a similar or shorter proportion of life in good health (shorter fractional healthspan).

These results have been controversial in part due to limitations such as study design (i.e. cross-sectional vs. longitudinal analysis) and phenotypes assayed biasing estimates of healthspan. As a result, it remains unclear whether decreased healthspan is a consistent property of contexts that extend lifespan. To study patterns of aging in individuals, we recently developed technology to track single individuals in high-throughput and measure multiple aging phenotypes with high temporal precision. By tracking several hundred wild-type (spe-9) individuals, we previously found that healthspan and lifespan were negatively associated in wild-type animals. Based on these results, we hypothesized that long-lived mutants should uniformly experience shorter fractional healthspan compared to wild-type. Surprisingly, we have found that some long-lived mutants experience significantly longer average fractional healthspan while others experience shorter average fractional healthspan. These results suggest that changes in healthspan vary across different backgrounds with altered longevity, but that there are physiological contexts in which lifespan and healthspan can be simultaneously improved.

1097A Longevity in C. elegans exposed to sulfur-containing chemicals. J. Hardin, D. Abuaqel, E. Carnalla, U. Patel, P.A. Smith Dept of Biological Sciences, Dominican University, River Forest, IL.

Many sulfur-containing compounds have demonstrated longevity enhancing effects on C. elegans. Our lab has tested dimethyl sulfoxide, dimethyl sulfone, dimethyl sulfide, diallyl sulfide, and diallyl disulfide for their ability to increase lifespan of wild type C. elegans and daf-16 and sod-defective strains. We have found that most of these compounds increase lifespan of wild type worms as well as daf-16 worms. For wild type worms, the addition of the pro-oxidant juglone generally decreased longevity compared similarly treated worms not exposed to the pro-oxidant. daf-16 worms exposed to the pro-oxidant generally survived longer than control daf-16 worms and the addition of a sulfur-containing chemical concurrently with the pro-oxidant increased lifespan further. We are testing if these conditions upregulate endogenous anti-oxidant genes in the nematode. We are also investigating the interactions between these sulfur-containing chemicals and worms lacking superoxide dismutase activity.


The 2,3-Pyridinedicarboxylic acid, widely known as quinolinic acid (QA), is a metabolite of tryptophan degradation in kynurenine pathway, which acts as a NMDA receptor agonist. Within the brain, QA is produced only by microglia and activated macrophages. Furthermore, QA has been described as a potent endogenous neurotoxin, when present at high levels, related to various psychiatric disorders and neurodegenerative processes. The nematode Caenorhabditis elegans has a nervous system
highly conserved with mammals and thus is an alternative animal model widely used in neurobiology research. However, there is no neurotoxin described that allows the study of glutamatergic system disturbance in \textit{C. elegans}. The aim of this study was verify if QA can induce neurotoxicity in \textit{C. elegans}, due to its action in glutamatergic system. Nematodes from N2 (wild type) and transgenic strains VM487 (nmr-1), VC2623 (nmr-2), TJ356(def-16::GFP), CL2070 (hsp-16.2::GFP), CL2166 (gst-4::GFP) and CF1553 (sod-3::GFP) at young adult stage were treated in liquid or agar containing QA in different concentrations (5, 10, 20, 50, 100 and 200 mM) or vehicle (M9 buffer) at 20°C for 1 hour. The analyses included evaluation of QA’s effects on survival, behavioral parameters (pharynx pumping and locomotion), subcellular DAF-16 localization, reactive species generation and expression of superoxide dismutase 3, glutathione-S-transferase-4, and heat shock protein 16.2. When used at high concentrations (50, 100 and 200 mM) QA can induced and increase in \textit{C elegans} mortality (~15%), although when used at low concentrations (10 and 20mM) QA altered some behavioral pattern of the nematodes. The QA can leave to an increase in the expression of \textit{hsp-16.2} (~15%) as well as \textit{gst-4} (~40%). However, \textit{sod-3} levels were not significantly different from control group. QA also activated DAF-16/FOXO signaling pathway and increased reactive species levels compared to control group. When used specific strains of glutamatergic system, the increase in reactive species production occur in a \textit{nmr-1}-dependent manner. Our data suggests that QA might be useful for neurotoxicological studies on glutamatergic system injuries associated with oxidative stress in \textit{C. elegans}.

1099C Prolifin 6 promotes longevity in \textit{daf-2}/\textit{insulin}/\textit{IGF-1} receptor mutants via acting together with heat shock factor 1 (HSF-1). H.G. Son1, 8, K. Seo1, 8, M. Seo1, 2, 4, 5, 8, H. Baek1, E. Choi1, S. Park1, E. Kim6, G.O. Ahn3, A.L. Hsu6, 7, H.G. Nam4, 6, S.K. Jang1, S.J.V. Lee1, 2 1) Department of Life Sciences; 2) School of Interdisciplinary BioScience and Bioengineering; 3) Division of Integrative Biosciences and Biotechnology, Pohang University of Science and Technology, Pohang, Gyeongbuk, 790-784, South Korea; 4) Department for aging Research, Institute for Basic Science; 5) Department of New Biology, DGIST; Daegu, South Korea; 6) Department of Internal Medicine, Division of Geriatric and Palliative Medicine, Ann Arbor, Michigan, United States of America; 7) Institute of Biochemistry and Molecular Biology, National Yang Ming University, Institute of Biochemistry and Molecular Biology, Taipei, Taiwan; 8) These authors contributed equally to this work. Heat shock factor 1 (HSF-1) is a master transcription factor that regulates cellular responses to proteostatic stresses. In \textit{C. elegans}, HSF-1 promotes long lifespan by acting as a downstream factor of insulin/IGF-1 signaling (IIS) pathway. Here we aimed at identification of novel factors that regulate lifespan acting together with HSF-1. We first carried out a genome-wide modifier RNAi screen and identified 17 RNAi clones that enhanced the sterile phenotype of reduction-of-function \textit{hsf-1(sy441)} mutants. Among them we found that \textit{pfd-6}/prolifin 6 was required for the longevity of \textit{daf-2}/\textit{insulin}/\textit{IGF-1} receptor mutants by using RNAi and \textit{pfd-6}/\textit{gk493448} mutations. We found that \textit{HSP-70}, one of direct target chaperones of HSF-1, physically interacted with PFD-6, by employing a split GFP system. Therefore, HSF-1 appears to increase the levels of \textit{HSP-70} that binds PFD-6, which contributes to the longevity of \textit{daf-2} mutants. We then showed that \textit{pfd-6::GFP} was expressed in the hypodermis and the intestine, where tissue-specific RNAi knockdown of \textit{pfd-6} significantly suppressed the longevity of \textit{daf-2(e1370)} mutants. Next, we asked how PFD-6 contributed to longevity. PFD-6 is a component of prolifin complex as well as R2TP (Rvb1, Rvb2, Tah1/TPR-containing protein, Pih1/Protein interacting with Hsp90)/prolifin-like complex, which have multiple functions including those as chaperones. We found that many components of R2TP/prolifin-like complex were partially but specifically required for the longevity of \textit{daf-2} mutants, whereas the majority of prolifin complex components were not. Thus, PFD-6 appears to act as a component of R2TP/prolifin-like complex to mediate longevity. In conclusion, our work suggests a novel mechanism by which PFD-6, a component of R2TP/prolifin-like complex, regulates longevity acting together with HSF-1 in IIS pathway.

1100A A vitellogenic open faucet causes senescent polymorphydism in \textit{C. elegans} hermaphrodites. T. Sornda, M. Eczurra, A. Benedetto, A. Gilliat, D. Gems Institute of Healthy Ageing, GEE, University College London, United Kingdom. According to ideas of G.C. Williams and M.V. Blagosklonny, a major cause of senescent pathology (i.e. aging) is harmful, late-life gene action, manifesting as deleterious run-on of developmental and reproductive functions (or hyper-function). In \textit{C. elegans} hermaphrodites, open faucet-type run-on of reproductive processes is a cause of several major senescent pathologies, including gonad atrophy, uterine tumor formation, intestinal atrophy and yolk accumulation. The latter seems to occur because during hermaphrodite selfing, vitellogenin (yolk protein) is synthesized in the intestine and taken up by oocytes; after depletion of hermaphrodite sperm, oocyte ceases, but yolk production does not. This causes yolky lipid pools to accumulate to high levels in the body cavity, and ectopic yolk deposition, a form of senescent steatosis. We have formally tested this model, according to which yolk accumulation reflects failure to switch off its source (synthesis) and loss of a sink (egg laying). Consistent with this, after self-sperm exhaustion, yolk protein (YP) levels accumulate continuously up to day 14 of adulthood. This causes YP170 levels to increase up to 7-fold relative to day 1 levels, and an overall YP level that is 30-40% of total protein content. These changes are fully suppressed in long-lived \textit{daf-2(e1370)} mutants. Moreover, provision of additional sperm by mating suppresses yolk accumulation, and spermless \textit{fog-2} mutants exhibit accelerated yolk accumulation. Yolk synthesis is coupled to intestinal atrophy by autophagy-dependent conversion of gut biomass into yolk, and blocking YP synthesis by combined \textit{vit-5}, \textit{vit-6} RNAi suppresses yolk steatosis and gut atrophy and extends lifespan. Notably, \textit{vit-5} RNAi increased VIT-6 protein levels, and \textit{vit-6} RNAi increased YP170 levels, but RNAi of \textit{vit-5} and \textit{vit-6}, alone or combined, had no effect on overall protein content per worm. Moreover, RNAi of \textit{vit-5} or \textit{vit-6} alone does not prevent gut atrophy. This suggests a model in which vitellogenin synthesis monopolizes intestinal protein synthesis in a way that leads to organ atrophy. We are currently testing this model.

1101B Regulation of a Holliday junction resolvase by an E3 ubiquitin ligase complex. Brett Spatola, Jackie Lo, Sean Curran Molecular and Computational Biology, University of Southern California, Los Angeles, CA.
Protein degradation is a fundamental part of proteostasis in which misfolded, aggregated, or nonessential proteins are presented to the proteasome for destruction. A major component of the ubiquitin-proteasome system (UPS) are E3 ubiquitin ligases, which concurrently bind E2 conjugation enzymes and specific substrates that are targeted for degradation. The most abundant class of E3 ubiquitin ligases are cullin-RING ligases (CRLs) that form multi-protein complexes consisting of a scaffold cullin, a RING domain, an adaptor protein, and a substrate receptor which directly binds to unique substrates. Presently, identification of specific substrates and their mechanism of interaction with substrate receptors in CRLs remain largely unknown.

Using the genetic tractability of *C. elegans* and the translational aspects of mammalian cell culture, we present the CRL-mediated regulation of a structure-selective endonuclease that resolves Holliday junctions during homologous recombination. First, after identifying a possible association through a yeast two-hybrid screen, we confirm the interaction biochemically, and we narrow down the amino acid residues of the resolvase that are responsible for this interaction. Second, we show that the CRL can polyubiquitylate the resolvase. Finally, we elucidate the spatial and temporal regulation of the resolvase’s stability using CRL mutants that we generated from an EMS screen in *C. elegans*. Although others studies have shown the involvement of CRLs in regulating other DNA repair components, these results unlock a novel regulatory role of a double-strand break repair protein that is paramount for the resolution of recombination intermediates to ensure proper chromosomal segregation.

1102C Understanding the toxicological effects of nutritional and non-nutritional sugars using *C. elegans*. Jessica Sponaugle, Amanda Harber, Julie Hall  Lincoln Memorial University, Harrogate, TN.

Sugars are carbohydrates taken into the body through eating or drinking, and are either used to make ATP or stored for a later use in the adipose tissue. The body needs an appropriate amount of sugar in order to function correctly. In fact, the daily recommended amount of sugar is only 32 grams. When the body has more sugar than needed it raises health risk factors including dental caries, obesity, diabetes, and heart diseases. There are two types of sugars: nutritive or nature sugars such as honey and stevia; and nonnutritive which are chemically made and include such things as sucralose, aspartame, and saccharin. Little research has been done on understanding the health risks of nonnutritive sugar. The toxicological effects of two nutritive and two nonnutritive sugars are being investigated in *C. elegans* to better understand their effects. Nematodes were exposed to the daily recommended amount (32g), two times the recommended amount, three times the recommended amount and the average daily consumption (72g) of sugar and tested for growth and reproduction. In regards to growth there was no change in the rate between any sugars or conditions. Higher concentrations of sugar did appear more sluggish and fatter. Currently reproduction is being investigated and preliminary data suggests that nutritive sugars increase the reproduction rates. These basic toxicological assays as well as aging can help in a better understanding of possible health risks, if any, related to different types of sugars.

1103A 2-Isopropyl-5-methylphenol ameliorates α-synuclein proteotoxicity and intracellular ROS accumulation in *Caenorhabditis elegans*. S. Srivastava, R. Pandey  CSIR-Central Institute of Medicinal & Aromatic Plants, Lucknow, India.

Parkinson disease (PD) is a progressive neurodegenerative disorder, leading to motor and non-motor symptoms such as bradykinesia, rigidity, and autonomic dysfunction. *Caenorhabditis elegans* serves as an excellent model system to study PD-related symptoms due to its genetic tractability and availability of PD models, including *dan-1, cch-1, pink-1, ubc-12, spp-9, grk-2, lrk-1 and lagr-1* whereas *pink-1, ube-2, spp-9, grk-2* and *pdr-1*, mRNA expression levels were augmented in *C. elegans* model of Parkinson’s disease. In wild-type worms, we perceived a significant up-regulation of *pink-1, ube-2, spp-9, grk-2, lrk-1 and lagr-1*. In the lead, staining with acridine orange, IPMP exhibited a significant decline in apoptosis. Altered lipid levels, another manifestation of PD were also found to normalize significantly in both wild type worms and NL5901 worms (Punc-54::a-synuclein::YFP::unc-119). Moreover, we furthered our studies towards deploying a qPCR technique for enumerating transcript expression of selected genes screened as α-synuclein modifiers from a genome-wide screen in *C. elegans* model of Parkinson’s disease. In wild-type worms, we perceived a significant up-regulation of *pink-1, ube-2, spp-9, grk-2, lrk-1 and lagr-1* whereas *pink-1, ube-2, spp-9, grk-2* and *pdr-1*, mRNA expression levels were augmented in *C. elegans* worms. In concise, we conclude imminent areas of investigation, about the emerging nature of IPMP that can be exploited to provide mechanistic perceptions toward therapeutic intervention for PD. We hypothesize that the observed beneficial effect establishes the potential of IPMP as a possible candidate for ameliorative or supportive roles in the management of Parkinsonism.

1104B The molecular basis of natural variation in cold stress response in *Caenorhabditis elegans*. Jana J. Stastna1, L. Basten Snoek2, Nell Nei1, Yiru Wang1,2, Joost A.G. Riksen2, Jan E. Kammenga2, Simon C. Harvey1  1) Biomolecular Research Group, School of Human and Life Sciences, Canterbury Christ Church University, Canterbury, CT1 1QU, UK; 2) Laboratory of Nematology, Wageningen University, 6708 PB Wageningen, The Netherlands.

Temperature regulation is generally a critical requirement for survival and proliferation, with the ability to survive both chronic and acute cold stress often being essential for wild-type fitness. Understanding how eukaryotic cells respond to low temperatures is also crucially important in aspects of biotechnology and medicine. In general, effects of low temperature depend
on the severity of the stress and has been implicated the genes and pathways that regulate membrane lipid composition in cold stress survival. In comparison to what is known about the response to high temperature stress, low temperature stress is less well understood.

Here we present an analysis of natural variation in cold stress resistance in the nematode C. elegans. Several approaches were taken to study this, these included testing in novel 4-parent recombinant inbred lines (RILs) and wild isolates. The results uncovered extensive variation in response to cold shock treatment and, for the first time, linked cold stress survival to the regulation of translation in C. elegans. Critically, genes previously identified in cold response cannot explain the variation detected. We have identified variation in eftu-2 (a homologue of human eEF-2), a gene important for the elongation step in protein synthesis, as the main genetic variant explaining the response to cold stress.

1105C Assessing collagen levels during aging and longevity. C.A. Statzer, C.Y. Ewald Department of Health Science and Technology, ETH Zurich, Switzerland.

Collagens are a major component of the extracellular matrix (ECM) and account for 30% of the total human protein mass. As we grow older our collagen mass declines 1% per year in our skin. This decrease is likely due to damage and degradation of collagen as well as transcriptional down-regulation of collagen expression with advanced age. Here, we determine the total collagen content and its decline during aging in C. elegans. We exploit the common post-translation modification of collagen fibers, which contain a hydroxylated form of the amino acid proline. The hydroxylation stabilizes the helical fold of collagens and can be utilized to specifically quantify their abundance. Interestingly, long-lived C. elegans show higher collagen protein levels compared to wild type. We are using publicly available expression profiles to identify collagens that show a progressive decline in mRNA expression levels during aging in wild type, but an upregulation in long-lived animals. We are employing the lifespan machine as developed by Dr. Nicholas Stroustrup to assess the requirements for these candidate collagens for longevity. Taken together, this work aims to elucidate how core ECM components are differentially regulated in normal and long-lived C. elegans and how the ECM itself is modulating the lifespan of an organism.

1106A Mitochondrial dysfunction mediated ECM degradation in C. elegans muscle cells. S Sudevan, M Takamura, Y Kubota, N Higashitani, A Higashitani Tohoku University, Sendai, JP.

Muscle atrophy is the loss of muscle mass or activity and is a common phenomenon in diabetes, cancer, aging as well as long duration bed rest. It has been observed in many studies that the mitochondrial function/activity decreases with the muscle atrophy or vice versa. For example, a study published in 2012 reported that mitochondria plays an important role in disuse muscle atrophy. Two other reports point towards the role of mitochondrial dysfunction in muscular diseases like Duchenne Muscular Dystrophy and Type II Diabetes. Although a connection between mitochondrial dysfunction and muscle damage has been established, the exact molecular pathway is still unclear. We found that treatment of wild type C elegans with Antimycin A (an Electron Transport Chain inhibitor) leads to paralysis as well as muscle damage. As muscle atrophy is often due to breakdown of muscle proteins via ubiquitin proteasome system (UPS), we analyzed the worms after treatment with MG132 (an UPS inhibitor). MG132 treatment could not rescue the muscle damage caused by Antimycin A. Surprisingly, overexpression of Emb-9 (Collagen IV) could suppress the motility defect as well as muscle damage (wavy myofilaments). On the other hand, similar wavy myofilaments were observed when the emb-9 ts mutant (g34) was kept under restrictive temperature. In addition, treatment with a Furin (Ca2+ proprotein convertase) inhibitor or a Matrix metalloproteinase (MMP) inhibitor could also rescue the muscle damage mediated by Antimycin A. We also observed decrease in Emb-9 protein level as well as an increase in muscle cytoplasmic Ca2+ concentration upon Antimycin A treatment, monitored through western blot analysis and GCaMP3.35 imaging system respectively. Ryanodine receptor (RyR, Ca2+ channel on ER, unc-68 in C elegans) mutant worms were resistant to Antimycin A treatment, indicating that the cytoplasmatic Ca2+ overload via RyR is essential for the muscle damage. This leads us to conclude that upon mitochondrial dysfunction, Ca2+ overload mediated activation of Furin and downstream activation of MMPs leads to degradation of extracellular matrix(ECM)component, Emb-9, which further results in muscle atrophy. Finally, we believe that ECM plays a very important role in maintaining muscle structure and activity, and mitochondria dysfunction could deteriorate ECM stability in muscle cells.

1107B A screen for protective drugs against delayed hypoxic injury. Chun-Ling Sun, Meng Liu, Huiliang Zhang, Wang Wang, C. Michael Crowder, 1) Department of Anesthesiology and Pain Medicine, University of Washington School of Medicine, Seattle, Washington 98109; 2) Department of Genome Science, University of Washington School of Medicine, Seattle, Washington 98109; 3) Department of Anesthesiology, The Second Military Medical University, People's Republic of China.

Despite longstanding efforts to develop cytoprotective drugs against ischemia/reperfusion (IR) injuries, there remains no effective therapeutics to treat hypoxic injury. The failure of traditional strategies at solving this problem suggests the need for novel and unbiased approaches that can lead to previously unsuspected targets and lead compounds. Towards this end, we report here a unique small molecule screen in the nematode C. elegans for compounds that improve recovery when applied after the hypoxic insult, using a C. elegans strain engineered to have delayed cell non-autonomous death. In a screen of 2000 compounds, six were found to produce significant protection of C. elegans from delayed death. Four of the compounds were tested in an ex vivo mouse heart ischemia/reperfusion model and two, mecloclycline and 3-amino-1,2,4-triazole, significantly reduced infarction size. Our work demonstrates the feasibility of this novel C. elegans screen to discover hypoxia protective drugs that are also protective in a mammalian model of hypoxic injury.
1108C  Lactate and Pyruvate influence longevity, stress resistance and neuronal physiology in C. elegans.  A. Tauffenberger1, L. Mottier1, H. Fiumelli1, J.A. Parker2, P.J. Magistretti1,2 1) Biology and Environmental Science and Engineering Division, King Abdullah University of Science and Technology, Thuwal, Makkah, SA; 2) EPFL, Lausanne, Switzerland; 3) CRCHUM, Montreal, Canada; 4) Neuroscience Department, Université de Montréal, Montréal, Canada.

The world population shifts to an older demographic and with it there is an increased incidence of age related disorders including neurodegenerative diseases. As it has become a necessity to find new therapeutics, we focus our research on the role of energy metabolism on neuronal physiology. Disrupted energy metabolism is one of a number of mechanisms that may contribute to neurodegeneration. Indeed, conditions that reprogram energy metabolism like dietary restriction are active areas of investigation in efforts to identify new therapeutic targets.

We sought to investigate the role of energy metabolism in neuronal protection using nematode diet supplementation with L-lactate and pyruvate, two important glucose metabolites. Although pyruvate has been extensively described as the glycolysis end product and first intermediate of the citric acid cycle, lactate has only recently emerged as an important oxidative metabolite and potential signalling molecule. Indeed, it has been known for many years as the end product of anaerobic glycolysis, but recent work1,2 has unveiled its role as a major factor in neuronal plasticity and protection.

We used the C. elegans model for both neuronal dysfunction (unc-47 mutant) and neurodegeneration (Amyotrophic Lateral Sclerosis – TDP-43). Both models mimic motor neuron dysfunction resulting in age-dependent paralysis. We performed different tests to measure aging rate and neuronal survival in nematode with supplemented diet and tried to identify signalling pathways using RNAi.

Our preliminary results suggest that both lactate and pyruvate increase resistance to oxidative stress, but reduce lifespan at high concentration. Interestingly, lactate but not pyruvate is able to reduce age-onset paralysis, suggesting a different mechanism than oxidative metabolism.

A summary of our results will be presented.

1) Pellerin L. and Magistretti PJ., 1994
2) Yang et al., 2014

1109A  Oocyte-specific insulin/IGF-1 signaling targets reveal mechanisms that regulate reproductive aging and oocyte quality maintenance.  N.M. Templeman, S. Luo, R. Kaletsky, C. Shi, J. Ashraf, W. Keyes, C.T. Murphy  Lewis-Sigler Institute for Integrative Genomics and Department of Molecular Biology, Princeton University, Princeton, NJ.

Reproductive decline is one of the earliest hallmarks of aging in both invertebrates and mammals, followed by a relatively long post-reproductive lifespan. The insulin/insulin-like growth factor-1 signaling (IIS) pathway plays a key, evolutionarily conserved role in regulating lifespan, and it also been shown to control reproductive span in Caenorhabditis elegans. While there has been ongoing characterization of the IIS transcriptional targets that influence somatic aging and lifespan, it is unclear whether the same molecular mechanisms are important for the regulation of reproductive aging. Moreover, although we have shown that disrupting IIS through mutating the IIS receptor (daf-2) extends reproductive span through continued maintenance of germline and oocyte quality with age, the oocyte-specific transcriptional targets of IIS had not yet been identified. Therefore, we compared the transcriptomes of isolated daf-2(-) oocytes to age-matched control oocytes. In addition, we identified soma-specific IIS targets by evaluating genes with expression levels that were altered by daf-2 downregulation in whole worms but not in isolated oocytes. Remarkably, IIS appears to regulate reproductive aging and somatic aging through largely distinct molecular mechanisms. We found striking discrepancies between oocyte-specific and soma-specific IIS targets with respect to: 1) differentially expressed genes, 2) GO terms and functions associated with those genes, and 3) DNA motifs enriched in the upstream promoter regions of the genes. Many of the tested oocyte-level IIS targets contributed to the extended reproductive span and maintenance of oocyte viability with age in daf-2 mutants, but did not exert consistent effects on lifespan. Several cathepsin B-like genes were significantly downregulated in the daf-2 RNAi-treated oocytes; we found that general inhibition of these cysteine proteases diminished the deterioration in oocyte quality with age, offering a potential mechanism to slow age-related reproductive decline. Oocyte-specific IIS transcriptional targets therefore represent excellent candidates for uncovering mechanisms that regulate reproductive aging.

1110B  Investigating the C. elegans matrisome during aging.  A. Teuscher1, J. Gebauer2, A. Naba3, C.Y. Ewald1  1) ETH Zurich, Health Sciences and Technology, Schwerzenbach-Zurich, CH; 2) University of Cologne, Institute of Biochemistry, Cologne, DE; 3) University of Illinois at Chicago, Department of Physiology and Biophysics, College of Medicine, Chicago, US.

Collagens represent one-third of all proteins in humans and collagen mass in the skin progressively declines during aging. Collagens are the major component of the extracellular matrix (ECM). Recent efforts have been directed towards characterizing all proteins in the ECM as well as ECM-associated proteins in humans and mice using bioinformatics and proteomics approaches. The compendium of these ECM genes and proteins is termed “matrisome”, which consists of core components (44 collagens, 195 glycoproteins, 35 proteoglycans) and about 750 matrisome-associated components in the human genome. In mammals, these 44 collagens form 28 different collagen types. Here for C. elegans, we used a similar bioinformatics approach and assembled an inventory list of the C. elegans core matrisome and matrisome-associated genes. With over 150 members, the group of collagens is the largest group in the core-matrisome of C. elegans. Moreover, we have identified bioinformatically 5 different collagen clades with varying numbers of subgroups. During development, different sets of collagens are expressed during the different larval stages, however, we are interested in adult expressed collagens and ECM components. The mRNA expression of most adulthood expressed collagens cease during aging. We are currently analyzing the spatial-temporal transcriptional expressing pattern of these ECM genes during aging. In parallel, we are analyzing the translational fusion of ECM
genes with GFP. For instance, COL-19::GFP, LON-3::GFP, ROL-6::GFP are expressed from the hypodermis and become integrated into the cuticle. Although their mRNA expression declines during aging, some of the respective GFP fusion protein are visible for many more days in the cuticle, suggesting that some of these collagens are long-lived, while others might be turned over. Taken together we made the first steps in order to generate data for a *C. elegans* matrisome and gained a better understanding of the role of certain collaging during the aging of *C. elegans*.

### 1111C Spontaneous nucleolysis occurs during organismal death in *Caenorhabditis elegans*.

Cuckoo Jetto, Agrima Nair, Vishnu Raj, Bejoy Vijayan, Anoopkumar Thekkuveettil Molecular Medicine, Sree Chitra Tirunal institute for Medical Sciences and Technology, Trivandrum, Kerala, IN.

Necrotic cell death has been extensively studied for its involvement in many human diseases. In *C. elegans* change in pH or temperature induces organismal death and is marked by a characteristic wave of necrotic cell death of worm’s digestive track; a phenomenon also marked with a blue fluorescence (called death fluorescence or DF) due to anthranilate release from stored vesicles. One of the critical events in this pathway is the increase in cytoplasmic calcium levels in the digestive tract, which is essential for both for the development of DF as well as for the organismal death. But whether the organismal death, being a spontaneous event, follows the necrotic cell death pathway involving calcium-triggered activation of calpain and cathepsin is not clear. Here we report a uniquely different mechanism in organismal death through calcium-regulated calpain proteases, bypassing the cathepsin/ aspartyl protease pathway. Our findings also show that nucleolysis is an immediate intracellular change occurs during organismal death and is a calcium-dependent process. These cellular responses during organismal death are unique, compare to necrotic neurodegeneration in *C. elegans* and could have significance in the onset of organismal death.

### 1112A FKH-9 modulates stress physiology and endoplasmic reticulum homeostasis during pathogen infection in *C. elegans*.


Animals maintain cellular homeostasis in response to environmental challenges, including microbial pathogens. Distinct stress response signaling pathways promote protein folding homeostasis in the cytoplasm, endoplasmic reticulum (ER), and the mitochondria, and a role for each has been implicated during infection of *C. elegans* with pathogenic bacteria. We previously demonstrated that the ER Unfolded Protein Response (UPR) is induced during infection of *C. elegans* with *Pseudomonas aeruginosa*, and that the UPR regulator XBP-1 has an essential role during infection and immune activation (1). To identify signaling mechanisms that could compensate for XBP-1 deficiency during pathogen infection, we conducted a forward genetic screen for suppressors of *xbp-1* mutant lethality on the pathogenic bacteria *Pseudomonas aeruginosa*. We recently reported that mutations in the eIF3k and eIF3l translation initiation factor subunits can suppress the lethality of the *xbp-1* mutant in the presence of *P. aeruginosa* (2). Here, we show that mutations in the Forkhead family transcription factor FKH-9 also suppress the lethality of the *xbp-1* mutant on *P. aeruginosa*. We observe that mutations in *fkh-9* confer improved intestinal ER morphology and organismal tunicamycin resistance, independent of previously defined UPR signaling pathways. To define how FKH-9 activity modulates ER homeostasis, we have conducted ChiP-seq analysis to define direct transcriptional targets of FKH-9, along with functional genetic analysis, which suggest a role for FKH-9 in integrative stress signaling. Our data suggest that XBP-1-independent pathways contribute to maintenance of ER homeostasis and survival during infection and innate immune activation in *C. elegans*.

References:


### 1113B Spatial relationship between mitochondria and the protein quality control system.

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Mitochondria play several important roles in the cell. Apart from being cell power plants that produce ATP and key depositories for calcium ions, they also take part in various cellular metabolism pathways, for instances heme or steroid synthesis. Malfunctions in any of these processes lead to defects in cell performance which in turns cause pathological states on the organismal level, such as muscular or neurological diseases. However, despite the importance of mitochondria, we still do not fully understand the processes responsible for keeping mitochondria in shape.

The aim of our study is to fully understand the link between healthy mitochondria and the proteasome. In particular, we investigate the spatial relationship between the mitochondria and a very recently discovered protein quality control mechanism, called the Unfolded Protein Response activated by mistargeted proteins (UPRam). We hypothesize that the part of the quality control for mitochondrial proteins exerted by the proteasome is accomplished on the surface of the mitochondria. We address this hypothesis by testing whether the defects in protein import into the mitochondria or in proteins homeostasis influence the abundance of the proteasome complex on the surface of that organelle. Our preliminary results showed increased proteasome activity in a mitochondria-enriched cellular fraction after depletion of DNJ-21 motor protein. We further investigate this phenomenon by using both biochemical and microscopic methods.

Results of this project will expand our knowledge on the process of mitochondrial health regulation. They will especially contribute to the understanding of the quality control of mitochondrial proteins exerted by the proteasome.
We are interested in the effects of diet and nutrition on the quality of aging. In the genetic model C. elegans, excess sugar (glucose) decreases lifespan, and a sugar mimic (2-deoxyglucose) promotes healthy aging. We are investigating whether sugar substitutes (aspartame, saccharin) might also influence C. elegans lifespan/healthspan. The study compares the effects of glucose levels and saccharin levels on the life span of C. elegans. As glucose substitutes, such as saccharin, become more prevalent in diets, it is important to understand their effects. Results from these studies should further understanding of how these common food ingredients impact human health.

The significance of cell protective stress response mechanisms is now widely appreciated to not just act at the level of single cells, but at the “multicellular” level. Evolutionary conserved stress responses initiate “transcellular chaperone signalling” that allows stress-responsive molecular chaperones, such as hsp-90 (daf-21), to be activated from one tissue to another. How this form of inter-tissue communication functions at a molecular level to allow systemic upregulation of protective chaperone expression however remains an open question to date. Using a systems-wide approach and further genetic analysis, we have identified the zinc finger transcription factor PQM-1 as a mediator of transcellular chaperone signalling (TCS) in C. elegans. We show how depletion of pqm-1 suppresses induction of TCS-mediated hsp-90 chaperone expression in several target tissues and provide evidence for a novel role of PQM-1 for proteostasis maintenance. We furthermore demonstrate that transcriptional activity of PQM-1 in the C. elegans intestine is increased during conditions that activate TCS. Our data suggests the intestine to be a key organ which “transduces” TCS-dependent protective responses across remote cell types and tissues in C. elegans.

Investigating the impact of sugar substitutes on healthspan in C. elegans. J. Ungarten, Brian Onken Centenary University, Hackettstown, NJ.

Mitochondrial unfolded protein response is required for the survival and lifespan of long-lived mitochondrial mutants. Megan Senchuk, Dylan Dues, Jason Cooper, Leira Lew, Emily Machiela, Claire Schaar, Jeremy Van Raamson Park Laboratory of Aging and Neurodegenerative Disease, Center for Neurodegenerative Science, Van Andel Research Institute, Grand Rapids, MI, USA.

Genetic mutations that mildly decrease mitochondrial function have been shown to markedly increase lifespan. In exploring the molecular mechanisms responsible for the increase in lifespan, it was shown that knocking down the expression of UBL-5, a ubiquitin-like protein that is required for the mitochondrial unfolded protein response (mitoUPR), reverts the long lifespan of the clk-1 and isp-1 mitochondrial mutants to WT (Durieux et al., 2011). It was subsequently demonstrated that knocking down the expression of ATFS-1, a transcription factor that acts with UBL-5 to mediate the mitoUPR, does not affect the longevity of isp-1 worms (Bennett et al., 2013). Thus, the role of the mitoUPR in mitochondrial mutant longevity remains unclear. In this work, we confirm that the mitoUPR is activated in clk-1, isp-1 and nuo-6 mitochondrial mutants during development and throughout adulthood. To examine the role of the mitoUPR in the longevity of these mutants, we crossed these worms to an atfs-1 deletion mutant (gk3094). We found that only the nuo-6;atfs-1 double mutants are viable and fertile, while isp-1;atfs-1 double mutants and the progeny of clk-1;atfs-1 double mutants fail to develop to adulthood. Similarly, when clk-1 and isp-1 worms are grown on atfs-1 RNAi from the parental L4 stage, the progeny do not develop to adulthood. Accordingly, we measured the lifespan of clk-1, isp-1 and nuo-6 worms on atfs-1 RNAi beginning at the experimental L4 generation. Under this paradigm, we found that atfs-1 is not required for the lifespan of any of these strains. In contrast, we found that knocking down atfs-1 beginning in the parental L4 generation decreases the lifespan of nuo-6 worms, while an atfs-1 mutation completely reverts nuo-6 lifespan to wild-type. Combined these results demonstrate that ATFS-1 is required for the long lifespan of nuo-6 worms and suggest that its effect on lifespan occurs during development. In characterizing the nuo-6;atfs-1 double mutants, we have identified multiple genes involved in stress resistance that exhibit an atfs-1-dependent increase in expression in nuo-6 worms. We are currently studying which of these changes in gene expression contribute to the longevity of nuo-6 mutants. Overall, this work suggests that the ability to activate the mitoUPR during development is crucial for the survival and lifespan of long-lived mitochondrial mutants.


Alzheimer’s disease (AD) is a progressive neurodegenerative disorder characterized by the presence of extracellular amyloid plaques formed by accumulated Amyloid-ß peptide (Aß) aggregates and neurofibrillary tangles, which are formed by aggregation of hypophosphorylated microtubule-associated protein Tau. We generated a new model of this disease by crossing existing Aß (CL2355) and Tau (BR5270, BR5271) pan-neuronal expressing transgenic strains of Caenorhabditis elegans. The lifespan and progeny viability of the double transgenic strain were significantly decreased compared with wild-type N2 (P<0.0001). We also observed that ectopic expression of these transgenes could interfere with neurotransmitter signaling pathways. Moreover, the double transgenic strain showed the most severe defects in chemotaxis associative learning when compared with other strains. In order to gain new insights into AD pathology mechanisms, we performed RNA-seq transcriptomic analysis. We observed 1053 genes to be regulated, of which 248 were up- and 805 were down-regulated in a comparison between the new AD model (UM0001, UM0002) and N2. Gene set enrichment analysis using David 6.7 indicated that up-regulated annotation clusters included UDP-Glucuronosyltransferase (6 genes, P<4.2E4), PapD-like (4 genes, P<1.8E-2), aging (5 genes, P<8.1E-2) and down-regulated included nematode cuticle collagen (36 genes, P<1.5E-21), metabolic process (65 genes, P<1.9E-8). Comparing the list of regulated genes from C. elegans to the top 60 genes related to human AD confirmed an overlap of 9 genes: patched homolog 1, PTC1 (ptc-3), the Rab GTPase activating protein, TBC1D16 (tbc-378
1118A The Role of SET-9 and SET-26 in Longevity and Germline Function. Wenke Wang, Amresh Chaturbedi, Siu Lee Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Epigenetic mechanisms, including histone modifications, have been found to regulate both aging and germline function. We discovered two highly homologous SET domain containing proteins, SET-9 and SET-26, to regulate lifespan and germline function in C. elegans. Specifically, lacking somatic set-26 extends lifespan, whereas deficiency of both set-9 and set-26 causes a severe germline defect. Both SET-9 and SET-26 have a PHD domain and a SET domain. We showed that the PHD domains of SET-9 and SET-26 bind to H3K4me3 in vitro and the genomic distributions of SET-9 and SET-26 are highly correlated with H3K4me3 marking in vivo, supporting a model that SET-9 and SET-26 are recruited to H3K4me3 marked regions in C. elegans. Although previous report suggested that the SET domain of SET-26 can deposit H3K9me3 in vitro, we found that loss of set-9 and set-26 does not affect global H3K9me3 levels. On the other hand, we detected elevated levels and expansion of H3K4me3 marking around SET-9 & SET-26 binding sites in the set-9 set-26 double mutant, suggesting that SET-9 & SET-26 normally help to restrict H3K4me3 marking. Interestingly, the spreading of H3K4me3 was not associated with gene expression changes. On the other hand, we revealed that some of the loci bound by SET-9 & SET-26 show gene expression changes in the set-9 set-26 mutants. We conclude that SET-9 & SET-26 may have dual roles in regulating gene expression and in restricting H3K4me3 deposition.

1119B Using free-living Caenorhabditis elegans as a model for anthelmintics study. K. J. Weaver, Y. Hu, C. J. May, B. L. Ellis 1) Department of Biology & Chemistry, Bethel College, South Bend, IN; 2) Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA.

Soil-transmitted helminths (STHs) are intestinal parasitic nematodes that infect humans, and are transmitted through contaminated soil. These nematodes include the large roundworm (Ascaris lumbricoides), whipworm (Trichuris trichiura), and hookworm (Ancylostoma ceylanicum, Ancylostoma duodenale, and Necator americanus). Nearly 1.5 billion people (~24% of the population) worldwide are infected with at least one species of these parasites, burdening the poor, in particular, children and pregnant women. To combat these diseases, the WHO only recognizes four anthelmintic drugs, including the preferred drug, albendazole, for mass drug administration (MDA). These four drugs have a total of two different mechanisms of action, and, as expected, resistance has been observed. This problem calls for new drugs with different mechanisms of action. Although there is precedence for the use of Caenorhabditis elegans (C. elegans), a free-living nematode, as a model for drug screening and anthelmintic testing, their usefulness for such anthelmintic study is not clear as past research has shown that C. elegans did not show a strong response to albendazole, the MDA drug of choice, in comparison with various STHs under similar treatment. To further examine if C. elegans has the potential to be a good model organism for anthelmintic drug study, we employed a health rating scale in order to tease out potential effects of albendazole, and other anthelmintics, that are missed when using a binary, dead/alive scale. Using the health-rating scale we found that although the worms may have not been dying, they were sick, showing dose responses to anthelmintic drugs, including albendazole, reinforcing the case for C. elegans as a useful model for anthelmintic study.

1120C Transgenerational effects of long-term dauer arrest. Amy K. Webster, James M. Jordan, Jonathan D. Hibshman, Rojin Chitrakar, L. Ryan Baugh Duke University Biology Department, Durham, NC.

Long-term effects of extended starvation during L1 arrest have been shown to persist to the F3 generation, suggesting transgenerational epigenetic inheritance. Though effects on lifespan and gene expression have been reported (Rechavi et al), we have only observed relatively subtle transgenerational effects on L1 starvation survival and heat resistance (Jobson, Jordan et al). Furthermore, we found that a minority of starved worms transmitted these effects, requiring phenotype-based sorting of the starved population to detect transgenerational effects among their descendants. In contrast to L1 arrest, larvae can survive dauer arrest for months and the majority of C. elegans in the wild are found as dauer. Persistent effects of dauer arrest have been reported for adults (Hall et al), but transgenerational effects have not been reported. We reasoned that dauer arrest is likely a more ecologically relevant and robust model for transgenerational effects of starvation. Here, we used a liquid culture system carefully controlling worm density and food availability to cause virtually 100% of N2 larvae to enter dauer arrest. These worms remain dauer for months and become reproductive adults when allowed to recover on plates. By assessing various life-history traits, we found that worms subjected to long-term dauer arrest exhibit apparent fitness costs upon recovery, including maternal effects in the F1 generation. However, L1 starvation survival was increased population-wide in the F3 generation (without phenotypic sorting of ancestors), suggesting potentially adaptive transgenerational effects of long-term dauer arrest. This phenotypic effect depended on the length of time the P0 generation spent in dauer arrest, suggesting that dauer formation alone is not sufficient. Notably, the transgenerational effects occurred in the absence of detectable gene expression changes, as if the presumed epigenetic effect on gene expression is distributed over many genes with a small effect on each. This work suggests that experiencing a stress such as starvation can initially be costly, but future generations may exhibit increased fitness in particular circumstances.

References:
1121A The Role of Sphingolipid Metabolism in NMJ Function and healthspan in C. elegans. C. Wentz, H. Hrobuchak, D. Servello, J. Chan Department of Biology, Juniata College, Huntingdon, PA.

Sphingolipids are vital to cellular function, which includes signal transduction, stress response, cell proliferation, differentiation, and survival. Sphingolipids are interconverted through enzymatic activity that regulates lipid content of a cell. Loss of sphingosine kinase (SK), an enzyme responsible for the conversion of sphingosine to sphingosine-1-phosphate, has been shown to decrease movement. We show that C. elegans mutants lacking SK (sphk-1) have shorter lifespans and exhibit a greater decline in thrashing and neuromuscular function with age. Using RNAi, we aimed to determine whether this decreased movement with age is a result of development or adult processes. For this, we analyzed worms treated with RNAi in different windows of development and adult life for changes in neuromuscular function. Neuromuscular function was analyzed through thrashing behavior and resistance to aldicarb, an acetylcholinesterase inhibitor. In particular, we will describe results of RNAi knockdown of sphk-1 occurred during days 1-3 and 3-7 of development. Furthermore, we examined changes to the neuromuscular junction (NMJ) structure with age using GFP-tagged synaptic markers. Finally, to further explore the role of other sphingolipid metabolism enzymes, we also analyzed knockdown of ceramide synthase genes (hyl-1, hyl-2) and a putative S1P lyase (tag-38). These results will help determine whether SK and S1P production might effect NMJ function and healthspan in aging animals.

1122B Interpreting an unbiased forward genetic screen: Characterization of novel longevity-genes in the nematode Caenorhabditis elegans. L.E. Wester1, 2, M.J. Derisbourg1, R. Baddi1, M.S. Denzel1 1) Max Planck Institute for Biology of Ageing, Cologne, North Rhine-Westphalia, DE; 2) Cologne Graduate School of Ageing Research, Cologne, North Rhine-Westphalia, DE.

Since Sidney Brenner paved the way for forward genetic screening in Caenorhabditis elegans (C. elegans) in 1974, genetic techniques have improved massively and nowadays allow the combination of chemical mutagenesis and whole genome sequencing. Here I present the systematic analysis of a direct whole genome screen for longevity genes by mutagenesis that uses the power of modern genomics to identify causative mutations. After an unbiased induction of mutations via ethyl methanesulfonate (EMS) on a parental population, F2 animals were collected, carrying homozygous mutations for a given allele. The clonal individual F3 populations were then tested for extended lifespan and in this event sequenced in a large-scale manner. Subsequently, all genomic variations within the long-lived EMS-mutant strains were mapped and identified, resulting in a list of potential longevity alleles that does not only encompass loss- but also gain-of-function mutations. Loci with at least two independent alleles of one gene were tested as candidate longevity genes. A total of 103 genomes were sequenced, resulting in over 240 candidate longevity alleles. Currently, I am validating the top candidate alleles and their predicted longevity phenotype in N2 wildtype animals, using the CRISPR/Cas9 technology to recreate the respective genotypes. The ultimate goal of my current work is the discovery of new verified longevity genes, that will be then further investigated. One such candidate is col-106: This gene encodes a small predicted cuticular collagen with a human homolog (COL1A1), mutations in which are implicated as cause of Ehlers-Danlos syndrome, a genetic connective tissue disorder. Collagens and the structural organization of connective tissues are thought to be signatures of conserved longevity pathways, however, a distinct link between collagens and longevity remains elusive. While the screen itself is almost completed and data concerning coding sequences are mainly analyzed, so far, non-coding parts of the genome remain unspected. One further core interest is the analysis of alleles found for instance in promotor regions. In these processes, the full potential of genetic forward screening combined with modern genomics will be set out. Finally, our overall aim is to contribute to the spectrum of potential mechanisms and modulators of ageing.

1122C Disruption of a specific extracellular matrix structure in the model organism Caenorhabditis elegans activates antioxidant, osmotic, and antimicrobial stress defenses. K.D. Wimberly, K.P. Choe Genetics Institute / Dept. of Biology, University of Florida, Gainesville, FL.

Extracellular matrices (ECMs) are dynamic structures that sense environmental stimuli and communicate with cells. It is now clear that there is dynamic and reciprocal cross talk between the ECM and underlying cells, and this cross talk is vital in many biological processes such as ECM remodeling, tissue morphogenesis, and wound healing. Molecular pathways that mediate these processes are well characterized, but very little is known about how ECMS interact with underlying cells following exposure to environmental stressors. Extracellular matrix barriers and cellular transcriptional responses form the first and second lines of defense against many chemical and microbial environmental stressors, respectively. The barrier in nematodes is a collagenous extracellular matrix called the cuticle. Disruption to cuticle collagen genes was previously shown to activate osmotic and antimicrobial response genes. We have learned that disruption of specific bands of collagen called annular furrows co-activates detoxification, hyperosmotic, and antimicrobial response genes without activating other core stress responses. The detoxification response to furrow loss is activated in epidermal cells via the SKN-1/Nrf transcription factor. Our results are consistent with an extracellular matrix associated sensor that coordinates antioxidant, hyperosmotic, and antimicrobial responses. We are now leveraging the genetic tractability of C. elegans to define signaling between the matrix and cellular...
stress responses and have identified candidate receptors, transcription factors, and protein kinases.

1124A  Global RNA-seq analysis of whole C. elegans animals overexpressing human α-synuclein.  L. Heikkinen¹, S. Asikainen², J. Peltonen³, M. Lakso³, G. Wong¹ ¹ University of Macau, Macau, Macau S.A.R., CN; 2) University of Helsinki, Helsinki, FINLAND; 3) University of Eastern FINLAND, Kuopio, FINLAND.

Parkinson’s disease (PD) is a devastating age-associated disorder characterized by slowness of movement and resting tremor. Neuropathologic hallmarks of the disease include degeneration of dopaminergic neurons and presence of Lewy bodies that contain α-synuclein. In our previous studies, we produced transgenic C. elegans animals over-expressing human α-synuclein under a pan-neuronal aex-3 promoter. Many behavioral effects that were similar to PD were observed in these animals. To gain insight into molecular mechanisms involved in synucleopathies we performed RNA-seq transcriptional analysis on total RNA isolated from whole transgenic animals over-expressing α-synuclein with A53T mutation that causes early-onset PD in humans via a dominant mode of inheritance. Greater than 1700 genes were identified as up-regulated and 92 genes were down-regulated when compared to N2 controls. Gene ontology analysis was performed to identify enrichment of gene-set groups in molecular function and biological processes that were affected by the transgenic expression. In comparison to human PD linked genes, we observed overlap of 4 neuronally expressed genes: DGKQ/GAK, diacylglycerol kinase (dgk-1); MAPT, microtubule-associated protein tau (ptl-1); SYT11/RAB25, synaptotagmin-11 (snt-1); and STK39, SPS1-related proline-alanine-rich protein (gck-3). Our results suggest that transgenic overexpression of human α-synuclein in C. elegans can alter gene expression in ways that may expose mechanisms and pathways that are also affected in human PD. These findings may ultimately provide novel pathways and insights into PD neuropathology.

1125B  Metabolic profiling of a SKN-1 gain-of-function Caenorhabditis elegans mutant.  Cheng-Wei Wu, Keith Choe Department of Biology and Genetics Institute, University of Florida, Gainesville, FL.

Stress-inducible SKN-1 and NRF proteins are a family of transcription factors that activate antioxidant and xenobiotic genes to promote stress resistance and longevity. Recent studies in C. elegans have demonstrated that SKN-1 regulates aging downstream of dietary restriction and nutrient-sensing interventions, and SKN-1 has also been shown to coordinate metabolic processes. Using Isotopic Ratio Outlier Analysis (IROA), we performed metabolic profiling of a SKN-1 gain-of-function mutant skn-1(k1023) which has a long-lived and stress resistant phenotype. Clustering analysis show that the skn-1(k1023) mutants have a distinct metabolic profile compared to the N2 wildtype worms, most notably, metabolite levels of the antioxidant glutathione and its precursor cystathionine and cysteine are highly elevated (~3.5-folds) in the skn-1(k1023) mutant. This suggests that skn-1(k1023) mutants promote stress resistance via both an increase in expression of detoxification genes as well as elevated levels of endogenous antioxidant metabolites. The skn-1(k1023) mutant also show change in levels of amino acids associated with oxidative stress, these include ~60% decrease in levels of phenylalanine, arginine, and tyrosine. We also show that compared to wildtype worms, skn-1(k1023) worms have distinct patterns of metabolic profiles during aging between day 1, 3, and 6 of adulthood. Notably, skn-1(k1023) mutants were able to resist age-related changes in many metabolites observed during aging in the wildtype worms. While SKN-1 is known to promote stress resistance and longevity via transcription of its downstream genes, this study provides new insights into how SKN-1 may also influence changes at the metabolome level to confer these phenotypes.

1126C  Toward understanding the network of transcriptional regulation in the daf-2 rsks-1 double mutant in C. elegans.  D. Wu, J. Lan, D. Chen Nanjing University, Model Animal Research Center, nanjing, Jiangsu, CN.

Insulin/IGF-1 and mTOR pathways play important roles in development, metabolism and aging. Previous studies showed that simultaneous inhibition of DAF-2 (IGF-1 receptor) and mTOR target RSKS-1 (ribosomal S6 kinase) results in synergistic life span extension, which is mediated by positive feedback regulation of DAF-16 (FOXO transcription factor) via the AMPK (AMP-activated protein kinase) complex. To better understand the molecular mechanisms of the super longevity produced by daf-2 rsks-1, we performed bioinformatics analyses of microarray data to identify genes that are differentially expressed only in the daf-2 rsks-1 double mutant, but not in daf-2 or rsks-1 single mutants. Genetic screens by RNAi helped to identify DDK-1 (daf-2 rsks-1 downstream kinase) as a tissue-specific mediator of the synergistic life span extension by daf-2 rsks-1. This pool of coregulated genes also allowed us to perform motif analysis to identify potential cis-regulatory elements and the associated transcription factors. Functional studies revealed novel transcriptional regulators that play an important role in life span determination in the daf-2 rsks-1 double mutant. Together, these findings may help to elucidate the transcriptional regulation network downstream of IGF-1 and mTOR pathways in aging.

1127A  SGK-1 acts downstream of TORC1, but not the insulin/IGF-1 signaling pathway, to regulate lifespan in C. elegans.  G. Wu, M. Zhu, WJ Li, YX Li, CX Fan, MQ Dong National Institute of Biological Sciences, Beijing, China.

SGK-1, the only C. elegans homolog of mammalian Serum- and Glucocorticoid-inducible Kinase, is an AGC kinase whose paralogs include two well-known kinases AKT-1 and AKT-2 in the insulin/IGF-1 signaling (IIS) pathway. Whether SGK-1 acts like AKT-1 and AKT-2 to regulate lifespan of C. elegans is controversial. Here we present experimental evidences to show that SGK-1 acts downstream of TORC1 (Target Of Rapamycin Complex 1), but not downstream of the IIS pathway, to regulate lifespan in C. elegans. Arguing against SGK-1 being functionally similar to AKT-1/2:
1. Multiple extensively backcrossed null alleles of sgk-1 all shortened the lifespan of wild-type worms, which was opposite to the loss-of-function (ll) phenotypes of akt-1 and akt-2 in comparison, independent sgk-1(gf) mutants lived slightly longer than the wild type.
2. Recombinant His-tagged DAF-16 was phosphorylated by immunoprecipitated AKT-2::GFP, but not SGK-1::GFP.
3. By IP-MS analysis, we found that SGK-1::GFP was associated with RSKS-1, the C. elegans S6 kinase and a component of the TORC1 signaling pathway. Verifying this finding, GST-SGK-1, but not GST pulled down RSKS-1::GFP (but not GFP alone) from worm lysates.

4. Null alleles of sgk-1 suppressed the longevity phenotype induced by eat-2(/), rapamycin (inhibitor of TOR), let-363 (TOR) RNAi, raga-1 RNAi, rsks-1(null), and AAK-2(1-321)::GFP overexpression, suggesting that TORC1 signaling limits lifespan by inhibiting SGK-1, at least in part.

5. Consistent with the reported finding that rsks-1(null) greatly enhances the daf-2 longevity, sgk-1(gf) also further extends the lifespan of daf-2(e1370) worms. This result strengthens the idea that there is a synergistic interaction between TORC1 and IIS signaling in lifespan regulation.

1128B An essential role for p38/ATF-7 pathway in survival under low potassium environment in C. elegans. Ziyun Wu1,2, T. Keith Blackwell1,2 1) Research Division, Joslin Diabetes Center, Boston, MA; 2) Department of Genetics and Harvard Stem Cell Institute, Harvard Medical School, Boston, MA.

Potassium ions are essential for all living cells. In humans, potassium levels have critical effects on action potentials, blood pressure, osmotic pressure, acid-base balance, metabolism and many other health parameters. Although C. elegans has frequently been employed to study the nervous system, genetics, development, metabolism and aging, how potassium levels affect C. elegans survival has not been investigated, the molecular mechanisms in regulation of survival in response to low potassium environment are unknown. Here we determined that the conserved p38 MAPK signaling pathway NSY-1(ASK1)–SEK-1(MKK3)–PMK-1(p38) is essential for C. elegans survival under low potassium conditions. Wild-type worms are not sensitive to low potassium environment and able to survive more than one month. By contrast, under these conditions animals in which this p38 pathway is disabled become paralyzed within two hours, and die within a few days. Through EMS mutagenesis screening of sek-1(km4) animals for resistance to low potassium, we determined that the p38 pathway mediates resistance to low potassium by regulating the transcription factor ATF-7. Additionally, pharmacological studies suggest that A-type K+ channels are critical for survival under low potassium conditions. The essential function of sek-1 in resistance to low potassium can be rescued by its expression specifically in the ASI chemosensory neurons and not the intestine, revealing that this function is distinct from the roles of sek-1 and p38 signaling in dietary restriction and pathogen resistance. Further genetic analyses in C. elegans may be invaluable for unraveling neuronal tissue-nonautonomous signaling mechanisms that maintain potassium homeostasis.

1129C Effects of Region-Specific Irradiation on Locomotion and Autophagy in C. elegans. Akira Yamasaki1,2, Michiyo Suzuki2, Tomoo Funayama2, Yashuiko Kobayashi2, Qiu-Mei Zhang-Akiyama1 1) Department of Zoology, Graduate School of Science, Kyoto university, Kyoto, Kyoto, JP; 2) Department of Radiation-Applied Biology Research, National Institutes for Quantum and Radiological Science and Technology (QST), Gunma, JP.

Irradiation generates reactive oxygen species, and causes damage to cell components including DNA and protein. In C. elegans, radiation sensitivity is different from germline-cell and somatic cell. When adult C. elegans are irradiated with ten-fold higher dose than the dose that leads to germline-cell death, worms are still alive1. Locomotion using motor neurons and body-wall muscles was reduced immediately after irradiation with 0.5 K Gy2. However the mechanism of reduction is not fully understood. In the present study, to investigate a tissue that is responsible for reduction of locomotion, we used region-specific microbeam irradiation. We used energetic carbon ions delivered from the AVF cyclotron at Takasaki Ion accelerators for Advanced Radiation Application facility of QST-Takasaki. After irradiation, a worm was replaced on NGM plate, and the locomotion was video-recorded and then the trajectory for 5-sec duration was derived by image processing of the movie. As a result, the same effects as whole-body irradiation were not observed after region-specific microbeam irradiation to pharynx or tail. This suggests that the radiation effects on locomotion depend on the size of irradiation area. To detect the dose whether the reduction of locomotion is restored or leads to individual death, and we investigated alternation of locomotion after irradiation. We found the dose that locomotion of worms were completely stopped immediately after irradiation, and had been reduced at least twenty-four hours. Less than the dose, locomotion of the worms was reduced in a dose dependent manner, and was partially restored after twenty-four hours. Protein damage generated by irradiation may be involved in this reduction and restoration of locomotion, so we investigated whether autophagy is induced after irradiation. Using GFP reporter assay of lgg-1, one of the autophagosome genes, the increased level of GFP::LGG-1 was detected seven hours after irradiation in somatic cells. This suggests that autophagy may be one of the reasons of restoration of locomotion after irradiation. 1. Ishii,N., Suzuki, K., Int. J. Radiat. Biol., 58:827-833. (1990) 2. Suzuki, M., et al., J. Radiat. Res. 50, 119–125. (2009)

1130A Epistatic regulation of DAF-16 via p38 MAPK signaling in molecular compensation of sod-genes and lifespan of C. elegans. S. Yanase1,2, N. Ishii2 1) School of Sports & Health Science, Daito Bunka University, Higashi-matsuyama, Saitama, JP; 2) Tokai University School of Medicine, Bohseidai, Isehara, Kanagawa, JP.

The nematode C. elegans SODs, which catalytically remove the intracellular O2- during normal aging, are encoded by sod-1 to sod-5 genes. Here we estimated whether expression levels of sod-1 gene increase in a deletion mutant of sod-5 gene encoding another Cu/Zn SOD isozyme. In spite of the lower expression levels of sod-5 gene in wild-type, sod-1 to sod-4 genes were induced several folds in the sod-5 deletion mutant. Interestingly, these inductions in other sod-genes were reduced in sod-5 mutant absent in also sod-1 gene. The molecular compensations were ultimately sufficient to recover each lifespan of these mutants, at least in part. It is known that expression of sod-3 and sod-5 genes is regulated via an ins/IGF-1 signaling pathway; expression of sod-1, sod-2 and sod-4 genes is regulated via a p38 MAPK signaling cascade, which related to longevity and oxidative stress resistance in C. elegans, respectively. Under the genetic induction of DAF-16 transcription factor that is
enzymes, such as DNA glycosylases and AP endonucleases. The molecular mechanism of BER has been elucidated so well,

Base excision repair (BER) is a repair pathway for relatively small damaged bases in DNA, and consists of various kinds of

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Aging and age-related diseases are associated with aberrant mitochondrial dynamics, however whether loss of mitochondrial

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Aging and age-related diseases are associated with aberrant mitochondrial dynamics, however whether loss of mitochondrial network homeostasis is causal to aging is not known. Here, we demonstrate that remodeling mitochondrial networks can promote longevity in C. elegans. We show that both activating AMP-activated protein kinase (AMPK), a master regulator of energy homeostasis, and dietary restriction (DR) increase lifespan by maintaining youthful mitochondrial networks. Inhibiting fusion via deletion of the mitofusin ortholog fzo-1 blocks AMPK- and DR-mediated longevity, and we find that fusion is specifically required in peripheral tissues. Promoting fusion directly does not increase lifespan, and inhibiting fusion also blocks AMPK and DR-mediated longevity. Strikingly however, co-inhibition of fusion and fission preserves mitochondrial network homeostasis during aging, and is sufficient itself to increase lifespan via metabolic reprogramming and coordination with peroxisomes. Together these data show that mechanisms that promote mitochondrial balance can be targeted to promote healthy aging.

Dietary restriction and AMPK increase lifespan via maintaining balanced mitochondrial dynamics in C. elegans. Heather Weir1, Kristopher Burkewitz1, Pallas Yao1, Frank K. Huynh2, Raymond Laboy1,3, Matthew D. Hirschey2, William B. Mair1 1) Department of Genetics and Complex Diseases, Harvard T. H. Chan School of Public Health, Boston, Massachusetts 02115, USA; 2) Duke Molecular Physiology Institute, Duke University Medical Center, 300 North Duke Street, Durham, NC 27701, USA; 3) Max Planck Institute for Biology of Ageing, Joseph Stelzmann Strasse 9b, 50931 Cologne, Germany.

Aging and age-related diseases are associated with aberrant mitochondrial dynamics, however whether loss of mitochondrial network homeostasis is causal to aging is not known. Here, we demonstrate that remodeling mitochondrial networks can promote longevity in C. elegans. We show that both activating AMP-activated protein kinase (AMPK), a master regulator of energy homeostasis, and dietary restriction (DR) increase lifespan by maintaining youthful mitochondrial networks. Inhibiting fusion via deletion of the mitofusin ortholog fzo-1 blocks AMPK- and DR-mediated longevity, and we find that fusion is specifically required in peripheral tissues. Promoting fusion directly does not increase lifespan, and inhibiting fusion also blocks AMPK and DR-mediated longevity. Strikingly however, co-inhibition of fusion and fission preserves mitochondrial network homeostasis during aging, and is sufficient itself to increase lifespan via metabolic reprogramming and coordination with peroxisomes. Together these data show that mechanisms that promote mitochondrial balance can be targeted to promote healthy aging.

Identification of regulators of the age-dependent activity of DAF-16. Kali Carrasco, Julia Morris, Matthew Youngman Department of Biology, Villanova University, Villanova, PA.

The insulin/IGF-1(IIS) DAF-2 signaling pathway confers stress-resistance and longevity in C. elegans by regulating the DAF-16/FOXO transcription factor, which in turn modulates the expression of immunity, detoxification, and repair genes among others. Inside the nucleus the DAF-16 complex cooperates with other proteins that act as co-regulators to specify its transcriptional output. Several of these proteins were identified in studies searching for suppressors of the extended lifespan and enhanced stress resistance of daf-2 mutants. We find that in wildtype animals DAF-16 activity increases not only in instances of stress but also in an age-dependent manner. This has allowed us to revisit the question of DAF-16 regulation in a more physiologically relevant context where its levels are not artificially elevated nor is its activity enhanced by a background mutation. Using a reverse genetic approach, we have identified thirteen putative regulators that influence the function of DAF-16 in adult but not larval stage worms. We tested candidate genes via RNAi knockdown screens using a chromosomally integrated in vivo GFP reporter for DAF-16 activity. The proteins encoded by these genes fall primarily into two functional categories: chromatin modifiers and phosphatase regulators. From the first category, six genes were implicated including swsn-4 and bar-1. From the second category, seven genes were implicated including ppfr-1 and ppfr-2. We expect that further characterization of the hits from our screen will reveal that loss-of-function of these candidate genes will phenocopy daf-16 in functional assays carried out during aging, confirming their role as positive regulators of DAF-16. While our studies reveal some overlap between regulatory modules governing the function of DAF-16 when it is hyperactivated and in adult wildtype animals, they also raise the intriguing possibility of distinct mechanisms of DAF-16 regulation that are exclusive to aging.

Phosphorylation by MPK-1/ERK regulates the SKN-1/Nrf1 proteasomal stress response. Peng Zhang1, Huimin Na1, John Hourihan1, Fang Zhang1, Meltem Isik1, Albertha J.M. Walhout1, T. Keith Blackwell1 1) Joslin diabetes center, Harvard Medical School, Boston, MA; 2) University of Massachusetts Medical School, Worcester, MA.

Protein degradation by the ubiquitin-proteasome system is vital to cellular homeostasis and survival, and mechanisms that promote healthy aging. The proteasome itself is also a critical therapeutic target in cancers that rely on the endoplasmic reticulum (ER) secretory system. An isoform of SKN-1 (SKN-1a; Nrf1 in mammals) regulates essentially all proteasome subunit genes, and mediates a pathway that maintains proteasome levels. Under conditions of proteasome inhibition, this recovery pathway generates additional proteasomes. In this little-understood pathway, SKN-1a/Nrf1 is processed through a complex mechanism that involves localization to and extrusion from the ER, and cleavage. Here, we performed a genome-scale RNAi screen in C. elegans to identify genes required to detect proteasome dysfunction, and activate SKN-1a/Nrf1. We show that a critical regulator in the proteasome recovery pathway is the kinase MPK-1/ERK, which responds to growth signals. We find that this pathway is regulated distinctly from the PMK-1/p38-dependent oxidative stress response that is mediated by a different SKN-1 isoform. MPK-1/ERK1 is activated by proteasomal stress, and inhibition of this kinase blocks the proteasome recovery pathway, and sensitizes C. elegans and human tumor cells to proteasome inhibition. In human tumor cells, phosphorylation by ERK on a single residue is required for Nrf1/SKN-1 to localize to nuclei and activate proteasome genes. Our data indicate that SKN-1a/ERK is directly regulated by MPK-1/ERK in the proteasomal recovery/synthesis pathway, suggesting that it is responsive to growth factor signaling. They also identify ERK/MPK-1 and possibly other hits from our screen as potential therapeutic targets for blocking compensatory proteasome synthesis in cancers that depend upon the proteasome.

C. elegans EXO-3 plays an important role for growth development and morphogenesis. Qiu-Mei Zhang-Akiyama, Masahiro Miyaji, Yuichiro Hayashi, Akihiro Tanaka, Yuichi Kato Department of Zoology, Graduate School of Science, Kyoto University, Kyoto, Kyoto, JP.

Base excision repair (BER) is a repair pathway for relatively small damaged bases in DNA, and consists of various kinds of enzymes, such as DNA glycosylases and AP endonucleases. The molecular mechanism of BER has been elucidated so well,
but its physiological roles in multicellular organisms are not fully understood. In this study, we investigated the relationship between DNA glycosylases and AP endonucleases in animal development and morphogenesis, using Caenorhabditis elegans (C. elegans). In C. elegans, two DNA glycosylases, nth-1 and ung-1, which remove oxidative pyrimidines and uracil respectively, and two AP endonucleases, apn-1 and exo-3, are conserved. We found that the exo-3 mutant showed developmental delay, and increased number of protruding vulva (pvl) under dut-1 RNAi, while the apn-1 mutant did not show either of them. The developmental delay was dependent on not ung-1 but nth-1 under normal conditions. However, when worms were treated with sodium bisulfite (NaHSO₃), which causes excessive uracil in DNA, the phenotype depended on ung-1. On the other hand, the pvl relied on ung-1, and was enhanced under additional oxidative stress conditions, such as ndx-1 RNAi, ndx-2 RNAi, or methyl viologen treatment. The enhancement of the pvl did not depend on nth-1. Moreover, both of these two phenotypes in the exo-3 mutant, developmental delay and increased number of pvl, were rescued by the lack of chk-2. These results suggest that deficient repair of AP sites has an adverse effect on development and morphogenesis through removing base damages such as oxidative base damages and uracils from genome and subsequently checkpoint activation.

1136C  DAF-16/FOXO interacts with TGF-β/BMP signaling to promote germline tumor formation in C. elegans. Qian Zhao¹, Wenjing Qi¹, Yijian Yan¹, Ralf Baumeister² 1) Bioinformatics and Molecular Genetics (Faculty of Biology), Albert-Ludwigs-University of Freiburg; 2) ZBMZ (Faculty of Medicine), Albert-Ludwigs-University of Freiburg.

Activation of the FOXO transcription factor DAF-16 by reduced insulin/IGF signaling (IIS) is considered to be beneficial in C. elegans due to its ability to extend lifespan and to enhance stress resistance. In the germline, cell-autonomous DAF-16 activity prevents stem cell proliferation, thus acting tumour-suppressive. In contrast, hypodermal DAF-16 causes a tumorous germline phenotype characterized by hyperproliferation of the germline stem cells and rupture of the adjacent basement membrane. Here we show that cross-talk between DAF-16 and the transforming growth factor β (TGFβ)/bone morphogenetic protein (BMP) signaling pathway causes germline hyperplasia and results in disruption of the basement membrane. DAF-16 may directly interact with both R-SMAD proteins SMA-2 and SMA-3 in the nucleus to regulate the expression of mTORC1 pathway and MADM/NRBP/ho-11 genes. Knockdown of BMP genes or each of the four target genes in the hypodermis was sufficient to inhibit germline proliferation, indicating a cell-non-autonomously controlled regulation of stem cell proliferation by somatic tissues. We propose the existence of two antagonistic DAF-16/FOXO functions, a cell- proliferative somatic and an anti proliferative germline activity. Whereas germline hyperplasia under reduced IIS is inhibited by DAF-16 cell-autonomously, erroneous activation of somatic DAF-16 in the presence of active IIS promotes germline formation and overrides repressive DAF-16 activity in the germline to induce tumor-like growth. Such cell-type specific differences may help explaining why human FOXO activity is considered to be tumor-suppressive in most contexts, but may become oncogenic, e.g. in chronic and acute myeloid leukemia. We will present our recent advances in our mechanistic understanding of this non-cellautonomous signaling mechanism.

1136A  PAR-1 plays an important role in C. elegans aging. L. Zou, J. Lan, D. Wu, D. Chen Nanjing University, Model Animal Research Center, Nanjing, Jiangsu, CN.

According to the evolutionary theory of aging, dysregulation of developmental processes during adulthood results in age-related functional decline and death. Previous studies have demonstrated that inhibition of certain developmentally essential genes during adulthood led to significant life span extension in C. elegans. PAR-1, a highly conserved serine-threonine kinase, functions as a key cellular polarity regulator during embryonic as well as larval development. However, it has not been clear whether and how PAR-1 affects aging. Here we show that inhibition of par-1 only during adulthood is sufficient to extend life span in a tissue-specific manner. Moreover, inhibition of par-1 improves health span, as indicated by increased stress resistance, decreased muscular function decline over time, and enhanced proteotoxicity resistance in human degenerative disease models. Genetic epistatic and molecular analyses revealed the effect of par-1 deficiency on life span requires the AMP-activated protein kinase (AMPK), but is independent of the DAF-16/FOXO transcription factor. Together, our studies revealed a novel function of PAR-1 during adulthood, which may help to understand the intrinsic link between development and aging.

Physiology - Dauer Larvae

1137B  Spatial analysis of transcriptional regulation of dauer tissue remodeling. U. Aghayeva¹, O. Hobert¹² 1) Department of Biological Sciences, Columbia University, New York, NY; 2) HHMI.

Transcription factors (TFs) that perform their functions on a systemic level are often broadly or ubiquitously expressed. Dauer formation in C. elegans is a developmental program that involves structural and physiological changes in all tissue types, and previous studies suggest that it is controlled in a systemic manner. TFs that are essential for execution of the dauer program - DAF-16/FOXO, DAF-12/NHR and DAF-3/Co-Smad - are indeed ubiquitously expressed, as evidenced by previously reported reagents (Antebi et al., 2000; Lin et al., 2001; Patterson et al., 1997) as well as fluorescent protein-tagged CRISPR alleles created in this work. However, it is still unclear how the dauer TFs act in different tissues to enforce the dauer program throughout the animal. Is there a signaling center from which molecular commands descend to instruct the rest of the tissues to remodel or are dauer TFs act strictly cell-autonomously? Previous studies (e.g., Libina et al., 2003; Huang et al., 2014) have not come to unanimous conclusions, and the role of the nervous system and the intestine as the signaling center has been debated. In order to address this question, we have generated conditional CRISPR alleles for all three dauer TFs based on the AID (auxin-inducible degron) system (Zhang et al., 2015), and crossed them into strains carrying single-copy insertions of TIR1 expressed under different tissue-specific promoters. All strains are in either daf-2(e1370) or daf-7(e1372) background, and penetrance and expressivity of dauer tissue remodeling is assessed at 25°C. Initial experiments show that whole-worm
conditional depletion of DAF-16 is as efficient as the daf-16 null mutation in suppressing daf-2(e1370). Pharyngeal and intestinal DAF-16 depletion result in partial suppression and formation of dauer-like larvae that have non-constricted pharynx that continues to pump, unlike daf-2(e1370) dauers. In ongoing experiments, the consequences of conditional dauer TF protein depletion in distinct tissues types is analyzed by assessing morphology and marker expression in the resulting dauers or dauer-like larvae.

1138C Characterization of a dauer constitutive mutant with dauer-specific molting defect in Pristionchus pacificus. H. R. Carstensen, R. M. Villalon, R. L. Hong Department of Biology, California State University, Northridge, Northridge, CA.

In contrast to the unsheathed dauer larvae (DL) in Caenorhabditis species, the DL of parasitic nematodes often retain their old J2 cuticles, which may prevent desiccation outside of the host. Ensheathed DL are also found in Pristionchus pacificus, a necromenic nematode associated with beetles. To gain a better understanding of the mechanism coordinating dauer entry and the dauer-specific molt, as well as the evolutionary developmental changes between free-living and parasitic nematodes, we have characterized the dauer-constitutive mutant tu391. We found that tu391 is a temperature sensitive daf-c allele that exhibits a molting defect only in the J2-dauer larvae molt. The small fraction of tu391 mutants that are stuck within loose cuticles exhibit significantly slower pharyngeal pumping than J3s of either strain, suggesting that these immobilized animals are dauer larvae. Detailed examination of the molting-defective animals shows the old J2 cuticle is too loose to allow locomotion on OP50, in contrast to the J2 cuticle of active wild-type or daf-c DL that is attached tightly to the head and tail. Furthermore, amphid neurons in tu391 mutants are Dil-filling defective, suggesting that defects in amphid neuron development contribute to its daf-c phenotype. To determine if tu391 also affects the glial cells that support the ontology and function of the amphid neurons, we are now examining the expression of the amphid sheath marker, Ppa-daf-6p::xfp.

A possible defect in the amphid organ may also affect sensing of host odors. The beetle sex pheromone (z)-7-tetradecen-2-one, function of the amphid neurons, we are now examining the expression of the amphid sheath marker, Ppa-daf-6p::xfp.

1139A The neuronal forkhead transcription factor, FKH-7, promotes dauer formation. Cynthia M. Chai, Paul W. Sternberg HHMI and Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA.

FKH-7 is one of 15 members of the C. elegans forkhead family of transcription factors. The orthologous FoxP in D. melanogaster and FOXP2 in mammals have been heavily implicated in synaptic plasticity and learning. Although this hints at an evolutionarily conserved role in neural circuit development and function, the precise function of FKH-7 in C. elegans remains to be elucidated. Previous transcriptomic studies have shown that fkh-7 expression is significantly upregulated during L1 arrest and the dauer stages, indicating that FKH-7 is an important mediator of starvation-induced states. To investigate the role of FKH-7 in C. elegans dauer diapause, we first generated fkh-7 knockout mutants by deleting regions of the DNA-binding domain sequence using CRISPR/Cas9 technology. We found that fkh-7 mutants exhibit a dauer-defective phenotype when assayed for dauer formation. Since several previously characterized dauer-defective mutants are also chemosensory defective, we wanted to determine if the fkh-7 mutant dauer-defective phenotype is directly due to deficiencies in the chemosensory apparatus. To this end, we conducted a screen for canonical chemosensory-related behaviors including locomotion, egg laying, ascarsis avoidance, olfactory chemotaxis, and sensory integration. We found that fkh-7 mutants perform comparably to wild type worms in all behavioral assays tested indicating that FKH-7 likely promotes dauer formation independently of a role in chemosensation. We then utilized a destabilized YFP transcriproal reporter strain to determine the expression pattern of fkh-7 during different life stages. We consistently observed fkh-7 expression in four neuron types in the head and three tail neurons throughout the larval stages. By combining Dil amphid staining with neuronal morphology examination, we identified the head neurons as the RID, RIA, URB, and AWB neurons. Interestingly, fkh-7 expression is restricted to the RID, RIA, and URB neurons from the young adult stage onwards. These results suggest that fkh-7 is expressed in a small subset of neurons that mediate the dauer entry decision and/or whole animal physiological changes in response to adverse environmental inputs from chemosensory neurons. We are currently building cGAL reagents to further dissect the functions of these neurons in the neural circuitry underlying dauer formation. We are also applying RNA-seq technology to identify the downstream genes and molecular pathways regulated by FKH-7 during the dauer stages.

1140B The role of DEX-1 in dauer formation and cuticle morphology. K. Flatt1, C. Beshers2, C. Unal3, N. Schroeder4 1) Neuroscience Program, University of Illinois at Urbana - Champaign, Urbana, IL; 2) Neuroscience Department, Oberlin College, Oberlin, OH.

The C. elegans dauer larvae undergo extensive tissue remodeling events that allow them to survive during times of unfavorable environmental conditions. These remodeling events include neuronal arborization and changes to the cuticle. To better understand these remodeling events we are currently investigating the role of the extracellular environment in dauer formation and morphogenesis. It has previously been shown that a set of sensory head neurons, the inner labial 2 (IL2) neurons, arborize in a dauer specific manner. To further investigate this phenotype, we looked at the extracellular matrix (ECM) protein,
DEX-1, that is required during embryogenesis for proper dendrite morphology and functions via interactions with zona pellucida (ZP) domain-containing ECM proteins. We observed that dex-1 mutant dauers exhibit wild-type IL2 arborization, but are sensitive to SDS treatments and have defects in radial constriction and lateral alae formation. A 5kb dex-1p::gfp promoter fusion is expressed in the seam cells, a set of 16 stem cell-like hypodermal cells, exclusively during dauer. Interestingly, a truncated version of this dex-1 promoter is expressed in the seam cells during all larval stages. During dauer, the seam cells undergo autophagy-mediated shrinkage that facilitates radial shrinkage and alae formation. We also observed that dex-1 mutant dauers are less responsive to mechanical stimulation compared with wild-type dauers. Closer examination of the dex-1 expression pattern revealed dauer-specific expression in the glial cells of the mechanosensory deirid neurons. Reporter analysis with an ajm-1::gfp and preliminary electron microscopy data indicate that dex-1 dauers have defects in seam cell shrinkage. The seam cell mediated radial shrinkage and subsequent alae formation are facilitated by a family ZP domain-containing ECM proteins called cuticulins (CUT). Disruption of CUT-1, CUT-5 and CUT-6 causes similar dauer morphology phenotypes as dex-1 mutants, and their ZP domains make them good candidates for DEX-1 interactions. Indeed, our preliminary data suggests possible genetic interactions between dex-1, cut-1 and cut-5. Our results indicate that DEX-1 plays an important role in ECM remodeling during dauer formation.

1141C One of neuropeptide receptors, NPR-17 modulates dauer arrest of C. elegans. Atsumi Harada1, Kenjirou Matsushita2, Yuka Kunimatsu2, Takashi Iwasaki1,2,3, Tsuyoshi Kawano1,2,3 1) Department of Bioresources Science, Graduate School of Agriculture, Tottori University, Tottori, Japan; 2) Department of Bioresource Science, Faculty of Agriculture, Tottori University, Tottori, Japan; 3) Department of Bioresources Science, The United Graduate School of Agricultural Sciences, Tottori University, Tottori, Japan.

Some of small peptides function as primary neurotransmitters through their receptors in diverse species of animals. C. elegans also has many of small neuropeptides and their receptors predicted by the genome sequence. Our screen for neuropeptide receptors (NPRs) relevant to dauer arrest revealed that disruption of npr-17 drastically reduced dauer arrest induced by dauer pheromones. NPR-17 is a GPCR and expressed in the ASI neurons and intestine. It has been reported that NPR-17 is a receptor of the neuropeptides, NLP-3 and NLP-24, which regulate withdrawal from noxious chemical stimuli (1). Interestingly, disruption of nlp-3 reduced dauer arrest, similar to that of npr-17, whereas that of nlp-24 did not. To elucidate molecular mechanism of NPR-17 on modulation of dauer arrest, we first carried out epistasis analysis on TGF-like and insulin-like signaling pathways. daf-7 and daf-2 are epistatic to npr-17. Next, we investigated relevance of NPR-17 to secretion of a TGF-β, DAF-7, and an insulin-like peptide, DAF-28, which are produced in the ASI neurons and regulate larval growth. In a npr-17(-) background, accumulation of DAF-7::mCherry in coelomocytes increased, while that of DAF-28::mCherry did not. In addition, we also investigated secretion of an insulin-like peptide, INS-35, which is mainly produced in intestine (2). In a npr-17(-) background, accumulation of INS-35::VENUS in coelomocytes scarcely increased. Together, these results suggest that NPR-17 promotes dauer arrest by suppressing secretion of DAF-7, not of insulin-like peptides, DAF-28 and INS-35.


1142A Functional investigation of transcription factors involved in early response to dafachronic acid. J. Lee1,2, P.Yin Shih1,2, O. Schaedel1,2, P. Quintero-Cadena1,2, P. Sternberg1,2 1) Biology and Biological Engineering, California Institute of Technology, Pasadena, CA; 2) Howard Hughes Medical Institute.

Under favorable conditions, C. elegans commits to reproductive development through positive amplification of dafachronic acid (DA) growth hormone throughout the body. DA affects a whole-animal developmental decision by acting on the vitamin D receptor homolog DAF-12 to promote entry into reproductive development, while inhibiting entry into the dauer dispersal stage. We sought to understand the mechanism of this decision by identifying the transcription factors that are differentially expressed during early response to DA. Under parallel growth conditions, we used synthetic DA to control the entry of animals into dauer or reproductive development. We performed whole-animal RNA-seq on pre-dauer L2d larvae that were not exposed to DA, and L2d that were exposed to a short pulse of DA. We identified up-regulation of the transcription factor genes bed-3, cky-1, dmd-6, efl-3, gei-13, nhr-41, nhr-71, and nhr-74 during early DA response. This observation is in line with the known developmental role of bed-3 in vulval organogenesis, nhr-41 in dauer SDS resistance, and nhr-71 in germline-dependent longevity. We investigated these genes using a pheromone-induced dauer entry assay, and identified increased dauer entry in bed-3(sy702), and decreased entry in dmd-6(gk287) and nhr-74(ok2751). These results suggest that bed-3 promotes reproductive growth, while dmd-6 and nhr-74 promote dauer development. The opposed effects of these transcription factors suggest a mechanism for fine-tuning the developmental decision in response to sensed conditions. We are investigating this hypothesis by testing the transcription factors in high temperature-induced dauer formation mutant backgrounds for changes in the dauer entry threshold. We will also use RNA-seq to investigate transcription factor mutants in daf-12(+ and daf-12(−)) backgrounds to test for genetic interactions with respect to target gene control.

1144C Peptidergic signaling is necessary for CO2 preference in dauer, and promotes dauer entry and coordinated nictation. P.Yin Shih1,2, J. Lee1,2, O. Schaedel1,2, P. Quintero-Cadena1,2, A. Rogers1, P. Sternberg1,2 1) Biology and Biological Engineering, California Institute of Technology, Pasadena, CA; 2) Howard Hughes Medical Institute.

Animals, including humans, can adapt to environmental stress through phenotypic plasticity. The free-living nematode Caeorhabditis elegans can respond to harsh environments by entering the stress-resistant dauer dispersal stage and demonstrating adaptive, dauer-specific behaviors. To better understand the dauer developmental process, we compared dauer and reproductive development at fine time points using whole-animal RNA-seq on pre-dauer L2d larvae, dauer-committed L2d,
L3-committing L2d, dauers, and L4 larvae. We detected differential gene expression from epithelial, muscular, intestinal, reproductive, and nervous tissues, as well as from single neurons, which we verified using fluorescent transcriptional reporters. In total, we detected 8,042 differentially expressed genes during dauer and reproductive development, and we observed strong enrichment of the neuropeptides in the genes up-regulated during dauer entry. This enrichment corresponded to the up-regulation of 51% of the neuropeptide genes in C. elegans, including 90% of the large family of FMRFamide-like (flp) neuropeptides. We used mutants null for the 7B2 homolog sbt-1 to knock down neuropeptide processing, and demonstrated that peptidergic signaling is necessary for CO2 preference in dauers, promotes the dauer entry decision, and promotes nictation coordination. We assayed 23 flp mutant alleles and observed dauer entry defects for 12 alleles. We also observed CO2 chemotaxis and nictation defects in flp-10 flp-17 double mutants, similar to sbt-1 nulls. Through a meta-analysis, we discovered flp up-regulation in the dauer-like infective juveniles of diverse parasitic nematodes as well, suggesting an evolutionarily shared strategy of phenotypic plasticity. We have therefore studied phenotypic plasticity in a whole organism using transcriptomics, functional genetics, behavioral studies, and comparative species analysis. Our findings reveal that C. elegans adapts to stress by using neuropeptides to enhance their decision-making and to expand their behavioral repertoire.

Physiology - Metabolism


Ascarosides are a family of small molecule signals that regulate a variety of important behaviors in C. elegans and other nematodes including mate attraction, aggregation, repulsion, and entry into dauer diapause under stressful conditions. Structurally, ascarosides are modular glycosides of the dideoxysugar ascarylose. Small structural changes in the fatty acid moiety attached to the first position of the sugar as well as the addition of other metabolically-derived components to the fourth position have been shown to elicit different behaviors. DAF-22, a thiolase responsible for the last step of peroxisomal β-oxidation of the ascaroside lipid side chain, is necessary for the production of biologically active ascarosides and is expressed in three different tissues in the worm: the intestine, the body wall muscle, and the hypodermis. We have transgenically rescued the expression of DAF-22 in each tissue individually within a DAF-22 knockout background. Although the intestine was initially hypothesized as the site of ascaroside biosynthesis, we have found that ascarosides are produced in all three tissues and rescue ascaroside-regulated behaviors.

1147C Pre- and probiotics modulate C. elegans fat storage and longevity. S. Akbari Alavijeh1,4, S. Soleimanian-Zad4, M. Sheikh-zeinoddin4, R. Shaddel3, Y. Wang1, R. Gaugler3, S. Hashmi1,2 1) Laboratory of Developmental Biology, Center for Vector Biology, Rutgers University, 180, Jones Avenue, New Brunswick, NJ 08901, USA; 2) Rutgers Center for Lipid Research, New Jersey Institute for Food, Nutrition, & Health, Rutgers University, New Brunswick NJ 08901, USA; 3) Department of Food Science, Rutgers University, 65 Dudley Road, New Brunswick, NJ 08901, USA; 4) Laboratory of Food Microbiology, Department of Food Science and Technology, Faculty of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran.

Functional foods are select foods that provide specific health benefits beyond the basic nutrition requirement. Pre- and probiotics are two known main functional food additives. Despite wide-spread dietary consumption of both pre- and probiotics, the biological effects of these food additives are still poorly understood. We studied the effects of Lactobacillus plantarum PTCC 1896 and Lactobacillus rhamnosus GG as probiotics and pistachio hull polysaccharides (PHP) and inulin as prebiotics on Caenorhabditis elegans longevity and fat storage. We also question what happens to the fecundity, growth and the rate of pharyngeal pumping of worms when they feed on media containing combinations of OP50, pre and probiotics. Further, to determine the role of pre- and probiotics in fat storage, we separately fed klf mutants (homozygous klf-2 (ok1043) V and klf-3 (ok1975) II) on pre- and probiotics and then measured the fat buildup by using Oil Red O staining. Both klf-2/3 mutants buildup extensive fats in their intestine. We found a dosage-related effect of pre- and probiotics on worm’s longevity; pre- and probiotics increased worm longevity by 20% over the worm fed on OP50. Pre- and probiotics positively modulated longevity independent of feeding behavior, reduced growth and reduced fat buildup in both mutants. We have identified an interplay between pre- and probiotics, klf-2/3 genes in fat storage. We are investigating the connections between functional food additives, life span, and obesity. We are using daf-2 (DR1574), daf-16 (GR1309), and daf-2/daf-16 (GR1307) double mutants in combination with other metabolic gene mutants to identify this connection. Mutation in daf-2 causes an extension in lifespan and increased fat storage and the FoxO/daf-16 is a negative regulator of insulin signaling in C. elegans. The similarity of the fat phenotype between the klf-2/3 and daf-2 mutant and the effects of klf-3 on the gene expression of daf-2 or daf-16 provide a basis for this study. Such an interesting relationship between life span and oral consumption of pre- and probiotics in worm opens up a new window to look into investigation of the potent use of these functional additives for delay aging and age-related disorders.

1148A daf-22 dark matter. A. Artyukhin, Y. Zhang, O. Panda, F. Schroeder Boyce Thompson Institute, Cornell University, Ithaca, NY.

Ascarosides, modular secondary metabolites common to many nematode species, are implicated in many aspects of worm behavior and development as well as in interaction of worms with other organisms. Over 150 ascarosides have been described in C elegans, but only a quarter of them are routinely detected in wildtype cultures, while others are long chain shunt metabolites that accumulate in specific mutants. DAF-22, thiolase that catalyzes the final step in peroxisomal β-oxidation, is required for biosynthesis of short chain ascarosides. Hence, daf-22 has been traditionally used as a control in various bioassays where ascaroside activity may be suspected. We set out to map the effect of daf-22 deletion on the entire worm exo-metabolome in an
oxidase and nitric oxide synthase activities were significantly increased during Cbl deficiency. Therefore, H2O2 and nitric oxide disease mechanisms are poorly understood. Our recent study indicates that atherosclerosis and neurodegenerative diseases. Such diseases are major symptoms of Cbl deficiency, although the underlying oxidative stress.

Cobalamin (Cbl) is the largest and most complex of all vitamins. After Cbl is taken up by living cells, it is converted into two metabolites – cobalamin and hydroxocobalamin. Oxidative stress is implicated in several human diseases, including infertility, prolonged life cycle (growth retardation), and reduced lifespan. These phenotypes resemble those of Cbl-deficient worms. In this exploratory study, we selected a dozen GT mutant strains, the majority of which belong to the GT-A fold protein (families 2, 7, 21, 27, and 13). 6 replicates of each strain (L1 stage) were cultured to about a population of 100-200 thousand for NMR metabolomics measurements, and animals were randomly selected from each sample for the measurement of population distribution using the large particle flow cytometer COPAS Biosorter. The Biosorter measures the extinction and time of flight of metabolites? We found that about 10% of detectable C. elegans metabolites disappear in daf-22. After excluding known ascarosides from the analysis, we identified about 80 of the remaining daf-22-dependent molecules as new ascarosides, which range from small modifications of known structures to new large modular assemblies over 900 Da. Surprisingly, we also discovered many more non-ascaroside compounds that were absent in daf-22 metabolome, which suggests that ascarosides are not the only compound class affected by peroxisomal β-oxidation. Its disruption has profound direct and indirect effects on numerous metabolic pathways and leads to rewiring of a large portion of the C. elegans metabolome. We repeated the targeted metabolomic comparison of wildtype and daf-22 in another worm species, P. pacificus and again, in addition to numerous new ascarosides, found a large number of unexpected structures. Overall, we demonstrate that disruption of a single gene can cause global metabolic perturbations and by using an untargeted metabolomics pipeline we can harness this information to extend our knowledge of the worm chemical language.

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C. elegans controls much of its behavior and development through the use of ascarosides, which are also present in many free-living and parasitic nematodes (Choe et al 2012). Some of the phenotypes mediated by ascarosides include aggregation, olfactory plasticity, dauer formation, attraction behavior, and hermaphrodite behavior. (Srinivasan et al 2008; Edison, 2009; Ludewig & Schroeder, 2013) Chemoatomically, ascarosides are glycosides of the dideoxy sugar ascarylose, attached to a fatty acid side chain, thus implicating GTs in their biosynthesis. In addition, the innate immune system in C. elegans utilizes a wide range of immune effectors and enzymes (including GTs) for microbial defenses and xenobiotics detoxification (Lindblom & Dodd, 2006; Stupp et al 2012). Stupp et al 2012 showed that C. elegans can detoxify two bacterial toxins, 1-hydroxyphenazine (1-HP), and indole via N- and O-glycosylation. Our research aims to discover the roles of specific GTs in biological processes like these.

In this exploratory study, we selected a dozen GT mutant strains, the majority of which belong to the GT-A fold protein (families 2, 7, 21, 27, and 13). 6 replicates of each strain (L1 stage) were cultured to about a population of 100-200 thousand for NMR metabolomics measurements, and animals were randomly selected from each sample for the measurement of population distribution using the large particle flow cytometer COPAS Biosorter. The Biosorter measures the extinction and time of flight of individual nematode which is used as a descriptor of developmental stages. Analysis of metabolic changes of some of the GT mutants and the association with the population distribution will be reported.

Cobalamin (Cbl) is the largest and most complex of all vitamins. After Cbl is taken up by living cells, it is converted into two coenzyme forms, 5'-deoxyadenosylcobalamin and methylcobalamin, which function as coenzymes for methylmalonyl-CoA mutase (EC 5.4.99.2) and methionine synthase (MS; EC 2.1.1.13), respectively. In particular, the failure of Cbl-dependent methionine biosynthesis leads to accumulation of homocysteine (Hcy), which has pro-oxidant activity and can activate NADPH oxidase to generate reactive oxygen species. Oxidative stress is implicated in several human diseases, including atherosclerosis and neurodegenerative diseases. Such diseases are major symptoms of Cbl deficiency, although the underlying disease mechanisms are poorly understood. Our recent study indicates that Caenorhabditis elegans grown under conditions of Cbl deficiency develops severe Cbl deficiency associated with various phenotypes that include decreased egg-laying capacity (infertility), prolonged life cycle (growth retardation), and reduced lifespan. These phenotypes resemble those of Cbl-deficient mammals. In this study, we report the effects of Cbl deficiency on various oxidative stress and damage markers in C. elegans to elucidate whether these phenotypes caused by Cbl deficiency are the result of the impaired MS reaction and are associated with oxidative stress.

Cbl-deficient C. elegans unusually accumulated homocysteine because of a significant decrease in MS activity. NADPH oxidase and nitric oxide synthase activities were significantly increased during Cbl deficiency. Therefore, H2O2 and nitric oxide were increased in Cbl-deficient worms. Lipid peroxide and protein carbonyl contents, indices of cellular oxidative damage, were approximately twice greater in Cbl-deficient worms than in the control worms. Whereas, reduced glutathione and ascorbic acid contents and superoxide dismutase and catalase activities were significantly decreased during Cbl deficiency. These results indicate that Cbl deficiency in C. elegans causes severe oxidative stress, leading to oxidative damage of various cellular components.

An NaCl chemotaxis associative learning assay indicated that Cbl deficiency did not affect learning ability but impaired memory.
retention ability, which decreased to approximately 60% of the control. When worms were treated with glutathione, ascobic acid, or vitamin E for three generations during Cbl deficiency, cellular malondialdehyde content as an index of oxidative stress decreased to the control level, but the impairment of memory retention ability was not completely reversed (up to approximately 50%). These results suggest that memory retention impairment formed during Cbl deficiency is partially attributable to oxidative stress.

1151A Comparative gene expression and imaging studies in *C. elegans* wild type and mutant strains in bacterial vs. axenic cultures. A. Bouyansif1,2,4, S. Jayarathne1,4, J.E. Hewitt3,4, S. Liyanage2,4, N. Abidi2,4, S.A. Vanapalli3,4, N. Moustaid-Moussa1,2,4 1) Department of Nutritional Sciences, Texas Tech University, Lubbock, TX; 2) Department of Plant and Soil Science, Texas Tech University, Lubbock, TX; 3) Department of Plant and Soil Science, Texas Tech University, Lubbock, TX; 4) Obesity Research Cluster, Texas Tech University, Lubbock, TX.

Obesity is a complex disease that is influenced by diet, physical activity, developmental stage, age, environmental, and genetic factors. An underlying feature of obesity is chronic low-grade inflammation and oxidative stress, which occur because of an imbalance between inflammatory substances and free radical production vs. anti-inflammatory and antioxidant factors produced. Bioactive compounds are key dietary components that reduce obesity-associated inflammation in animals and humans. However, limited studies have explored their cell and molecular mechanisms in model organisms.

*C. elegans* has gained tremendous interest for studying lipid metabolism, obesity and longevity. The main goal of this study is to use this model organism to study changes in lipid accumulation, inflammation and oxidative stress markers using dietary bioactive compounds. As a first step, we developed and compared *C. elegans* models in bacteria-based vs. axenic liquid cultures. We hypothesized that specific genes involved in inflammation and lipid metabolism would be comparable between these two culture systems.

We used WT (N2) strains and two mutants (*tub-1, fat-3*) *C. elegans* strains and cultured them in agar cultures or CeMM (*C. elegans* maintenance media) cultures. The worms were then used for IR imaging studies; and to extract RNA for gene expression studies. Target genes tested included Fatty Acid Synthase, Oxidative Stress Induced gene, Monocyte Chemoattractant Protein 1 as well as 18S and/or Glyceraldehyde Phosphate Dehydrogenase as internal housekeeping controls. Preliminary results indicate that mutant strains expressed significantly lower levels of the above markers compared to WT. Furthermore, FTIR analyses revealed that, in addition to lipids and proteins that are detected in all *C elegans* cultures, the intense vibrations in the spectra of WT are characteristics of omega-3 fatty acids produced by the worms. These vibrations are very small in the IR spectra of tub-1. These findings indicate that FTIR imaging is a very useful technique to apply for macromolecular studies in *C. elegans*. In addition, CeMM culture studies are likely to provide gene expression data that are directly relevant to *C. elegans* responses, independent of bacterial effects. Studies are currently ongoing with application of these tools to determine responses of these *C. elegans* to bioactive compounds, including omega-3 fatty acids.

1152B NMR-based metabolomic characterization of ascaroside production in recombinant inbred lines (RILs) of Caenorhabditis elegans. T. Carter1, A.S. Edison1,2, G. Gouveia1, F. Tayyari1, F. Ponce1, E. Andersen2 1) CCRC, University Of Georgia, Athens, GA; 2) Molecular Biosciences, Northwestern University, 2205 Tech Drive, Evanston, Illinois.

Organisms from all kingdoms of life communicate in extremely diverse ways. One such example is the unique and complex chemical signaling of small molecules used by *Caenorhabditis elegans*, known as ascarosides. Ascarosides are glycosidic derivatives of 3,6-dideoxyascarylose that are attached to fatty acid side chains that can be oxidized to various lengths, as well as many other modifications. Many ascarosides work synergistically at dose-dependent concentrations in concert with one another to communicate a variety of signals. Adding to this complexity is the fact that variable concentrations of the same ascaroside can elicit different behaviors. Although ascarosides are conserved in nematodes, different strains produce and respond to different mixtures ascarosides and the variable “bouquet” of concentrations that work together have yet to be fully addressed.

We analyzed a subset (10) of the hundreds of existing recombinant inbred lines (RILs) of *C. elegans* and their parent strains, the laboratory strain (N2) and a wild isolate from Hawaii (CB4856). Our primary goal in analyzing the RILs and parental N2 and CB4856 strains is to relate metabolite profiles and ascaroside variation to the complex phenotypic variation. Using an NMR (Nuclear Magnetic Resonance) Spectroscopy based metabolomic approach we will present the preliminary analysis revealing global metabolic changes among the parent strains and their recombinant progeny. These results will determine the feasibility of extending these studies to larger numbers of RILs. With a more robust sample size, we aim to isolate and identify ascaroside variance within and between the RILs using both NMR and LC-MS (Liquid Chromatography-Mass Spectrometry). Once isolated, the ascarosides will be used to test the behavioral response of N2 and CB4856 to the different RIL ascaroside bouquets using simple bioassays.

1153C Effects of animal density on gene expression during L1 larval starvation. I.L. Chan, O.J. Rando, C.C. Conine Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA.

The ability of developing *C. elegans* larvae to sense unfavorable environmental conditions and respond by developmental arrest has provided key models for studying regulation of metabolism and development. In addition to the classic model dauer larvae, recently there has been increased interest in characterizing developmental arrest during other developmental stages. Notably, developmental arrests are reversible, enabling researchers to dissect environmental inputs and molecular mechanisms involved in both entering and exiting the arrest. Worms enter the first larval stage (L1) upon hatching from the egg and are able to commit to L1 developmental arrest solely based on the availability of food. A routine method performed by *C. elegans*
researchers is to make use of a short period of L1 arrest to generate synchronous populations for experiments. However, variation in the density of resuspended L1 larvae in buffer during synchronization is often overlooked. Here, we use genome-wide analysis of mRNA abundance to identify molecular phenotypes in arrested L1 animals that are dependent on L1 density. We further characterize physiologically-relevant phenotypes that are associated with L1 density during starvation. These observations indicate that environmental sensing mechanisms during L1 arrest are not limited to food availability, and suggest external signaling may regulate gene expression and metabolism during L1 starvation. The observed L1 density phenomenon may have multiple implications for experimental design for virtually all aspects of C. elegans research, but especially for researchers who study environmental effects on worm physiology.

1154A An Emerging Role for DBL-1/BMP in Lipid Metabolism in C. elegans. J.F. Clark1,2, M. Meade2, G. Ranepura2, J.L. Watts3, D.H. Hall4, C. Savage-Dunn1,2 1) PhD Program in Biology Department, The Graduate Center, CUNY, New York, NY; 2) Biology Department, Queens College, CUNY, Queens, NY; 3) School of Molecular Biosciences, Washington State University, Pullman, WA; 4) Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY.

Transforming Growth Factor-beta (TGF-ß) Superfamily is a large family of peptides that control cell functions such as differentiation, proliferation, and regulation of the immune system. Misregulation of TGF-ß family members is implicated in many diseases. Recently, roles in metabolism have been emerging for TGF-ßs, BMPs, and GDFs related to obesity, the browning of white adipose tissue, and insulin resistance. In C. elegans, DBL-1/BMP is a major regulator of body size, cell patterning, and innate immunity. However, a role in lipid metabolism has not yet been shown. To analyze the function of DBL-1 in lipid metabolism, we observed overall lipid stores, biochemical analysis, and lipid droplet dynamics in dbl-1 mutants and sma-3 mutants defective in components of the DBL-1 pathway. Using Oil Red O, a neutral lipid dye, we observe an overall decrease in the levels of triglycerides in DBL-1 pathway mutants, dbl-1(II), sma-3(III), and dbl-1++(oe). Genetic epistasis analysis places the DBL-1 pathway upstream of DAF-2/IR, a well-known regulator of lipid metabolism. Preliminary gas chromatography mass spectrometry data confirms the Oil Red O trend, with sma-3 mutants exhibiting over a 25% decrease in triglycerides. Using a GFP tagged lipid droplet associated protein, DHS-3::GFP, we analyzed the lipid droplet dynamics within dbl-1 and dbl-1++ mutants. We show that lipid droplet size positively correlates with the level of DBL-1 signaling in the worm, dbl-1 mutants have a decrease in average lipid droplet diameter, while dbl-1++ mutants have an increase in diameter. A decrease in lipid droplet size is also seen in electron micrographs of dbl-1, sma-3, and sma-9 mutants. However, any change in DBL-1 signaling decreases the overall number of lipid droplets in the worm, with both dbl-1 and dbl-1+++ mutants having reduced numbers of lipid droplets compared to control animals. Finally, we show that proper lipid levels are dependent on expression of SMA-3 in the hypodermis, as hypodermal expression of SMA-3 is necessary and sufficient to rescue the low-fat phenotype of sma-3 mutants, while intestinal or pharyngeal expression cannot, reinforcing the idea that the DBL-1 pathway signals in a non-cell autonomous manner. Together these data describe a definite role in lipid regulation for the DBL-1/BMP pathway in C. elegans. These findings implicate that DBL-1/BMP signaling plays a role in maintenance of proper metabolic homeostasis and development.

1155B Overactive EGF signaling suppresses lipid synthesis via the transcription factor SBP-1. Matt Crook1,2, Allison Bogisich5, Miranda Huang6, Brenda Zarazua6, Lizzi Wong6, Lindsay Schwartz2 1) Biology, Texas A&M University San Antonio, San Antonio, TX; 2) Biology Department, Queens College, CUNY, Queens, NY.

Epidermal Growth Factor (EGF) signaling in C. elegans controls development, healthspan and ovulation. We have discovered a role for overactive EGF signaling in suppressing lipid synthesis. A gain-of-function mutation in let-23, the sole C. elegans EGF receptor, results in pale worms. We used Quick Oil Red O staining to measure the total lipid content of L4 larvae/ young adults and found that let-23(sa62gf) animals had a significantly lower total lipid level compared with wildtype animals. We saw the same reduction in lipid levels in let-60/ Ras gain-of-function animals, suggesting a role for LET-60/ Ras signaling in LET-23 mediated control of lipid synthesis. Lipid synthesis in C. elegans is controlled by the cellular localization of the transcription factor SBP-1. SBP-1 is retained in the endoplasmic reticulum until lipid synthesis is required, upon which it is processed and enters the nucleus to activate expression of lipid synthesis genes. We used a SBP-1::GFP translational fusion to determine the effect of overactive EGF signaling on SBP-1 cellular localization. We found that SBP-1::GFP localization was biased to the inactive cytoplasmic form in let-23(sa62gf) mutants, establishing a connection between EGF signaling and lipogenesis. Finally, we used GFP fluorescence imaging to identify individual lipid species and compare their relative quantities at different levels of EGF signaling. We found a large flux in polyunsaturated lipids and multiple lipid species with a greater than two fold difference between let-23 mutant and wildtype, but no association between EGF signaling levels and relative abundance of a specific lipid class. Thus the let-23(sa62gf) mutation results in the suppression of lipid synthesis, at least in part through LET-60/ Ras signaling, and does so by restricting SBP-1 to the cytoplasm, resulting in a marked change in the lipidome. EGFR and Ras gain-of-function mutations are found in many cancer types, especially lung cancer, and changes in metabolism are a hallmark of tumorigenesis. An better understanding of the role of overactive EGF signaling in the control of lipid metabolism will further our understanding of how overactive EGF signaling leads to cancer.

1156C Using simulated anaerobiosis in C. elegans as a platform for anthelmintic drug discovery. S. Del Borrello1,2, M. J. Lautens1,2, M. Spensley1,2, M. Schertzberg1,2, A. A. Caudy1,2, A. G. Fraser1,2 1) Molecular Genetics, University of Toronto, Toronto, Ontario, CA; 2) Terrence Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Ontario, CA.

Parasitic helminth infections affect ¼ of the world, and result in thousands of deaths every year as well as economic losses in agriculture. This is in part due to increasing anthelmintic resistance, thus fueling a need for the discovery of novel anthelmintics. During host infection, parasitic helminths are in an oxygen-deprived environment, during which they use a special form of anaerobic respiration to survive – malate dismutation – which requires rhoquoine, a ubiquinone-related electron carrier. This
pathway is absent in mammals, but exists in the nematode worm C. elegans, making it an ideal drug target and the worm a good model of parasitic helminth metabolism. We established an image-based assay of worm movement—the acute assay—and found that worms treated with potassium cyanide (KCN) become paralyzed, however additional treatment with 2-Deoxy-D-glucose (2DG) recovers movement. KCN prevents oxidative phosphorylation in the aerobic electron transport chain (ETC), and 2DG is a glucose analog that blocks glycolysis. A key question is how worms are producing ATP when both glycolysis and the ETC are blocked.

We used RNAseq to investigate the transcriptional response to KCN + 2DG, and found our treatment activates the hypoxia response. Mutant analysis of genes required for this response has shown this response is required for recovery. Furthermore, RNAseq data shows that treatment with KCN + 2DG recapitulates anaerobiosis. Interestingly, we also see inhibition of Complex I with Rotenone prevents recovery. Therefore, we conclude that recovery from paralysis requires at least two processes: a working hypoxia response, and the anaerobic electron transport chain. My project goal is to better understand these and other anaerobic pathways to uncover new anthelmintic targets. Our long-term goals include conducting a drug screen for new anthelmintics using simulated anaerobiosis in C. elegans, and a forward genetic screen to uncover alternative metabolic pathways required for recovery.


Adenylosuccinate lyase (ADSL) deficiency is caused by mutations in ADSL, an enzyme of de novo purine biosynthesis. Like other Inborn Errors of Purine Metabolism, ADSL deficiency is associated with devastating neurological symptoms and distinctive behavioral phenotypes, including life-threatening seizures, degenerative muscular ataxia, autistic-like behaviors, and compulsive laughter. Both the lack of purine biosynthesis and the accumulation of toxic intermediary metabolites are proposed to contribute to symptoms. However, accurate etiologies have remained elusive because the disease is rare and understudied. We developed a C. elegans model for the study of the neuromuscular, developmental and behavioral aspects of ADSL deficiency using adsl-1(tm3328) and adsl-1(RNAi). Our aim is to contribute novel ideas for patient therapies and to probe fascinating links between purine metabolism and specific neurological deficits. Reduction of adsl-1 activity results in neuromuscular and behavioral dysfunctions that parallel those of the human disorder. adsl-1 mutants differ in both the quantity and quality of muscle contractions as measured by crawling speed, thrashing rate and body bending intensity. Levamisole and aldicarb sensitivity assays reveal that the locomotion defects are primarily a result of neural dysfunction. adsl-1 animals also have a novel associative learning phenotype, involving an altered response to a learning paradigm, and reproductive phenotypes resulting in sterility. Using RNAi, we demonstrated an acute need for adsl-1 during oogenesis. Metabolic profiling reveals an accumulation of ADSL substrates when ADSL activity is decreased but little change in steady state purine levels. These measurements parallel observations in patients. Despite the lack of depressed purine levels, supplemental purines restore fertility to adsl-1 animals. Purine supplementation has no effect on muscle function. In contrast, drug treatments that prevent accumulation of intermediary metabolites prevent neuromuscular dysfunction but have no effect on fertility. Thus, we have shown that phenotypes associated with ADSL deficiency differ in etiology, suggesting a novel approach to therapy involving both inhibition of purine biosynthesis and purine supplementation. We also present metabolomics data to assess the effect of the toxic substrate. In summary, we have established a productive model for study of a rare metabolic disorder, have attributed phenotypes to specific metabolic perturbations, and have generated a novel therapeutic strategy for ADSL deficiency.

1158B  Comparative approach to understanding neuromuscular deficits in de novo purine biosynthesis mutants.  L. Franklin, C. A. Moro, W. Hanna-Rose  Biochemistry, Microbiology and Molecular Biology, Pennsylvania State University, State College, PA.

Caenorhabditis elegans is a powerful model organism which enables us to study, in vivo, the physiological properties of the rare metabolic disorder adenylosuccinate lyase (ADSL) deficiency. This disorder is caused by the lack of the enzyme ADSL resulting in a block in the de novo purine biosynthesis pathway. Symptoms of ADSL deficiency resemble those of an autistic patient, which can include repetitive movements, mild-to-moderate cognitive impairment, and sound sensitivity. ADSL deficiency is a relevant disease to study in C. elegans because purine biosynthesis in general and adsl-1 gene activity in particular is conserved with humans. Moreover, probing the neurological effects of ADSL deficiency may shed light on a variety of neurological disorders given the similarity of ADSL deficiency to aspects of autism. We have identified a motor deficit caused by neural dysfunction as a phenotype in adsl-1 mutant animals. Adenylosuccinate Synthase (ADSS) is an enzyme which is also found in the de novo purine biosynthesis pathway. C. elegans adss-1(RNAi) has a comparable phenotype to adsl-1 deficient C. elegans in terms of motor deficits. We hypothesize that using a comparative approach, between adss-1 and adsl-1 will provide insight on how the lack of a functional de novo purine biosynthesis pathway affects motor skills and sterility. We used thrashing assays as well as aldicarb and levamisole sensitivity assays to compare the motor function of adss-1(RNAi) to adsl-1(RNAi) C. elegans. Compared to controls, adss-1(RNAi) and adsl-1(RNAi) showed reduced thrashing as well as decreased sensitivity to levamisole or aldicarb. Our results suggest the ADSS-1 enzyme is a plausible candidate for helping us to understand ADSL-1 deficiency. In the future, we plan to compare the metabolic effects of adss-1(RNAi) to that of adsl-1(RNAi) to gain mechanistic insight into the cause of the neuromuscular deficit.

1159C  Isolation of genes required for cold acclimation.  Mayu Fujita1, Shiori Sakai1, Misaki Okahata1, Yohei Minakuchi2, Atsushi Toyoda2, Akane Ohta1, Atsushi Kuhara1 1) Institute for Integrative Neurobiology, Graduate School of natural science,
and School of Science and Engineering, Konan University, Kobe, Japan; 2) National Institute of Genetics, Japan.

Animals can sense and memorize environmental temperature to survive and proliferate in variety of temperature condition. In this study, we are studying about molecular mechanisms for temperature acclimation and its memory by using cold acclimation. Wild-type animals can not survive at 2 degree after cultivation at 25 degree, whereas they can survive at 2 degree after cultivation at 15 degree. After 25 degree-cultivated animals were transferred to and stayed at 15 degree for 3 hours, these animals can survive at 2 degree, as previously reported (Ohta, Ujisawa et al., *Nature commun.*, 2014). This phenomenon is regulated by CREB that is a transcription factor in mammalian memory, suggesting that gene expression is involved in the regulation of cold acclimation or temperature memory (Fuji et al. this meeting).

(1) We quantified gene expression-changes depending on temperature shift, by using RNA sequencing analysis with next generation sequencer. We collected mRNA of the animals cultivated at various temperature conditions. By the RNA sequencing analysis, expression levels of about 950 genes were strongly changed, in which mutants of about 520 genes were available in the stock centers. So far, abnormal cold acclimation was observed in *hacd-1* mutant defective in HADH, 3-hydroxyacyl-CoenzymeA dehydrogenase. HADH is involved in beta-oxidation regulating fatty acid metabolism in a mitochondrial matrix. GFP reporter analysis indicated that *hacd-1* was expressed in neurons and intestine. We found that abnormal cold acclimation of *hacd-1* mutant was rescued by the expression of *hacd-fcDNA* in neurons and intestine. We are planning to determine which neuron is required for *hacd-1*-dependent cold acclimation.

(2) We also used DNA microarray analysis on temperature shift to identify more genes involved in cold acclimation. We found that RB2549 *sms-3(ok3540)* showed abnormal cold acclimation. When 25 degree-cultivated animals transferred to 15 degree and stayed for 3 hours, RB2549 mutant can survive at 2 degree, while wild-type can not survive at 2 degree. However, another null mutant *sms-3(tm4022)* did not show abnormal phenotype. Therefore, we hypothesized that background mutation(s) excepting *sms-3* mutation in RB2575 causes abnormal cold acclimation. To identify the responsible gene, we decoded whole genome DNA sequences by using next generation sequencer (NGS). So far, the candidate responsible genes were narrowed down to 28 genes. We are isolating recombinant strains, and are introducing SNP analysis to determine the responsible gene for abnormal cold acclimation of RB2575 strain.

1160A Assessing the influence of PI3K/Akt signaling integrity on metabolic activity and oxidative status in *C. elegans*. T. Gibbons, T. Kao, S.J.B. Freatham Dept of Biology, Luther College, Decorah, IA.

Altered oxidative state and impaired energy metabolism are hallmarks of many neurological diseases. Several of these diseases have been linked to aberrant insulin/insulin-like signaling including DJ-1 and PINK1 mutations in Parkinson’s Disease and emerging evidence connecting insulin resistance to Alzheimer’s pathology. This study examines the role of phosphatidylinositol 3-kinase (PI3K)/Akt, a downstream effector of insulin/insulin-like receptor signaling, in regulating cellular metabolism and oxidative state. The PI3K/Akt signaling cascade integrates extracellular and intracellular signals such as nutrient availability, growth factor signaling, and ATP levels to regulate cellular metabolism, cytoskeleton structure, and gene expression through several downstream effectors including inactivation of the FOXO homolog DAF-16. To better elucidate the relationship between PI3K/Akt signaling integrity and oxidative and metabolic state, synchronous populations of young adult *Caenorhabditis elegans* (*C. elegans*) were cultured on NGM agar plates seeded with OP-50 *E. coli* and collected for ATP, glutathione, and MTT reduction potential assessment. *age-1* (PI3K) and *akt-1* mutants with reduced upstream PI3K/Akt signaling had elevated ATP levels relative to wild type (WT) animals. Conversely, increased upstream PI3K/Akt activity and reduced FOXO activity in *daf-18 (PTEN)* and *daf-16 (FOXO)* mutants resulted in decreased ATP levels relative to WT. Furthermore, total glutathione, an important antioxidant tripeptide, and MTT reduction potential were altered by mutations in the PI3K/Akt/FOXO pathway, however unlike ATP levels these oxidative alterations did not show a consistent pattern relative to PI3K/Akt activity. These observations suggest that in standard culture conditions ATP levels and oxidative status depend on PI3K/Akt activity.

1161B Selfish mitochondrial genomes exploit nuclear-encoded pathways to propagate. Bryan Gitschlag1, Maulik Patel1,2 1) Biological Sciences, Vanderbilt University, Nashville, TN; 2) Cell & Developmental Biology, Vanderbilt University, Nashville, TN.

Hundreds to thousands of mitochondrial DNA (mtDNA) molecules exist per cell. Mutations that arise therefore coexist with wildtype mtDNA copies, a state referred to as heteroplasmy. Because replication of new mtDNA copies occurs independently of the cell cycle, mutant mtDNA copies can occur at varying frequencies, and mitochondrial dysfunction results when pathogenic mtDNA variants exceed a frequency threshold. Approximately one out of 5,000 individuals carries a disruptive mtDNA mutation at pathogenically high levels. How are mutated mtDNA copies able to reach pathogenically high levels despite the paradoxical fact that this process is damaging to cell fitness? I have characterized a heteroplasmic *C. elegans* strain harboring the *uaDf5* deletion, which removes essential genes. Remarkably, mtDNA *uaDf5* is stably co-transmitted with wildtype mtDNA across generations and can rise in frequency. Thus, mtDNA *uaDf5* behaves as a selfish genetic element, propagating at the expense of host fitness. Using droplet digital PCR, I discovered that high mtDNA *uaDf5* levels result in a transcriptional imbalance between mtDNA and nuclear-encoded genes and induces mitochondrial unfolded protein response (UPRmt) activation. Furthermore, loss of UPRmt activation suppresses mtDNA *uaDf5* levels. This suppression is dependent on mitophagy, suggesting that the protective function of UPRmt paradoxically promotes mutant mtDNA proliferation by shielding the mutant from degradation. Ongoing studies have expanded the investigation to UPRmt-independent mechanisms that underlie the propagation of mutant mitochondria, including the role of energy metabolism.

1162C HIF-1-independent hypoxic resistance involves a novel mechanism of mitochondrial adaptation in *C. elegans*. I. Gikas1,2, I. Daskalaki1,2, E. Lionaki1, N. Tavernarakis1,3 1) Institute of Molecular Biology and Biotechnology,
A continuous increase in obesity and obesity-related diseases, such as metabolic syndrome and type-II diabetes, has led to a global health crisis. Therefore, the understanding of molecular mechanisms controlling fat metabolism is crucial to identify potential new therapeutic targets. Key transcription factors involved in lipid metabolism, such as SBP-1/SREBP, LPD-2/C/EBP and MDT-15, are conserved from nematodes to mammals, and C. elegans has proven to be a powerful model for obesity and metabolic research. 

The C. elegans RNase REGE-1 is closely related to the mammalian Regnase-1/MCPIP1/Zc3h12a, an extensively studied regulator of innate immunity. The loss of REGE-1 causes a dramatic decrease in overall body fat, accompanied by increased expression of lipid metabolic and innate immunity genes. Using Exon-Intron Split Analysis (EISA), we found that REGE-1 controls fat by targeting mRNA encoding a fat loss-promoting transcription factor, ETS-4, previously implicated in ageing. While REGE-1 degrades ets-4 mRNA via an endonucleolytic cleavage within its 3′ UTR, ETS-4, in turn, promotes the transcription of rege-1. Thus, ETS-4 and REGE-1 form an auto-regulatory module (ERM), affecting fat by controlling the transcriptional output of ETS-4. The ERM is a novel mechanistic paradigm in fat regulation. Posttranscriptional mechanisms are well suited to a rapid and reversible control of gene expression. Similarly, REGE-1–mediated mRNA degradation might be a way to rapidly re-wire lipid metabolism in response to environmental changes, including change of diet, temperature or exposure to pathogens.

1165C The C-box region of MAF1 regulates transcriptional activity and protein stability. A. Pradhan1, A.M. Hammerquist1,2, A. Khanna1,2, S.P. Curran1,2  1) Leonard Davis School of Gerontology, University of Southern California, Los Angeles, CA; 2) Department of Molecular and Computational Biology, Dornsife School of Letters, Arts, and Sciences, University of Southern California, Los Angeles, CA.

MAF1 is a conserved negative regulator of RNA polymerase (pol) III and intracellular lipid homeostasis across species. Here, we show that the MAF1 C-box region negatively regulates its activity. Mutations in Caenorhabditis elegans mafr-1 that truncate the C-box retain the ability to inhibit the transcription of RNA pol III targets, reduce lipid biogenesis, and lower reproductive
output. In human cells, C-box deletion of MAF1 leads to increased MAF1 nuclear localization and enhanced repression of ACC1 and FASN, but with impaired repression of RNA pol III targets. Surprisingly, C-box mutations render MAF1 insensitive to rapamycin, further defining a regulatory role for this region. Two MAF1 species, MAF1L and MAF1S, are regulated by the C-box YSY motif, which, when mutated, alters species stoichiometry and proteasome-dependent turnover of nuclear MAF1. Our results reveal a role for the C-box region as a critical determinant of MAF1 stability, activity, and response to cellular stress.

1166A 10 days to hatch! – a severely delayed hatching phenotype.  J. Blaszkiewicz, N. LaGanke, M. DeGennaro, E. Eberly, V. Huso, M. McReynolds, W. Hanna-Rose  The Pennsylvania State University, University Park, PA.

We use C. elegans as a model to study the biological roles of NAD⁺ biosynthetic pathways. Vitamin B₃ precursors for NAD⁺ biosynthesis, such as nicotinamide riboside (NR), have garnered a lot of attention recently because of their ability to significantly raise NAD⁺ levels when supplemented to animals or cells, to apparent therapeutic benefit. To investigate the normal biological roles of this NR pathway, we have examined animals with mutations in the gene coding for nicotinamide riboside kinase (nmrk-1 / T27A3.6), an enzyme required to process NR for NAD⁺ biosynthesis. We frequently use UV-killed OP50 as a food source to prevent the bacterial food from metabolizing vitamin B₃ supplements that we add to cultures. Upon growth of nmrk-1(ok2571) on UV-killed OP50, but not live OP50, we noted a surprising phenotype. Hatching is delayed by up to 10 days. The phenotype is specific to NAD⁺ biosynthesis from NR and is not a feature of mutants that affect other NAD⁺ biosynthesis pathways or combinations of pathways. The phenotype is leaky, with approximately 25% of the brood hatching in the expected time frame.

25% of the brood hatches between 3 and 10 days after egg laying, and about half of the brood never hatches. Embryogenesis is not delayed and animals maintain normal embryonic movements over the course of their time in the eggshell. By switching conditions of the mothers or the eggs from live to UV-killed OP50, we found that the mother must experience the UV-killed environment for the embryos to manifest the phenotype. Using global metabolomics profiling to compare populations of animals grown on live versus UV-killed OP50, we have identified metabolic signatures, including increased oxidative stress, associated with growth on UV-killed food. Thus, we tested if this feature was relevant to the delayed hatching phenotype. We find that treatment with paraquat can substitute for the requirement for UV-killed food to reveal the delayed hatching phenotype of the nmrk-1 mutants. Our observations are consistent with a hypothesis that the animals are physically unable to hatch, perhaps because of an altered eggshell.

1167B Assessing variability in pharyngeal pumping frequency in the nematode Caenorhabditis elegans.  Abanoub Akhnoukh1, Akhnoukh Maria1, Christopher Barrientos1, Pierre Fahmy1, Marina Kaldes1, Brianna Ortiz2, Vanessa Valdez1, Joshua Hincks2, Barbara Taylor3, Michael Harris1  1) Biology, California State University Long Beach, Long Beach, CA; 2) Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, AK.

In C. elegans, feeding is achieved through a well-characterized behavior, pharyngeal pumping. Pharyngeal pumping is stimulated by the presence of bacterial food (E. coli) and/or the endogenous neuromodulator of feeding behavior, serotonin (5HT). Age-related changes in tissue morphology and function, and a decline in C. elegans health are correlated with reduction in the rate of pharyngeal pumping. As such, pharyngeal pumping frequencies have been used to exemplify health and age-related decline in this model organism. Traditionally, pharyngeal pumping has been assessed by eye, and reported as a pump rate, computed from the number of pumps observed divided by total recording time. Although rhythmic, pharyngeal pumping is both variable and periodic, traditionally pharyngeal pumping rate assessment does not consider underlying variability of pumping behavior. The recently developed NemaMetrix ScreenChip System automates the process of accurately resolving pharyngeal pumping by recording an electrical signature, the electropharyngeogram (EPG). As EPG recordings allow for precise detection of individual pump events, we aimed to assess patterns of variability in EPG measurements to test the hypothesis that underlying variability could be a relevant and insightful index of functional decline in this organism. In the context of directed undergraduate research, we use the ScreenChip System to resolve individual pump cycle durations and assess variability over conditions including age, 5HT stimulation and experimental disruption. Findings suggest relatively consistent patterns of variability, and changes in variability coincide with reductions in pharyngeal pumping frequencies. We suggest it may be possible to resolve more subtle aspects of functional decline from assessments of changing variability, than are possible from pharyngeal pumping frequencies alone.

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1168C daf-16/FoxO promotes trehalose synthesis during starvation to increase survival.  Jonathan Hibshman1, Alexander Doan1, Brad Moore1, Rebecca Kaplan1, Anthony Hung1, Dhaval Bhatt1, Amy Webster1, Matthew Hirsche1y2, Ryan Baugh1  1) Biology Department, Duke University, Durham, NC; 2) Duke Molecular Physiology Institute, Duke University, Durham, NC.

Starvation is a common environmental stress. Survival during starvation requires pervasive transcriptional, metabolic, and physiological changes. The forkhead box transcription factor daf-16/FoxO is known for its essential role in mounting a starvation response. However, effectors of daf-16/FoxO are poorly understood. We define a set of genes regulated by daf-16/FoxO during starvation in C. elegans L1 larvae. We find that daf-16/FoxO functions early in starvation to transcriptionally control metabolic enzymes, promoting a metabolic shift to utilize the glyoxylate shunt, gluconeogenesis, and synthesize trehalose, a disaccharide of glucose. Defective trehalose synthesis reduces starvation survival, and trehalose supplementation during starvation increases survival. Ablation of the trehalose 6-phosphate synthase enzymes fps-1 and fps-2 demonstrates they are required to use other sugars and maintain the steady state pool of trehalose, contributing to survival. RNA-seq analyses reveal that trehalose
supplementation does not fully complement elimination of trehalose synthase activity, suggesting a physiological role of the phosphorylated intermediate, trehalose 6-phosphate, or a secondary function of trehalose synthase enzymes. While trehalose is well known for its ability to buffer macromolecules during stress, our results also suggest it functions as an energy source to extend starvation survival. As an energy source, trehalose can fuel cell division in at least two different tissues. These observations and others support the model that trehalose is produced in the intestine during starvation and is used to fuel glycolysis in other tissues. This work reveals an integral role for trehalose in organismal energy homeostasis, and it demonstrates that 

**1169A Loss of Caenorhabditis elegans W02B12.15, mammalian cisdl, alters iron homeostasis, mitochondrial electron transport complexes and lipid metabolism.**  
Kuei-Ching Hsiung, Kuan-Yu Liu, Mei-Ling Cheng, Bertrand Tan, Szecheng Lo  
Department and Institute of Biomedical Sciences, Chang Gung University, TaoYuan, Taiwan, 333.

Iron-sulfur cluster (ISC) has indispensable functions in many biological processes; including mitochondrial respiratory chain and numerous enzymatic functions, due to its unique molecular geometry. Three species of CDGSH iron sulfur domain-containing proteins, Cisd1, Cisd2, and Cisd3 have been identified in mammals. Cisd1 and Cisd2 contain a CDGSH domain and a mitochondrial transmembrane domain. Cisd1 might play a role in transferring ISC to an apo-ferredoxin in vitro and obesity-associated dysfunction adiposeness in human VAT while Cisd2 mediates mitochondrial integrity and life span in mammals. Cisd3 has two CDGSH domains but no transmembrane domain. In. C. elegans, one CDGSH coding gene, W02B12.15, was found and based on a phylogenetic tree analysis, W02B12.15 is closer to the Cisd1 clade. Furthermore, deletion mutant (tm4993) showed no defects in life span, motility, and development. Thus, C. elegans W02B12.15 was designated as a Cecisd1 gene. Cecisd1 was found primarily in the mitochondrial membrane fraction and the Cecisd1::GFP was colocalized with Mitotracker Red. The deficiency of Cecisd1 showed the abnormal mitochondrial morphology. Although the basal level of oxygen consumption of tm4993 was higher than wild type, the spare respiratory capacity did not reflect this phenomenon. Noticeably, the ATP-linked respiration and ROS production were increased in tm4993. The mitochondrial electron transport chain complexes were altered in tm4993, which lead to proton leakage and loss of mitochondrial membrane potential (???m). It might cause the increasing of AMP/ATP ratio that activated the AMPK phosphorylation. Subsequently, fatty acid oxidation genes acs-2, cpt-5 and ech-1 were activated by AMPK to react in decreasing of the total amount and the average size of lipid droplets. The lipidomic and metabolic sets enrichment analyses indicated the increasing of branched chain fatty acids oxidation and beta-oxidation of long chain fatty acids. Without the ISC transferring from Ccisd1 to cytosolic aconitate, the iron regulatory proteins increase the uptake of iron, which leads the overloading of cytosolic total iron (especially ferrous iron) in the tm4993. Collectively, our data suggest that depletion of Ccisd1 causes the imbalance of mitochondrial energy and lipid metabolism through interruption of cellular iron homeostasis.

**1170B Starving in the midst of plenty: effects of food perception on gene regulation and development.**  
Rebecca E.W. Kaplan1, Amy K. Webster1, Joseph A. Dent2, L. Ryan Baugh1  
1) Duke University, Durham, NC; 2) McGill University, Montreal, QC.

Chemoreception cues play important roles throughout the C. elegans lifespan, helping the organism find food, avoid pathogens, and otherwise evaluate its environment. Upon hatching, L1 larvae arrest development unless fed. The effects of actual feeding versus food perception have not been delineated. We wanted to examine how much of the organism’s response to food is due to perception independent of feeding. To examine this question we used ivermectin in a genetic background where the drug affects only the pharynx, blocking feeding by paralysis. 

**daf-16**, a transcriptional effector of insulin-like signaling, is known to be localized largely in the nucleus during starvation and more in the cytoplasm during feeding. After one hour of exposure to food without feeding GFP::DAF-16 localization is largely cytoplasmic, closely resembling that of a fed worm. However, after 24 hours of exposure to food without feeding GFP::DAF-16 returns to the nucleus and resembles a starved worm. This observation suggests food perception alone can alter DAF-16 localization initially, but actual feeding is required to maintain cytoplasmic DAF-16 localization. We used RNA-seq to examine transcriptional changes that occur in response to food perception without feeding. We found that 1501 genes are differentially expressed in response to perception and that this set significantly overlaps with the transcriptional response to normal feeding. Interestingly, the genes in this overlap are mostly involved in metabolic processes, while those genes that alter expression only in response to normal feeding are involved in developmental processes. This is in line with our observation that L1 larvae exposed to food without feeding do not exit L1 arrest. The most striking phenotype observed from our studies is that almost all L1 larvae exposed to food without feeding for 24 hours fail to recover to adulthood upon return to normal culture conditions. Repression of transcription or translation during exposure to food without feeding significantly rescues recovery. We hypothesize that the gene expression response to food perception alters the metabolism of the organism to prepare for development, but that this response is detrimental to the worm if feeding does not actually occur.

**1171C Sulfolipids drive C. elegans defensive responses to a predator.**  
Z. Liu1, M. Kariya2, C. Chute3, S. Leinwand1, A. Tong1, A. Pribadi1,4, K. Curran4, N. Bose5, F. Schroeder2, J. Srinivasan3, S. Chalasani1  
1) Molecular Neurobiology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037; 2) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853; 3) Department of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA 01605; 4) Division of Biological Sciences, University of California, San Diego, La Jolla, CA 92093.

Animals respond to predator threats by implementing characteristic behavioural and physiological changes, but it is not known how the underlying neuronal ensembles process these threats. The simple, well-defined nervous system of the nematode, Caenorhabditis elegans facilitates uncovering conserved principles in neuronal processing. Here we show that C. elegans responds to excretions from a nematode predator, Pristionchus pacificus with instantaneous escape behaviour and a prolonged
reduction of oviposition. Chemical analysis of the *P. pacificus* exo-metabolome revealed a series of novel sulfated lipids that instruct these *C. elegans* responses. These sulfolipids are specific to the predator and originate from a biosynthetic pathway that ties into endocrine signalling required for predacious behavior.

**1172A Mitochondrial DNA copy number in *C. elegans* is regulated by a functional output.** Cait S. Kirby, Maulik R. Patel Vanderbilt University, Nashville, TN.

Besides the nuclear genome, eukaryotic cells also contain a circular mitochondrial DNA (mtDNA). mtDNA encodes essential components of the mitochondrial electron transport chain, which produces most of the cellular ATP. Cells typically contain hundreds to thousands of copies of mtDNA. While it is known that the number of mtDNA copies per cell is tightly regulated, the cellular and molecular mechanisms underlying this mtDNA copy number control remain poorly understood. A major difficulty in studying mtDNA copy number control arises from the lack of genetically tractable systems with evidence of robust mtDNA regulation, as well as lack of quantitative methods to accurately determine mtDNA copies. To overcome these challenges, I have developed protocols to accurately and precisely measure mtDNA copy number from single *C. elegans* individuals at all stages of development. My analyses reveal that the adult *C. elegans* germline, which harbors most of the mtDNA content, employs active homeostatic mechanisms to regulate mtDNA copy number. In addition, I have discovered that mtDNA encodes a functional feature, which is sensed by the cell to “count” mtDNA copy number. Consistent with this hypothesis, my mutational analysis shows that mutations in mtDNA that disrupt this functional output fail to get counted, resulting in an overabundance of mtDNA copies. Taken together, my results provide fundamental insights into mechanisms of mtDNA copy number control. I am now poised to determine the molecular machinery that counts mtDNA copies, and determine how it is mechanistically coupled with mtDNA replication.

**1173B Investigating Complex I activity and mitochondrial protein import in the multi-cellular model organism *C. elegans*.** A. Knapp-Wilson, I. Collinson, P. Kuwabara University of Bristol, GB.

Mitochondria are small cytoplasmic organelles essential for meeting the majority of cellular energy demands via the oxidative phosphorylation (OXPHOS) pathway. [1, 2]. They feature a double membrane that separates the organelle into four distinct compartments [3].

The vast majority of the work on mitochondria has been carried out in *Saccharomyces cerevisiae*, a single-celled genetically tractable eukaryote [4]. Although biochemically competent; the model has limitations in its applicability to humans. The mitochondrial structure, metabolism and bioenergetics of the nematode are very similar to the mammalian counterpart [5], this conservation of mitochondrial function and the technological and anatomical advantages offered by the nematode make it an excellent model organism to explore mitochondrial function and activity. [1, 6]

My project is multidisciplinary, using a broad range of techniques including whole animal physiology, biochemical and structural analysis, and molecular dynamic simulations to gain a better understanding of the mitochondrial respiratory machinery, and the impact dysfunction of this machinery can have.

Mitochondrial dysfunction leads to many serious human diseases and a better understanding of the mechanisms involved is essential in future medical applications.


**1174C The search for novel anthelmintic targets: Characterizing alternative metabolic pathways in *Caenorhabditis elegans*.** Margot Lautens1, Samantha Del Borrello1, Amy Caudy1,2, Andy G. Fraser1,2 1) Molecular Genetics, University of Toronto, Toronto, Ontario, CA; 2) Donnelly Centre for Cellular and Biomolecular Research, Toronto, Ontario, CA.

A quarter of the human population is infected by parasitic helminths and they place a large economic burden on the agricultural industry. Unfortunately anthelmintic resistance is a growing problem. Although helmiths are able to survive long periods of time of hypoxia in their hosts, their anaerobic metabolism has yet to be fully characterized. These alternative pathways are a promising, selective target for new drugs. The Fraser lab has developed a movement assay that allows for quantitative response to drugs and genetic perturbations to be measured in *Caenorhabditis elegans*, a free living relative who can also survive hypoxia. When the electron transport chain (ETC) is blocked by high doses of cyanide (KCN), worms are unable to move. However, if glycolysis is blocked at the same time by 2-deoxyglucose (2DG), the worms are able to recover. This two drug combination recapitulates the hypoxia response on a transcriptional level and this recovery requires the key hypoxia transcription factor, HIF-1. Furthermore, Complex I, an established hallmark of anaerobic metabolism and a target of existing anthelmintics, has been shown to be essential for this recovery. Together this strongly suggests that *C. elegans* recovers from...
KCN-induced paralysis in the presence of 2DG using anaerobic metabolism. Steady state LCMS has already revealed that the addition of 2DG allows for a buildup of succinate in worms treated with KCN alone to be depleted while the glycolytic shunt and the propionate shunts have been found to be nonessential for recovery. Two metabolic mutants in opposing pathways in the rate-limiting step TCA cycle show hyper- and hypo-recovery (idh-2 and idh-2 respectively) from KCN in the presence of 2DG. These findings show that this system will allow us to find inhibitors of anaerobic metabolism and so, hopefully anthelmintic targets.

1175A Persistent and multi-generational metabolic effects of developmental inorganic arsenic exposure in C. elegans deficient in electron transport chain proteins.  T. Leuthner, A. Luz, J. Meyer  Nicholas School of the Environment, Duke University, Durham, NC.

Perturbations in energy metabolism during development result in persistent effects lasting into adulthood. However, it is not well understood how exposure to pollutants during development may drive these persistent metabolic changes. Arsenic, a ubiquitous drinking water contaminant, is known to inhibit metabolic enzymes, induce ROS production, and shift cells to a more glycolytic state. These metabolic effects may explain diseases observed in people worldwide consuming unsafe levels of arsenic, including immune and endocrine system dysfunction and cancer. Therefore, we use C. elegans as a model to study arsenic-induced mitochondrial dysfunction in vivo. We hypothesize that arsenic-induced mitochondrial stress from early life exposure could result in persistent, altered metabolic profiles in C. elegans later in life, as well as in progeny, and that this effect could be exacerbated in mitochondrial-deficient mutant strains. We exposed C. elegans strains with mutations in electron transport chain complex proteins (mev-1, ncu-6, isp-1, and atp-2) to inorganic arsenic, iAs, during larval development and measured growth, reproduction, lifespan, steady state ATP levels, and oxygen consumption rates following exposure and until 10 days of age. Though mutants varied in sensitivity, all strains showed a significant decrease in ATP levels after a 48hr exposure to 0.5mM sodium arsenite. Interestingly, decreased ATP levels persist throughout life in complex III deficient mutants (isp-1), as does a significant decrease in maximal respiratory capacity. To understand the potentially mito-hormetic effect of an early life exposure, developmentally iAs-exposed adult C. elegans were exposed to heat as a secondary stressor. Response varied among strains, but there was an increase in heat sensitivity in complex II and complex III mutants that were exposed to iAs during development. We also found a strain-specific variation in lifespan, in which wildtype C. elegans and complex V mutants (atp-2) exhibit decreased lifespan, while complex III mutants (isp-1) have a slight increase in lifespan after developmental arsenic exposure. Finally, in order to test for multi-generational effects, relative ATP levels were measured in progeny. Surprisingly, we found that progeny of parental generation to secondary stressors indicates a mechanism other than hormesis. Nevertheless, these results provide insight into potential mechanisms of health effects observed in human populations exposed to arsenic during sensitive developmental time points.


C. elegans has recently been advanced as a premier metazoan model organism for the study of metabolism, with the publication of two whole-genome metabolic models (1, 2). Using these models together with -omics data allows the in-depth data-driven exploration of systems-level metabolism using in silico simulations. In a GENie workshop to be held April 2017 at the Babraham Institute, Cambridge, UK, the relationships between these two existing metabolic models will be explored with the objective of generating a consensus model. Because the two reconstructions are still incomplete, and certain important pathways and areas of metabolism are currently under-annotated, we aim to identify specific areas that are relevant to the C. elegans community and prioritise them for further annotation in a follow-up community-driven “annotation jamboree” workshop. This poster will describe the main objectives set by the first workshop and opens the invitation to the C. elegans metabolic research community to contribute to the follow-up annotation efforts.


Maintenance of lipid homeostasis is crucial for cell in response to lipid requirement or surplus. The transcription factors SREBPs play essential roles regulating lipid metabolism, and are associated with many metabolic diseases. However, the SREBP regulation in lipid metabolism is still less understandable. Here, we show that SBP-1, a homologue of SREBPs in C. elegans, represses zinc level to maintain the activity of its target stearoyl-CoA desaturase (SCD) and lipid homeostasis. Reciprocally, reduction of zinc bypasses SBP-1 to directly activate SCD through iron overload, promoting lipid biosynthesis and accumulation; whereas it may feedback to reduce the transactivation of SBP-1 on SCD. Additionally, acyl-CoA synthase (ACS-1) acts upstream of SCD for zinc-reduction induced lipid accumulation. Collectively, we reveal a zinc-mediated regulation of
SREBP-SCD axis in lipid metabolism, distinct from the negative regulation of SREBP-1 or SREBP-2 by phosphatidylcholine (PC) or cholesterol, respectively, thereby providing novel insights into regulation of lipid homeostasis.

1178A  Serotonergic regulation of fat metabolism. N.K. Littlejohn1,2,3, E.B. Vansstrun1,2,3, S. Srinivasan1,2,3 1) The Dorris Neuroscience Center; 2) Department of Molecular Medicine; 3) The Scripps Research Institute, La Jolla, CA.

The increasing prevalence of obesity is a global health problem. Reversing obesity at any age is strongly associated with many beneficial health outcomes including ameliorating metabolic diseases such as liver disease, diabetes, heart disease, increased lifespan, and remarkably, improved cognitive function. The Srinivasan Lab has determined that the neuromodulator serotonin is a major regulator of whole body metabolism. Elevated endogenous serotonin (5-HT) released from sensory neurons or exogenous 5-HT treatment induces fat loss. Importantly, 5-HT significantly increases adipose triglyceride lipase (ATGL-1) expression in the intestine, and 5-HT-induced fat loss is dependent on ATGL-1. 5-HT-induced fat loss stimulates the release of an FMRF-Like Peptide, FLP-7, which resembles the mammalian tachykinin peptides. FLP-7 is a neuroendocrine peptide that is released from the nervous system and activates its cognate receptor, NPR-22, in the C. elegans intestine. Since mutants lacking FLP-7 or NPR-22 fail to respond to 5-HT, the reduced fat caused by 5-HT treatment is dependent on FLP-7 and NPR-22 signaling. NPR-22 activity regulates ATGL-1 via transcriptional and post-translational mechanisms to effect fat loss in the intestine. From an RNAi-based screen, our lab has identified two transcription factors, HLH-11 and NHR-76, required for the transcriptional regulation of ATGL-1 by 5-HT. In preliminary studies, we determined that the intestinal AMPK a1 kinase subunit, AAK-1, and the p38 MAP kinase, PMK-1, are required for 5-HT-mediated fat loss. Mutants lacking AAK-1 do not exhibit 5-HT induced fat loss, and pmk-1 null mutants have elevated fat content that is only slightly reduced with 5-HT treatment. Our hypothesis is that these kinases regulate the activity of enzymes that convert fat to energy.

We are currently unraveling the precise molecular mechanisms by which FLP-7/NPR-22 signaling acts in the intestine to stimulate fat loss, and the ultimate consequences of this pathway for the lifespan of C. elegans. All the molecular players of this pathway are ancient and conserved. Thus, discovering previously unknown mechanisms of lipolysis control has major implications for our understanding of metabolic diseases.

1179B  Exploring the effect of glycine metabolism on the life history of C. elegans. Yasmine Liu, Arwen Gao, Reuben Smith, Riekelt Houtkooper Laboratory Genetic Metabolic Diseases, Academic Medical Center, Amsterdam, The Netherlands.

Amino acid, fatty acid and carbohydrate metabolic processes compose three major nutrient metabolisms in a living organism and dysregulations of these processes are considered as the culprits of aging and aging-related diseases. In recent years, to untangle the roles of them in aging, extensive studies have been performed especially in C. elegans, yet the questions about how they contribute to aging individually and interactively are still not fully answered. In this study, we focus on amino acid metabolism with the ultimate goal to elucidate its role in aging. Utilizing a sensitive mass spectrometry platform, we analyzed the amino acid profiles throughout the worm’s life history. Most amino acids peaked during development, except aspartate and glycine, which exclusively accumulated in aged worms. We aimed to elucidate what is the causal relation between the glycine/aspartate accumulation and lifespan. We tested gene expression levels of genes involved in glycine and aspartate metabolism, and found that genes involved in glycine degradation or consumption are decreased with age, while synthesis appears unchanged, which fits with the glycine accumulation. We also found in BXD recombinant inbred mouse strains that the abundance of glycine in the liver positively correlated with the lifespan of the cohort. Finally, and in line with these associations, supplementation of glycine in the worm diet remarkably extends the lifespan, further validating its beneficial role in aging. Therefore, we are currently looking into the different glycine pathways to figure out how glycine exerts this influence on worm longevity.

1180C  Investigating Interactions between Steroid Hormone Signaling and Nuclear Receptors DAF-12 and NHR-8: Key Regulators of C. elegans development. P. Loi1, P. Gudibanda1,2, A. Ludewig1, P. Mahanti1,3, A. Antebi4, F. C. Schroeder1,3 1) Boyce Thompson Institute, Ithaca, NY; 2) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY; 3) Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY; 4) Max Planck Institute for Biology of Ageing, Cologne, Germany.

Nuclear hormone receptors (NHR) are evolutionarily conserved, ligand-modulated transcription factors that interact with coregulatory proteins to control development, metabolism and adult lifespan. In C. elegans, NHR-8, a homolog of the mammalian LXR and FXR nuclear receptors is implicated in regulating cholesterol and bile acid steroid homeostasis, and fatty acid metabolism. NHR-8 shares conserved sequence homology with another NHR, DAF-12, which acts as the central modulator to mediate both larval development and lifespan. In unfavorable conditions, interactions between unliganded DAF-12 with its co-repressor DIN-1 promotes entry to the developmentally arrested dauer state, while in favorable conditions, steroidal ligands, the dafachronic acids (DAs), prevent co-repressor binding to promote reproductive development via the let-7 family of microRNAs. In contrast to DAF-12, the endogenous ligands of NHR-8 remain unknown. Previous work has identified three chemically distinct endogenous DAs as ligands of DAF-12 through comparative metabolomics and NMR spectroscopic analysis, and recently, DAs have been shown to interact with NHR-8 signaling by counteracting the population-density-dependent acceleration of development in vivo. Additionally, independent of DAF-12, NHR-8 has been demonstrated to be required for DA-mediated lifespan extension under dietary restriction. We show that NHR-8 and DAF-12 form heterodimers via the DNA-binding domains and that dimerization is not affected by ligands in vitro. Furthermore, we demonstrate in vitro that, like DAF-12, the ligand-binding domain of NHR-8 binds to the co-repressor DIN-1. Utilizing a library of C. elegans metabolome fractions, we have initiated a screen to identify NHR-8 ligands using a high throughput cell culture based bioactivity assay and a bead-based affinity AlphaScreen assay. In addition, we are testing metabolome fractions for potential effects on heterodimer formation and DIN-1 recruitment by NHR-8. Based on preliminary
results, we propose a model for DAs and other small-molecule ligands to regulate reproductive development and diapause via DAF-12 and NHR-8.

1181A  

*Paullinia cupana* and caffeine reduced the triacylglycerol content of *Caenorhabditis elegans* supplemented with glucose.  


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Obesity is characterized by an excess of adipose tissue and is associated with a series of lipid disturbances, such as hypercholesterolemia and high triglyceride levels. Many methods are used to treat obesity, most of them are pharmaceuticals, which might cause collateral effects. *Paullinia cupana* (guarana) is widely consumed in Brazil, and has high concentration of caffeine, which is known for its stimulant and thermogenic properties in vitro. These compounds are usually consumed in energy drinks or used as diet supplementation for weight reduction. Thus, the aim of this study was to investigate chronic exposure effects of *Paullinia cupana* or caffeine *in vivo* on triglyceride levels of *Caenorhabditis elegans* supplemented with glucose. We treated N2 wild-type from L1 stage to young-adult with *Paullinia cupana* at 1 mg/mL or caffeine at 50 uM. To perform the assay of worms with more lipid accumulation, 15 mM of glucose was added to the medium. The parameters analyzed were antimicrobial activity of the compounds, quantification of triglycerides levels, pharyngeal pumping rate, defecation cycle and brood size. The compounds analyzed did not present antimicrobial activity. *Paullinia cupana* or caffeine did not alter the triglycerides content of N2 worms, but when the worms were treated with 15 mM glucose, the triglyceride levels were increased about 94% compared with control group, and the treatments with *Paullinia cupana* or caffeine showed a decrease in triglyceride levels about 40%. It was observed that neither *Paullinia cupana* nor caffeine presented any effect on baseline triglyceride levels, but when the animals were supplemented with glucose, caffeine and *Paullinia cupana* prevented the increase of triglyceride levels. Furthermore, *Paullinia cupana* and caffeine did not alter pharyngeal pumping rate, defecation cycle and brood size. So, the use of drinks with stimulating properties could be used as modulator of fat storage in order to control obesity, a worldwide problem.

1182B  

O-GlcNAC cycling and mitochondrial function.  

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O-linked-N-acetylglucosamine (O-GlcNAC) modified proteins are critical in myriad cellular processes and functions. Abnormal O-GlcNACylation is associated with a spectrum of diseases, including Alzheimer’s disease, cancer, cardiovascular disease, and diabetes. Mitochondrial protein O-GlcNACylation is emerging as a key regulator of cellular energetic metabolism, redox signaling and cell survival pathways, but the mechanisms involved are largely unknown. O-GlcNAC transferase (OGT) is the enzyme responsible for the addition of O-GlcNAC to target proteins while O-GlcNACase (OGA) catalyzes the removal of the modification from target proteins. OGT and OGA are encoded by single genes in *C. elegans* (ogt-1 and oga-1, respectively). OGT and OGA modulate lifespan, stress susceptibility and oxidative stress resistance. We hypothesize that O-GlcNAC cycling mediated by OGT and OGA plays a role in regulating mitochondrial metabolism and morphology. To that end, we measured oxygen consumption rate (OCR), a proxy for mitochondrial oxidative phosphorylation function, in N2, ogt-1 and oga-1 null mutants. OCR was measured using the Seahorse Bioscience XF24 Extracellular Flux Analyzer and normalized by number of worms for each individual well. Our results suggest that altered O-GlcNAC cycling reduces oxygen consumption by negatively impacting mitochondrial oxidative metabolism. In addition, O-GlcNAC cycling mutants have altered sensitivity to rotenone, an inhibitor of NADH ubiquitone reductase (Complex I). These results suggest that modulation of mitochondrial function by O-GlcNAC cycling may underlie some of the phenotypes of O-GlcNAC cycling mutants.

1183C  

Screening for temperature sensor in ASJ sensoryneuron regulating cold tolerance.  

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Temperature is important environmental information for biological reaction. Animals have acclimation mechanism to environmental temperature changes. *C. elegans* has cultivation temperature-dependent cold tolerance. Wild-type animals cultivated at 20-degree died after 2-degree cold stimuli. By contrast, 15-degree cultivated-animals can survive after 2-degree. We are using the cold tolerance as a model for studying temperature signaling in animal. Previously reported, ASJ sensory neuron that is known as light and pheromone-sensing neuron senses temperature through trimeric G-protein pathway, which is required for photo signaling (Ujisawa et al., PLOS ONE, 2016). However, photoreceptor LITE-1 was not involved in cold tolerance and temperature sensation in ASJ(Ohta, Ujisawa et al., Nature commun, 2014). To identify the temperature sensing receptor upstream of trimeric G protein in ASJ, we are screening G protein-coupled receptor (GPCR). Mutant animals defective in G protein signaling in ASJ showed abnormal increase of cold tolerance after 20-degree cultivation. We performed exhaustive RNAi screening for about 1000 GPCR genes, as a result, about 40 gene-knocked down animals respectively showed strong abnormality in cold tolerance. We observed expression patterns of these GPCR genes by using GFP, and at least 14 genes including srg-37, str-45, str-92, srd-48, str-19, srd-244 and srt-72 were expressed in ASJ sensoryneurons. We constructed knockout mutants of respective GPCR genes by using CRISPR/Cas9 system, and analyzed cold tolerance. However, these mutants did not show abnormal cold tolerance. We are therefore constructing mutant animals defective in multiple GPCR genes, and measuring cold tolerance of these mutants. So far, we have constructed the septuple mutant, and we are measuring their cold tolerance.
Fluorouracil (5-FU) is a fluoropyrimidine and uracil analogue commonly used in the treatment of colorectal cancer. 5-FU exerts cytotoxicity by inhibiting nucleotide synthesis and by misincorporating into RNA and DNA, leading to cancer cell damage and death. Despite the widespread use of this drug, new approaches are needed to boost efficacy and decrease toxicity. Furthermore, 5-FU treatment outcome is variable between patients and cannot be completely explained by genetic factors, suggesting that environmental factors could contribute to its efficacy. The gut microbiota is defined as the collective community of microbes that reside a host gastrointestinal tract, and have recently been linked to other elements of host fitness, such as immunity and metabolism. However, not much is known about the interplay between drug, gut microbes and the host. To unravel the mechanism by which the microbiota is affecting the metabolism of 5-FU, we used the nematode C. elegans as a host model and microbe E. coli as a gut microbe model. Via high-throughput screens and metabolomic approaches we found that microbes can affect drug conversion and efficacy in the host. We discovered this effect to be influenced by bacterial metabolism and synthesis of ribonucleotides and vitamins B₉ and B₆. Additionally, we find deoxynucleotide pools imbalances in bacteria increased autophagy and cell death on host cells upon 5-FU treatment. Interestingly, this effect was found to be dependent and regulated by the host gene nucleoside diphosphate kinase ndk-1. In summary, our data suggests bacteria can contribute to drug efficacy on host metabolism through two distinct but complementary processes. These findings emphasize the importance of the gut microbiome in the co-metabolism of host-targeted drugs. Moreover, this study reveals the potential therapeutic power of manipulating gut microbial communities to help treat disease and improve treatment outcomes.

References:

Diet Influences Stress and Innate Immune Resistance in C. elegans

The opportunistic, multi-host pathogen Pseudomonas aeruginosa causes a wide-variety of hospital acquired infections, as well as being a chief contributor to morbidity and mortality in cystic fibrosis patients. Unfortunately, this bacteria is notoriously resistant to antimicrobials and readily acquires additional resistance mechanisms. To simplify identification of novel anti-Pseudomonal compounds, a liquid-based C. elegans-P. aeruginosa pathogenesis assay (called Liquid Killing) was developed. In previous publications, we have reported on the characterization of this assay, and the identification of pyoverdine, the main siderophore (or iron-acquiring molecule) of P. aeruginosa, as the key determinant of pathogenesis.

During our characterization of pyoverdine-mediated pathogenesis and C. elegans' defense against it, we performed conventional RNAi studies using the HT115(DE3) strain. To our surprise, we noted a substantial increase in resistance to Liquid Killing when compared to OP50. Since pyoverdine-mediated killing involves the removal of host iron, we initially hypothesized that HT115 more effectively mobilized and provided iron to C. elegans. Mass spectrometric and fluorometric analyses disproved this hypothesis. We also ruled out weak OP50 pathogenesis; we compared pathogenesis using RNAi targeting innate immune genes in either the OP50 or HT115 backgrounds and saw no differences from wild-type worms. Further experimentation revealed that worms reared on HT115 also show increased resistance to other abiotic stresses (e.g., heat shock, oxidative stress) as well.

In the absence of any other obvious candidates, we turned to microarray transcriptional profiling to gain an unbiased picture of the differences that result from feeding C. elegans with HT115 instead of OP50. To our surprise, there were a relatively small number of genes differentially expressed between worms fed OP50 and HT115. Amongst them were several mitochondrially-associated genes, suggesting that the relevant difference may involve this organelle. Using a variety of approaches, we determined that worms fed HT115 exhibit increased mitochondrial health than their OP50 counterparts. Furthermore, supplementation of growing OP50 with cobalamin recapitulated virtually all of the HT115 phenotypes.
storage. Interestingly, both mtch-1 depletion and overexpression causes complete sterility indicating that tight regulation of mtch-1 levels is required to allow reproduction. mtch-1 mutants live longer than wild type or mtch-1 heterozygotes, suggesting that mtch-1 could cause moderate mitochondrial dysfunction.

In mammals, MTPCH2 regulates estrogen receptor 1 (ESR1) activity and we find that nhr-88 and nhr-252 could be functional ESR1 homologs since their RNAi rescues the reduced lipid phenotypes of the mtch-1 mutant worms.

1187A Mitochondrial stress elicits a metabolic cytoprotective response by coordinated upregulation of multiple branches of the mevalonate pathway. S. Levin, O. Oks, A. Sapir Department of Biology and the Environment, Faculty of Natural Sciences, University of Haifa, Oranim, Tivon, 36006 Israel.

Fundamental for human health and disease, the mevalonate pathway catalyzes the biosynthesis of cholesterol, mitochondrial electron carriers, a lipid anchor for N-glycosylation, and isoprenyls for protein prenylation. Recent data have linked the mitochondrial stress response (MSR) with mevalonate pathway metabolism but the molecular basis of this effect remains largely unknown. We hypothesized that the MSR regulates specific enzymes of the pathway as part of the protective response to mitochondrial stress. Surprisingly, we found that the MSR does not affect the levels of the pathway’s rate-controlling enzyme, HMG-CoA reductase, but rather upregulates the first enzyme of the pathway HMG-CoA synthase. This upregulation relies on the activity of three transcription factors, ATFS-1, DVE-1, and SKN-1 whose coordinate activation can protect the organism from mitochondrial dysfunction. Extending our analysis to the entire pathway, we identify two additional regulatory mechanisms: 1) The MSR upregulates enzymes of electron carriers biosynthesis, probably to alleviate mitochondrial dysfunction. 2) The MSR upregulates enzymes participating in the geranylgeranylation of small GTPases whose activation is requisite for MSR execution. To identify the GTPases that play a role in this process, we screened the C. elegans genome and identified two GTPases, RHEB-1 and RAL-1, as critical mediators of the MSR. Our study explains, at the molecular level, how the mevalonate pathway is upregulated during mitochondrial stress and uncovers the physiological significance of this upregulation as a mechanism to protect the organism from mitochondrial dysfunction.

1188B A function of mtDNA effects copy number regulation in C. elegans. B. Saunders, C. Kirby, M. Patel Vanderbilt University.

Eukaryotes contain both a nuclear genome and a mitochondrial genome (mtDNA). Generally organisms contain hundreds to thousands of copies of mtDNA per cell. It is known that a mechanism exists that closely regulates the copy number of mtDNA within the cell, but this mechanism is still not well understood. The mtDNA encodes essential parts of the electron transport chain (ETC). The ETC is responsible for generating most of the ATP within the cell. We have characterized C. elegans strains whose mtDNA copy number is increased by up to 2 orders of magnitude. We hypothesize that some function of mtDNA is coupled with its copy number regulation. I am currently developing assays to measure concentrations of a number of different metabolites that might play a role in mtDNA copy number regulation. I will present data from these experiments.

1189C Assessing the role of the Forkhead-8 transcription factor in dopamine metabolism. D.A. Shelton1, B. Cawthon2,1, B. Nelms1,2 1) Biology, Fisk University, Nashville, TN; 2) Cellular and Molecular Biology, Vanderbilt University, Nashville, TN.

Rationale: Dysregulation of dopamine signaling is a common hallmark for Parkinson’s disease, schizophrenia, and depression. Studies of aberrant dopamine signaling linked to human disease have shown that systemic loss of dopaminergic neurons, mutations in the dopamine transport protein (DAT-1), and loss of dopamine receptors can be involved in disease onset. However, less is known about mediators involved in regulation of the dopamine metabolism machinery. Thus, there is a need to further elucidate new targets involved in regulating dopamine signaling and metabolism.

Hypothesis: The forkhead domain-containing protein, Forkhead-8, in Caenorhabditis elegans (C.elegans) is a transcription factor required for dopamine function that may act on the dopamine metabolism machinery, including genes encoding catechol-o-methyltransferase (COMTs) and monoamine oxidases (MAOs).

Methods: Using wild-type worms (N2) and deletion mutants for the genes encoding FKH-8, and the monoamine oxidase AMX-2, I am assessing changes in the dopamine-related swimming induced paralysis (SWIP) assay after COMT and MAO inhibitor treatments. The drugs clorgyline and tolcapone were used to inhibit MAO and COMT, respectively. Each worm genotype is separated into 3 groups: clorgyline only (treated with 25uM clorgyline), tolcapone only (treated with 100uM tolcapone), and dual treatment (25uM clorgyline/100uM tolcapone). Preliminary data suggest that deletion of FKH-8 is involved in perturbations of dopamine metabolism via AMX-2 and COMT enzymes.

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Zinc is essential for proper cell function as it plays important catalytic and structural roles in many proteins. However, exposure to high zinc doses is toxic and causes cell stress. To maintain homeostasis, levels of zinc detoxification genes are adapted through transcriptional regulation mechanisms that play an essential role in allowing the organism to deal with environmental changes. The key players in transcriptional regulation are transcription factors (TF), their regulatory DNA elements, and co-regulators such as the Mediator complex. We previously showed that the Mediator complex subunit MDT-15 is required for the regulation of many gene sets in Caenorhabditis elegans, including zinc responsive genes. Our preliminary data show that the
Mediator subunit CDK-8 also plays an important role in this response. While zinc is an essential trace metal, the mechanism of high zinc detoxification is shared with those that protect against cadmium, a non-essential toxic metal. However, the TF and DNA elements interacting with these Mediator subunits in zinc homeostasis regulation remain unknown. Here, we examine which factors collaborate with MDT-15 and CDK-8 to regulate zinc homeostasis and cadmium stress response, and study the physiological role of these Mediator subunits. By using the promoter of the zinc and cadmium responsive gene, cdr-1, we found that cdr-1 induction by cadmium and zinc depends on mdt-15 and cdk-8, as well as the transcription factors nhr-33/hizr-1 and elt-2. We also found that NHR-33 physically interacts with MDT-15. Moreover, the basal mRNA levels and metal-inductions of several zinc responsive genes are strongly mdt-15 dependent and partially cdk-8 dependent. To assess zinc storage in Mediator mutants, we studied the gut granules of C. elegans, necessary for zinc homeostasis, in mdt-15 and cdk-8 mutants. Lastly, we explored the regulatory conservation of this new mechanism. The insulin-secreting β-cells of the pancreas require appropriate amounts of zinc for proper insulin storage in insulin granules, and deregulated zinc metabolism and mutations in zinc transporter genes are linked to a higher diabetes risk. Using mice that lack the mdt-15 ortholog Med15 in the pancreatic β-cells, we found that Med15 is required to express the Slc30a8, the ortholog of the mdt-15-regulated transporter cdfl-2. Collectively, our data suggest that mdt-15 and cdk-8 are required for normal zinc storage, and that this may be a conserved regulatory mechanism.

1191B The role of DAF-2 in the transmission of maternal and paternal nutritional status during embryogenesis. Jeff Simske, Yi Dong Rammelkamp Ctr, Cleveland, OH.

Proper maternal nutrition is essential for embryonic viability and normal healthspan, but the mechanisms that transmit nutritional information to the embryo are not fully described. Several mutants show sensitivity to elevated dietary glucose, including agl-1 (glycogen debranching enzyme), aak-2 (AMPK alpha subunit), and mutants identified in a conditional glucose-sensitized screen, gin-1(cv10) and gin-2(cv11). Generally, embryonic lethality of these mutants is suppressed by growth on a rich bacterial diet (HB101), and enhanced by growth on a restrictive diet (DA837). agl-1 sensitivity to elevated glucose is dependent on insulin signaling, as mutations in daf-2 suppress the agl-1 glucose-dependent embryonic lethality. Involvement of insulin signaling pathways during embryogenesis is also supported by the observations that certain daf-2 alleles alone or in combination with daf-16 display embryonic defects. Analysis of daf-2; daf-16 animals identified defects in polar body extrusion, cytokinesis and cell polarity, assessed by NMY-2::GFP and PAR-6::GFP localization. To monitor DAF-2 embryonic expression, CRISPR/Cas9 was used to introduce a GFP tag into the daf-2 locus, creating a functional C-terminal tagged GFP fusion protein. DAF-2::GFP is expressed in the early embryo, and expression appears to be influenced by nutritional status, as previously observed. Specifically, conditions of both elevated glucose or dietary restriction reduce DAF-2::GFP levels in the nervous system as well as in embryos; certain diets appear to result in an increase in cytoplasmic versus membranous DAF-2::GFP. Surprisingly, we discovered that DAF-2::GFP is expressed in sperm, and is localized at centrosomes in the zygote during early cell divisions, suggesting paternal DAF-2 functions in embryogenesis. Consistent with this observation, we found that nutritional information could be transmitted paternaly; for example, agl-1 males grown on a rich diet rescued embryonic lethality in progeny from hermaphrodites grown on a poor diet. Similarly, males with a poor diet mated to well-fed hermaphrodites conferred increased embryonic lethality. Taken together, these results suggest that at least some nutritional status is transmitted via sperm, possibly by DAF-2 itself. Given the disparate daf-2; daf-16 phenotypes, it appears likely that nutritional status, cell polarity, polar body extrusion, and other embryonic developmental processes may involve distinct daf-2 signaling pathways.

1192C Metabolomics and fluxomics reveal a metabolic fingerprint of the long-lived mutants daf-2 and eat-2. R.L. Smith, A.W. Gao, M. van Weeghel, R.H. Houtkooper Genetic Metabolic Disease, Academic Medical Center Amsterdam, Amsterdam, NL.

The revival of metabolism as a major focus in healthcare and ageing research has yielded many new techniques and insights. We developed and applied state-of-the-art metabolomics techniques on the Q-Exactive platform to analyze the long-lived mutants, we studied the gut granules of C. elegans, necessary for zinc homeostasis, in mdt-15 and cdk-8 mutants. Lastly, we explored the regulatory conservation of this new mechanism. The insulin-secreting β-cells of the pancreas require appropriate amounts of zinc for proper insulin storage in insulin granules, and deregulated zinc metabolism and mutations in zinc transporter genes are linked to a higher diabetes risk. Using mice that lack the mdt-15 ortholog Med15 in the pancreatic β-cells, we found that Med15 is required to express the Slc30a8, the ortholog of the mdt-15-regulated transporter cdfl-2. Collectively, our data suggest that mdt-15 and cdk-8 are required for normal zinc storage, and that this may be a conserved regulatory mechanism.

1193A Xanthine Dehydrogenase in neuron is required for cold tolerance. Natsune Takagaki1, Akane Ohta1, Yohei Minakuchi2, Atsushi Toyoda1, Atsushi Kuhara1 1) Graduate school of Natural Science & Institute for Integrative Neurobiology, Konan University, Kobe, Japan; 2) National Institute of Genetics, Japan.
Animals can survive and proliferate under continual environmental temperature changes, by receiving temperature information and processing previous temperature experience. We are studying about molecular physiological mechanism of cold tolerance. Wild-type animals can not survive at 2 degree after cultivation at 25 degree, however, 15 degree-cultivated animals can survive at 2 degree.

In order to isolate the molecules involved in cold tolerance, we used DNA microarray analysis. The expression level of flp-17 gene was significantly changed by temperature stimuli, and then RB2575 flp-17(ok3587) animals showed defective cold tolerance. However, abnormal cold tolerance of flp-17(ok3587) was not restored by the expression of flp-17 genomic gene. Besides, another null mutant flp-17(n4894) showed normal cold tolerance. We therefore hypothesized that background mutation(s) excepting flp-17(ok3587) causes defective cold tolerance. We decoded whole genome sequence of RB2575 flp-17(ok3587) mutant, by using next generation DNA sequencer. Through the SNP analysis, we found that responsible gene of abnormal cold tolerance in RB2575 was F55B11.1 gene. F55B11.1 protein is highly homologues to Xanthine dehydrogenase (XDH) and then we officially named this gene xdh-1. RB2575 strain has a severe mutation in xdh-1, and xdh-1 null mutant showed abnormal cold tolerance. Defective cold tolerance of both RB2575 and xdh-1 mutants were rescued by genomic xdh-1 gene. Rescuable genomic xdh-1::GFP reporter was expressed in some neurons in the head, intestine and excretory cell. To determine the responsible cells and tissues for defective cold tolerance in xdh-1 mutant, we performed a cell-specific rescue experiment by expressing xdh-1cDNA under driven by specific tissue promoters. We found that abnormal cold tolerance of xdh-1 mutant was rescued by expressing XDH in neurons but not in excretory cell or intestine. We are trying to identify specific neurons for XDH-dependent cold tolerance. Since xanthine dehydrogenase (XDH) produces uric acid known as an antioxidant causing ROS accumulation, we speculated that ROS accumulation in specific neuron causes abnormal cold tolerance.

**1194B Characterization of the yq187 mutant that affects mitochondrial homeostasis in *C. elegans*.** R. Tang¹, ³, J. Zhou¹, ³, Q. Gan¹, ³, C. Yang¹, ²  ¹) Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China; ²) Center for Life Sciences, School of Life Sciences, Yunnan University, Kunming, China; ³) University of Chinese Academy of Sciences, Beijing, China.

Mitochondria play essential roles in ATP production, amino acid and lipid metabolism, as well as metal homeostasis. Mitochondrial dysfunction may lead to apoptosis, aging and neuronal degeneration diseases. Thus maintenance of mitochondrial homeostasis is pivotal for their functions and individual health. However, the mechanisms governing mitochondrial homeostasis are not fully understood. To explore this issue, we established *C. elegans* hypodermis as a system to study mitochondrial homeostasis using genetic approaches. We first generated an integrated array (yqls157) that expresses mitochondrion-targeted GFP (mito-GFP) in the hypodermis of *C. elegans*, and then performed genetic screens to look for mutants that exhibit morphological change of mitochondria. By screen of more than 10,000 haploid genomes we obtained 22 mutants. Among them, the yq187 mutants showed abnormally enlarged mitochondria. However, yq187 did not cause obvious mitochondrial changes in other tissues. The enlarged mitochondria in yq187 animals had abnormal membrane potential and greatly reduced ATP production. In addition, yq187 worms had shorter body length than the wild type. These findings suggest that the hypodermal mitochondria in yq187 mutants were dysfunctional. Genetic analysis indicated that the enlargement of mitochondria in yq187 mutants requires mitochondrial fusion machinery. By SNP mapping, we determined that the gene affected by yq187 is located on chromosome X. We are in the process of cloning this gene and investigating its role in mitochondrial homeostasis.

**1195C Effects of vitamin B₁₂ deficiency on fertility, growth and survival of *Caenorhabditis elegans* cultured in axenic medium.** Surafel Tegegne, Mattew Flavel, Markandeya Jois  Department of physiology anatomy and microbiology, La trobe University, Melbourne, Victoria, Australia.

Animal models of vitamin B₁₂ deficiency have been proven to be difficult due to the storage of the vitamin and the need to maintain animals on deficient diet for extended periods. *C. elegans* has emerged recently as a suitable alternative due to its short lifespan. *C. elegans* show signs of B₁₂ deficiency including poor growth rate, low fertility and a shortened lifespan when fed E. coli of low B₁₂ content for five successive generations. However, bacterial diets can impose confounding effects on treatments as they modify nutrients by their metabolism. Aim of the present study was to investigate the effects of vitamin B₁₂ deficiency under axenic conditions to avoid the confounding effects of bacteria. Wild type *C. elegans* (N2) were conditioned to *C. elegans* Habitation and Reproduction (CeHR) medium for three generations before culturing in either complete or deficient (no added B₁₂) medium for an additional three generations. Fertility of wild type *C. elegans* (N2) in deficient CeHR declined significantly (P<0.05) by 52%, 74% and 85% in the first, second and third generations respectively compared to complete CeHR. Body length measured at day 2 from L1 of *C. elegans* maintained in deficient CeHR was reduced by 63%, 70% and 64% in the three subsequent generations compared to complete CeHR. At day 6 from L1, where all the worms attained their maximum body length, worms in deficient CeHR showed 17%, 27% and 38% reduction in the three subsequent generations respectively compared to worms in complete CeHR. Survival of B₁₂ deficient worms was not affected in the first two generations with a significant reduction (P<0.05) of 13% in the third generation. The results show that *C. elegans* maintained under axenic media of low B₁₂ develop signs of deficiency more rapidly when compared previous reports of deficiency under monoxenic conditions.

**1196A Parallelized single animal measurement of growth and reproduction: Do worms optimally control their rates of growth and aging?** B.D. Towbin, H Grosshans  Friedrich Miescher Institute for Biomedical Research, FMI, Basel, CH.

The juvenile growth rate of worms differs depending on the environmental conditions, such as the quality of the food source.
What limits the growth rate of worms? Do worms grow as fast as possible in each environment, or do worms attenuate their growth rate under some conditions, e.g. to improve their performance at other tasks?

Naively, a fast developmental growth rate may seem beneficial for organismal fitness. However, fast juvenile growth may have adverse effects later in life with negative impact on reproduction, aging, or survival. We hypothesize that worms actively adjust the allocation of their resources to growth to their environment, and that worms thereby optimize their life history to maximize their fitness in different environmental conditions. To test this hypothesis and understand the mechanisms involved, we measure growth and reproductive schedules of hundreds of individual animals in parallel. Exploiting the endogenous variability among individuals, we aim at measuring how the reproductive fitness of worms depends on their juvenile growth rate, and how the optimal growth rate changes between different environments.

1197B The GRP170 Genes of Caenorhabditis elegans.  G. Wadsworth1, Y. Li2, A. Rockwell3  1) Biology, SUNY Buffalo State, Buffalo, NY; 2) Biological Sciences, SUNY Binghamton, NY; 3) Biology, Clarkson University, Potsdam, NY.

GRP170 functions as an ER co-chaperone by acting as a nucleotide exchange factor for BiP. It also directly mediates ER protein metabolism by binding to protein folding intermediates. In the nematode order Rhabditida, a gene duplication has generated two highly diverged GRP170 loci, in C. elegans grp170a (T24H7.2) and grp170b (T14G8.3). The structure of the gene family combined with the genetics of C. elegans provides a unique opportunity to study the physiological role of GRP170.

In worms treated with tunicamycin, an N-glycosylation inhibitor, grp170b’s mRNA was induced 6 fold while grp170a’s expression did not significantly change. Tunicamycin induction of grp170b was dependent on the Unfolded Protein Response IRE-1 pathway. A deletion allele for grp170a (tm3109) had only minor phenotypic effects, most notably delayed development. Under standard culture conditions, no phenotypic effects were observed for the deletion allele of grp170b (ok502). Although, deletion of grp170b did provide a slight increase in resistance to tunicamycin. There was a maternal effect on the phenotype of worms homozygous for deletion alleles at both loci. Lack of maternal GRP170a resulted in embryonic arrest (as evidenced by the lack of double mutant larva) while lack of maternal GRP170b resulted in early larval arrest. These data suggest that GRP170a and GRP170b share a critical overlapping function that is masked by a functional allele at either locus and is only apparent in the double mutant. Analysis of deletion alleles at individual GRP170 loci showed that grp170a plays a more important role in the general physiology of worms. Induction of grp170b mRNA by the unfolded protein response and its association with tunicamycin sensitivity suggests grp170b may play a stress-related role.

1198C Dissecting glucose metabolism’s involvement in mating behavioral decay during aging process in C. elegans males.  Y. Wan, L. Garcia  Department of Biology, Texas A&M University, College station, TX.

Motor execution of male C. elegans mating behavior decays after 2 to 3 days of adulthood. However, the mechanism by which aging drives this decay is not clear. Our previous studies found that copulation decay correlated with increased spicule intromission circuit excitability and altered metabolism in aged males. We hypothesized that the altered metabolism induces the increases of neuromuscular circuit excitability, resulting in the decay of mating behavior in aged males. To test this hypothesis, we investigated a hexokinase paralogue, hxk-1, whose expression dramatically increases and hypothetically contributes to the altered glucose metabolism in aged males. Using CRISPR-Cas9 technique and Cre-lox system, we generated a genetic tool that allows us to spatiotemporally knockout hxk-1 and design assays to evaluate the effects on mating behaviors of the knockouts. Consistent with the hypothesis that increased expression of hxk-1 contributes to the decay in mating potency during aging, aged males with homozygous knockout of hxk-1 outperformed control males with wild-type hxk-1 in a mating competition assay. To further dissect the functions of hxk-1, we characterized the expression patterns of hxk-1 by constructing fluorescent reporters driven by hxk-1 promoters. We further induced tissue-specific knockouts of hxk-1 and evaluated the effects on mating behavior. To our surprise, aged males with hxk-1 knockout in tissues directly involved in the intromission circuit such as tail muscles and nerves did not improve their mating efficiency when competing with control males with wild-type hxk-1. On the contrary, hxk-1 knockout specifically in intestines and pharynx displayed similar phenotype as hxk-1 homozygous knockout in enhancing mating efficiency in aged males. These results suggest glucose metabolism regulates mating behavior in aged males through cell-non-autonomous mechanisms.

1199A LETM-1 is essential for mitochondrial function in C. elegans.  F. Wang1, S. Yu1, C. Yang1,2  1) Institute of Genetics and Developmental Biology, Chinese Academy of Sciences; 2) Center for Life Sciences, School of Life Sciences, Yunnan University, Kunming 650091, China.

Mitochondria are critical cellular organelles best known for their role in energy production through oxidative phosphorylation (OXPHOS). Mitochondrial dysfunction leads to many human disorders, including Alzheimer’s disease (AD), Parkinson’s disease (PD), and amyotrophic lateral sclerosis (ALS). To understand how mitochondrial normal function is maintained, we carried out a genetic screen to search for mutants that display abnormal mitochondria. A mutant, yp203, was identified to exhibit abnormally enlarged mitochondria in multiple tissues. Using SNP mapping and whole genome sequencing, we found that yp203 caused a point mutation of the letm-1 gene. C. elegans LETM-1 is a homolog of human LETM1 (leucine zipper and EF-hand containing transmembrane protein 1), a mitochondrial inner membrane protein associated with Wolf-Hirschhorn Syndrome (WHS). WHS is a complex multigenic disease caused by haploinsufficiency of the WHSCR1 and WHSCR2 regions on human chromosome 4.

We found that the formation of abnormally enlarged mitochondria in letm-1 (yp203) mutants requires mitochondrial fusion machinery. We are now investigating the mechanism by which LETM-1 regulates mitochondrial dynamics and functions.

1200B The mitochondrial signal to non-canonical HIF-1 activation in Caenorhabditis elegans.  G. Wang1, M. Abbott1, S. Brook1, S. Pyonteck2,3, J. Xue1, C. Rongo2,3, M. Driscoll1  1) Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ; 2) Department of Genetics, Rutgers University, Piscataway, NJ; 3) The Waksman Institute, Rutgers
University, Piscataway, NJ.

Transcription factor hypoxia inducible factor-1 (HIF-1) modulates energy metabolism and is well known to be stabilized by hypoxia. However, more and more studies demonstrate that HIF-1 can be stabilized by non-canonical factors, such as oxidative stress, believed to be mediated by hydrogen peroxide ($\text{H}_2\text{O}_2$). Most $\text{H}_2\text{O}_2$ is biologically derived from superoxide ($\text{O}_2^-$), but $\text{O}_2^-$ is poorly studied as a signaling molecule due to variety of challenges. Superoxide dismutases (SODs) are the only known enzymes to remove $\text{O}_2^-$ in distinct subcellular locations (cytosol, mitochondria or extracellular matrix) of most organisms. Interestingly, deletion of the mitochondrial $\text{sod}$ gene in either fly or mouse causes neonatal lethality, but all SODs are dispensable in $\text{C. elegans}$ [1], so the worm is a great animal model to study the regulation of life and death by $\text{O}_2^-$. We found that the HIF-1 signaling pathway is activated in absence of either cytoplasmic or mitochondrial but not extracellular SODs. Moreover, cytoplasmic or mitochondrial $\text{O}_2^-$ showed a synergistic effect on HIF-1 activation. HIF-1 is normally degraded by cytoplasmic prolyl hydroxylases (PHDs) in the presence of $\text{O}_2^-$, a-ketoglutarate (a-KG) also influences activity. Endogenous a-KG is only known to be produced in mitochondria, and it is exported into cytosol via the Malate-Aspartate Shuttle. When we use RNAi to knockdown the two essential Shuttle components, cytoplasmic and mitochondrial malate dehydrogenase, MDH-1 and MDH-2, respectively, we observe activation of the HIF-1 pathway. We speculate that HIF-1 may monitor the change of mitochondrial metabolism via responding to the homeostasis of a-KG, and $\text{O}_2^-$ may stabilize HIF-1 via a similar mechanism, possibly by interacting with positively charged co-enzymes. We examined the pathophysiological role of HIF-1 activation in sod mutants. We found the compartmentalized $\text{O}_2^-$ had totally opposite effects on worm locomotor activities. The thrashing rate is increased in absence of cytoplasmic SODs, but decreased in the absence of mitochondrial SODs. None of these changes are mediated by HIF-1, but mitochondrial $\text{O}_2^-$ can cause more damage without HIF-1. Currently, we are conducting an RNAi screen of the mitochondrial metabolism network genes to map out the mitochondrial signal to non-canonical HIF-1 activation, and testing approaches that reverse $\text{O}_2^-$ mediated HIF-1 stabilization.

References:

1201C Ancient metformin response pathways uncovered by $\text{C. elegans}$ genetics. L. Wu$^{1,2}$, A. Soukas$^{1,2}$ 1) Center for Genomic Medicine, Mass General Hospital, Boston, MA; 2) Department of Medicine, Harvard Medical School, Boston, MA, USA.

Metformin serves the first-line therapy for type 2 diabetes, and more recently it has become clear that metformin has both anti-cancer and pro-longevity effects. In spite of these remarkable health benefits, its mode of action remains elusive. In a recent study, we uncovered an pathway responsible for both the anti-cancer and pro-longevity effects of metformin. This ancient pathway is conserved from $\text{C. elegans}$ to human, and involves ordered signaling through mitochondria, the nuclear pore complex (NPC), mechanistic target of rapamycin complex 1 (mTORC1) and the BIGuanide Resistant gene-1 ($\text{bigr-1}$, previously named CeACAD10 based on its functional similarity to the human acyl-CoA dehydrogenase 10) (Wu et al., Cell, Dec. 15, 2016). The anti-cancer and pro-longevity responses to metformin involve restriction of transport through the nuclear pore in a manner dependent upon the mitochondrial action of metformin. Restricted nuclear pore transport in response to metformin blocks cell growth by preventing activation of mTORC1. This is achieved by limiting access of the small GTPase RagC to the nucleus, locking both RagC and mTORC1 in the “off” state. In spite of these advances, many questions remain in metformin’s mode of action. In the present work, we have used forward genetics to attempt to address the mechanism by which metformin action at the mitochondria is communicated to the NPC. In an unbiased $\text{C. elegans}$ genome-wide mutagenesis screen, we identified 5 additional unique genetic mutants that participate in a mitochondrial-NPC signaling relay mediating the effects of metformin. Animals carrying loss of function mutations in any of these 5 genes show metformin-mimicking effects on $\text{Bigr-1}$ induction without presence of metformin. Our ongoing work will identify the mechanisms for these effects, specifically how NPC transport is modulated, and how this pathway can be leveraged to promote and enhance metformin beneficial effects on aging. We also anticipate that we will be able to exploit this pathway to mimic metformin’s health promoting effects and as potential avenues for cancer and diabetes treatment.

1202A The identification of genes affecting iodide toxicity in $\text{C. elegans}$. Z. Xu, Y. Hu, Y. Deng, Q. Nie, Q. Pan, L. Ma State Key Laboratory of Medical Genetics, School of Life Sciences, Central South University, Changsha, Hunan, CN.

Iodine is an essential trace element for life. Iodide deficiency can lead to defective biosynthesis of thyroid hormones and is a major cause of thyroid disorders. On the other hand excess iodide intake has been linked to different thyroidal diseases. How excess iodide causes harmful effects is not well understood. We use $\text{C. elegans}$ to study the molecular mechanism underlying the xenobiotic effect of excess iodide. We found that wildtype animals exhibit dose-dependent growth defects in iodide medium. 5 mM iodide could cause developmental arrest and other pleiotropic defects including partial cuticle shedding and premature accumulation of intestinal auto fluorescence. To identify genes involved in these processes, we performed forward genetic screens and isolated 35 mutants that could survive in excess iodide. These mutants define at least five complementation groups, three of which we determined as $\text{bli-3}$, $\text{doxa-1}$ and $\text{tsp-15}$. $\text{bli-3}$, $\text{doxa-1}$ and $\text{tsp-15}$ encode $\text{C. elegans}$ homologs of the mammalian dual oxidase DUOX, the DUOX maturation factor, and the tetraspanin protein, respectively, which were previously shown to form a dual oxidase complex. We also detected an increased biogenesis of reactive oxygen species (ROS) in animals treated with excess iodide, which can be partially suppressed by mutations in $\text{bli-3}$ or $\text{tsp-15}$. We identified missense mutations in $\text{skn-1}$ and $\text{wdr-23}$ from the other two complementation groups. $\text{skn-1}$ encodes a bZip transcription factor orthologous to the mammalian nuclear
factor erythroid 2-related factor (Nrf) and wdr-23 encodes a WD40 repeat-containing protein that negatively regulates SKN-1 activity. Previous studies suggest that SKN-1 is activated by oxidative stress in C. elegans. Based on these results, we propose that the BLI-3/TSP-15/DOXA-1 dual oxidase complex and the SKN-1 transcription factor might act together in affecting the xenobiotic effects of excess iodide in C. elegans.

1203B Late endosomes and lysosomes are potential target sites for a diabetes drug metformin. J. Kim¹, H-Y. Lee¹, J. Ahn², M. Hyun², I. Lee², K-J. Min¹, Y-J. You²,² ¹) Department of Biological Sciences, Inha University, Incheon, South Korea; ²) Department of Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond, VA; 3) Graduate School of Science, Nagoya University, Nagoya, Japan.

Diabetes is one of the most impactful diseases worldwide. The most commonly prescribed anti-diabetic drug is metformin. Metformin lowers blood glucose level by decreasing hepatic glucose production in the liver and by increasing glucose uptake in the muscle. In the cell, it activates AMP dependent kinase (AMPK) and inhibits mTOR, creating a low-energy state. Recent studies show that metformin induces cell death in certain cancer cell lines by interfering with the metabolism of the cancer cells. Therefore, understanding of the action mechanisms for metformin will provide insights into the pathophysiology of diabetes and other metabolic disorders as well as better ways to treat them. Metformin has many known targets, yet none of them were shown to directly bind to metformin. Given metformin’s broad effects on metabolism, it could act on multiple targets. In order to identify new targets, we performed an unbiased screen in C. elegans using a metformin-induced phenotype in L1 longevity. It has been shown that in Drosophila, metformin treatment (100 mM) reduces life span. We discovered that metformin (100 mM) also reduces a certain type of lifespan, L1 longevity. An unbiased screen for a mutant that survives better in the presence of metformin identified a mutation in an endosomal Na+/H+ exchanger (eNHE), NHX-5. The NHES exchange one extracellular (or extra-organell) Na+ for one intracellular (or intra-organell) H+, making electroneutral exchange. The movement of H+ results in pH changes critical for controlling cell volume and intracellular organelle trafficking. Metformin inhibits oxygen consumption in C. elegans in NHX-5 dependent manner, suggesting a conserved action mechanism of metformin in mitochondria in C. elegans. The same NHE homolog exists in flies where it too mediates the effects of metformin. Interestingly, knockout mice that lack NHE6, the mammalian homolog of C. elegans NHX-5, show Angelman-like syndrome in which the lysosome becomes dysfunctional, mimicking lysosomal storage disorders. Consistent with the lysosomal defects shown in NHE6 knockout mice, metformin treatment misregulated autophagy during L1 starvation, suggesting that metformin could act on the endo-lysosomal compartment via endosomal NHES and regulate the endocytic cycle and autophagy. Considering the newly discovered roles of endo-lysosomes in metabolism, such as in mTOR or AMPK activation, our study could suggest a novel link between metabolic disorders and the endocytic cycle.

1204C Identifying bacterial metabolites that affect C. elegans growth. Jingyan Zhang, Brent Horowitz, Safak Yilmaz, Albertha J. Walthout Program in Systems Biology, University of Massachusetts Medical School, Worcester, MA, USA.

Diet is crucial for the development and survival of all organisms, and it is well established that changes in the composition and abundance of food alter metabolism, development, and physiology. However, the molecular mechanisms by which changes in diet affect these processes are largely unknown. We conducted a genetic screen in E. coli to identify bacterial genes that affect C. elegans development. We fed animals the Keio E. coli BW25113 collection, which contains 3,985 single gene deletion mutants, and quantified the body size of animals by ImageJ. We identified 14 bacterial mutants that increased C. elegans body size and 286 bacterial mutants that decreased animal body size. Most of the latter set of mutants slowed C. elegans development, indicating that specific bacterial metabolites are required as nutrients to promote animal growth. We are currently integrating our findings with both C. elegans and bacterial metabolic network models to identify those metabolites and plan to test these by supplementation.

1205A An approach to integrate 2D flow cytometry data with Nuclear Magnetic Resonance data in Caenorhabditis elegans developmental studies. S. Zhang, F. Tayyari, F. V. Ponce, R. Borges, R. Tajuale, G. Gouveia, A. Edison Complex Carbohydrate Research Center, University of Georgia, 315 Riverbend Road, Athens, Georgia, USA 30602.

Nuclear magnetic resonance (NMR) is a useful tool to study changes in Caenorhabditis elegans metabolomics as a function of development. NMR is highly reproducible and provides atom-specific information that helps in the identification of unknown compounds. We typically use between 50k-200k worms per sample. When we add biological replicates for statistical significance, it is difficult to investigate each developmental stage independently. Therefore, we have developed an approach that integrates data from the large particle?nematode?Biosorter with NMR data. This method allows for the correlation of a specific NMR feature with a specific developmental stage. We transform the flow cytometry dataset into the same dimension as NMR spectra dataset. This is accomplished by creating a 1D vector that contains the experimental worm population distribution along the axis length (TOF) and optical density (EXT) axes. Upon doing this, we can use Statistical Total Correlation Spectroscopy (STOCSY), a widely used statistical tool for finding correlated features in NMR spectra. In our application, we obtain correlations between NMR and biosorter datasets. The result is a correlation of a subset of worm population with a specific peak, which can also show whether a metabolite changes its concentration during the organism’s development or not. We have tested this method by integrating a dataset from flow cytometry and NMR from the same samples. The dataset came from 28 samples of C. elegans collected at 5 time points. After picking peaks from ascarosides that showed up in specific developmental stages as driver peaks for STOCSY, we constructed a statistical biosorter map that indicated how highly correlated (positively or negatively) the NMR peak was with the distribution. This approach is general and should be easily applied to other flow-cytometry-based quantitative analysis.
Physiology - Novel Technologies

1206B  A novel high-throughput whole-genome RNAi screening technology utilized to investigate the molecular pathways of exopher production.  M. Abbott, I. Melentijevic, R. Guasp, M. Driscoll Rutgers University, Piscataway, NJ.

RNA interference is a powerful tool for the exploration of gene function in C. elegans. Past whole genome RNAi screens in C. elegans have provided fascinating insights into nematode biology, however, current screening protocols can be prohibitively resource and time intensive. We are developing a highly automated, high-throughput whole-genome RNAi screening platform that can be implemented at a fraction of the time and cost of manual screens. Our screening protocol employs robotic dispensers and aspirators, coupled with a high content imaging system for animals grown and measured in a 96-well plate format. Multiple fluorescent channels and pattern recognition algorithms enable the analysis of multiple parameters simultaneously. We have developed methods for animal growth and imaging that should enable screening of the entire genome in approximately one week, fast enough to make epistatic studies feasible.

Our initial experiments investigate genes that influence production of exophers, large extracellular vesicles that can mediate expulsion of protein aggregates and damaged organelles from neurons\(^1\). If conserved, exophers may play important roles in neurodegenerative diseases characterized by protein aggregation, including Parkinson’s and Alzheimer’s. In our model, we track an aggregating mCherry protein that is expelled in exophers. The mCherry protein exits touch neurons as exopher cargo, transits through the hypodermis, and can later be found concentrated in coelomocytes. When coelomocyte or hypodermal uptake is genetically disrupted, fluorescent exopher contents accumulate in the body in a phenotype we call “starry night.” We will present preliminary screening data and images demonstrating the viability of the technology to screen suppressors and enhancers of the stary night phenotype.


1207C  TrakBox: A new modular option for behavioural monitoring of freely moving C. elegans.  F. Calahorra\(^1\), C. James\(^2\), V. O’Connor\(^3\), L. Holden-Dye\(^4\) 1) Biological Sciences, University of Southampton, Southampton (UK), Southampton, GB; 2) Warwick Engineering in Biomedicine, University of Warwick (UK).

Tracking the movement of C. elegans is a well-stablished route to identify and refine understanding of microcircuits that control behaviour. Over several years a number of different experimental platforms and associated software for video analysis have been developed to facilitate this\(^1\). To contribute to this we have designed a relatively low cost modular system for real-time tracking and analysis of worm locomotory behaviour.

TrakBox (EMbody Biosignals Ltd\(^2\)) is assembled from 3-D printed components in which a robotic arm moves a USB camera to maintain the animal being tracked within a target site. Reverse kinematics is used to decode the coordinates of the worm as it moves around the plate with continuous tracking being possible for at least 24 h. Videos may also be captured but are not required for the behavioural analyses. Advanced signal processing filters and corrects the worm position over time and constantly updates a series of behaviour parameters calculated ‘on the fly’. TrakBox software derives parameters over the entire course of tracking that describe worm position, instantaneous velocity, instantaneous direction of travel, dwelling and roaming times and number of reversals. The user may define the behavioural parameters of interest and they may be displayed as a graphical representation.

As a proof of concept we compared the behaviour of N2 and a mod-1 mutant. mod-1 encodes a serotonin-gated chloride channel and a loss of function mutant exhibits increased exploration and extended roaming time\(^3\). TrakBox efficiently extracted this phenotype by analysing roaming/dwelling fractions of time, velocity and reversal events and their duration. Thus, we show TrakBox permits discrete real-time analysis of C. elegans locomotory behaviour that circumvents the need for post-hoc analysis of videos. This experimental platform also has applications for investigation of the effects of drugs on behaviour, may be applied to analysis of other microscopic nematodes including pest species, have uses in field studies and provide an affordable option for classroom demonstration of C. elegans biology.

2) http://embody-biosignals.com/  

1208A  Transcriptomic profiling of the Caenorhabditis elegans intestinal bacteria.  J.P. Chan, Justin Wright, Anastasia Ardesheva, Jamey Brumbaugh, Christopher McLimans, Regina Lamendella Biology, Juniata College, Huntingdon, PA.

Gut bacteria impact many essential functions in host organisms, ranging from the regulation of nutrient mobilization, animal development, and immune responses amongst others. Recently, the use of Caenorhabditis elegans has become an advantageous model organism to better understand how bacteria, and its metabolic and gene networks, regulate host physiology and life history traits. For example, bacteria alter development, lifespan, and pathogen resistance in worms; furthermore, high-throughput sequencing has helped identify different bacterial communities in varying host genotypes and environments. Together, this has broadened our knowledge of host-bacteria interspecies interactions. However, less is known regarding the functional metabolites and transcriptional profile of bacteria that may affect host physiology. To better examine this, we developed an RNaseq protocol and bioinformatics pipeline to elucidate bacterial gene expression differences in known
genetic models of aging. Briefly, *E. coli* (OP50) fed worms were isolated and RNA extracts were subject to ribosomal RNA subtraction, cDNA synthesis, Nextera library preparation, and sequencing using the Illumina Hiseq platform. Our bioinformatics pipeline utilizes the most robust programs for quality filtering, assembly, annotation, and differential gene analysis. We discuss preliminary bacterial transcriptome data found in metabolism biosynthetic and virulence pathways in *N2* worms fed OP50. Furthermore, we compare the transcriptomic profile of wild type to that observed in the long-lived *daf-2*(*e1380*)/InsR and the short-lived *daf-16*(*mu86*)/FOXO mutants, which may contribute to aging phenotypes. These methods will help us understand interspecies interactions and identify bacterial metabolites that impact host physiology.

1209B Automation of the *Caenorhabditis* Intervention Testing Program. A.L. Coleman-Hulbert\(^1\), C.A. Sedore\(^2\), E. Johnson\(^3\), M. Lucanic\(^3\), T.W. Plummer\(^3\), E. Chen\(^2\), G. Harinath\(^2\), A.C. Fulger\(^3\), B.W. Blue\(^3\), S. Banse\(^3\), R. Falkowski\(^2\), D. Hall\(^3\), A.B. Crist\(^1\), M.P. Presley\(^3\), T. Garrett\(^3\), J.L. Kish\(^3\), G.J. Lithgow\(^1\), M. Driscoll\(^2\), P.C. Phillips\(^1\) \(^1\) University of Oregon, Eugene, OR; \(^2\) Rutgers University, Piscataway, NJ; \(^3\) Buck Institute for Research on Aging, Novato, CA.

The *Caenorhabditis* Intervention Testing Program (CITP) is a multi-institutional consortium supported by the National Institutes of Aging and tasked with identifying robust life-extending compounds using nematodes from the genus *Caenorhabditis*. CITP partners at Rutgers University, the University of Oregon, and the Buck Institute for Aging Research have streamlined efforts and reproduced findings from 22 diverse strains of *Caenorhabditis* under ten separate compound interventions. Our work highlights the necessity of replication of longevity analysis, and our intention is to greatly expand the scale of our assays in order to keep up with demand from the aging research community. The *C. elegans* Lifespan Machine developed by Nicholas Stroustrup (Fontana Lab, Harvard) holds a great deal of promise as a high-throughput longevity approach. The *C. elegans* Lifespan Machine uses typical office scanners modified to hold petri plates containing nematodes. Through hourly image capture and later image processing, the Lifespan Machine automates the process of gathering lifespan data while providing tight temporal scaling. Here, we compare manual and automated lifespan measurements for multiple compounds across dozens of strains within each of the three laboratories. Overall, there are distinct differences in phenotype between manual and automated approaches for some strains and, especially, for some compounds. Lab to lab variation can also be a challenge. Nevertheless, the automated approach provides a great deal of value to the workflow of the CITP, and we plan to incorporate it into our future work, coupled with verification via manual lifespan assays.

1210C Of Worms and Drugs. Mostafa Elfawal\(^1\), YouMie Kim\(^1\), Anand Sitaram\(^1\), William Fenical\(^2\), Makedonka Mitreva\(^3\), Raffi Aroian\(^1\) \(^1\) Molecular Medicine, UMASS Medical School, Worcester, MA; \(^2\) Center for Marine Biotechnology and Biomedicine, University of California, San Diego, CA; \(^3\) School of Medicine, Washington University, St. Louis, MO.

Parasitic nematodes are major causes of diseases for humans, livestock, pets, and plants worldwide. Anthelmintics are drugs or treatments used to cure or limit these infections. All anthelmintics currently in use were found screening against parasitic nematodes but clearly *C. elegans* could offer major advantages in finding new and urgently-needed drugs. We are one of the few laboratories that have significant efforts both with *C. elegans* and parasitic nematodes such as *Ancylostoma ceylanicum*, *Necator americanus*, and *Trichuris muris*.

Here we investigate two areas. First, we ask if *C. elegans* is in fact a good model for drug screening. For this test, we have screened at multiple doses a drug library of 1200+ compounds against multiple stages of parasitic nematodes and *C. elegans*. By making key assumptions as to what constitutes a good "hit", we are able to deduce that experimental parameters dictate when *C. elegans* is the most appropriate model. We will present a decision tree representative of these conclusions. Second, we are using *C. elegans* to study how anthelmintics work (mode-of-action). We are using forward genetic screens to discern how *C. elegans* defends against a variety of anthelmintic agents. We will discuss how the creative development and use of toxicity assays and genetic screens and the choice of worm stages used for such screens, although complex and different for each bioactive, makes all the difference between success and failure. We will also discuss our results from mode-of-action studies.

1211A Determining the biomechanical properties of *C. elegans*. Muna Elmi\(^1\), Vijay Pawar\(^1\), Michael Shaw\(^1\)\(^2\), David Wong\(^1\), Haoyun Zhan\(^1\), Mandayam Srinivasan\(^1\)\(^3\) \(^1\) UCL TouchLab, Department of Computer Science, University College London, London, London, UK; \(^2\) National Physical Laboratory, Hampton Road, Teddington, Middlesex, UK; \(^3\) MIT TouchLab, Massachusetts Institute of Technology, Department of Mechanical Engineering and the Research Laboratory of Electronics, Cambridge, USA.

The ability to sense touch is a fundamental perceptual mechanism that nearly all organisms use to explore and manipulate with their surroundings. Despite the importance of touch to everyday life, the process of sensing touch (mechanotransduction) whereby a mechanical stimulus gives rise to a neuronal response is still poorly understood. Touch sensation is a chain of events beginning with mechanical loads applied to the skin, which result in stresses that deform the skin's surface, sub-layers and underlying mechanoreceptors. In response to this mechanical input, the mechanoreceptors activate a neuronal signaling network that encodes the physical contact as information, which we perceive as 'touch'. To address the question of mechanotransduction we present an investigation of the biomechanical properties of skin using *C. elegans*. We have developed an experimental platform consisting of a custom built high resolution optical microscope and robotic force sensing micromanipulation systems to measure the mechanical loads as well as the spatial deformation of the animal in response to an indentation range. Furthermore, we used the captured empirical data to create a generalized multi-layer biomechanical computational model for *C. elegans*, based on its anatomy, and derive material properties such as the elastic modulus and
poisson’s ratio. By interrogating this model, we discuss first estimates for both the strain energy required to activate an individual mechanoreceptor, and the number of mechanoreceptors required to elicit a touch-evoked behavioural response.


Laser microsurgery has long been a powerful tool for creating specific cellular or circuit lesions in C. elegans. Laser ablations have been typically performed by using a nanosecond or femtosecond pulsed laser to induce local plasma formation in the target cell. In our experience, aberrations in microscope objectives limit our ability to ablate deep structures in animals larger than L2 larvae using traditional laser ablation systems. In addition, the small size of plasma formation makes it difficult to lesion larger structures such as the ventral nerve cord. Here, we demonstrate that C. elegans cells can be efficiently ablated by using a pulsed infrared laser to damage tissues via locally elevated temperature. This method, while less spatially precise than ablation by nanosecond or femtosecond lasers, is highly effective in thick samples like L4 and adult C. elegans. We demonstrate that a single, 0.8 ms pulse from a laser with a wavelength of 1480 nm and peak power of 400 mW, is ideal for killing a targeted neuron but not its immediate neighbors. At this dosage, a neuron 2.5 µm away from the target has a ~45% chance of being killed, and a neuron 5 µm away has a ~10% chance of being killed. We show that a short train of pulses can reliably sever the ventral nerve cord in adult C. elegans. Hence, our method is a tool that can be used to lesion cells and other structures in C. elegans in cases where traditional microsurgery methods are not practical.


The accumulation of misfolded protein aggregates within cells is a common cause of tissue injury and degenerative disease (e.g., Alzheimer’s, Huntington’s, ALS and alpha-1-antitrypsin (AT) deficiency). To better understand the genetic factors that influence pathogenesis of protein aggregation disorders and facilitate discovery of therapeutic drugs, we developed a C. elegans model of AT-deficiency (ATD). Transgenic animals expressing wild-type AT efficiently secreted the protein. In contrast, animals expressing the aggregation-prone mutant AT failed to secrete the protein and instead accumulated toxic protein aggregates. Using an automated high-content, high-throughput assay, we screened small molecule libraries (n >150,000) and identified hit compounds that significantly reduced the accumulation of misfolded protein aggregates. Remarkably, these compounds were efficacious in reducing protein aggregation and fibrosis in a mouse model of ATD. Characterization of these compounds using mKate::LGG-1 and SQST-1::GFP reporter lines revealed that these drugs exert their effects by enhancing autophagy. Since autophagy plays an important role in the pathogenesis of numerous degenerative diseases, we hypothesize that these drugs may be effective in reducing tissue injury associated with other protein aggregation disorders such as Alzheimer’s and Huntington’s. Studies are currently under way to test the effect of these drugs on C. elegans models of b-amyloid and polyQ accumulation.

1214A A System for High Throughput Monitoring of Healthspan and Lifespan. R.A. Kerr, C. Kenyon  Calico Life Sciences, South San Francisco, CA.

Investigations of genes and environmental conditions that promote healthy aging have been hindered by the relative difficulty of performing assays that monitor health as opposed to lifespan. This difficulty manifests itself in two ways. First, it limits the number of genes and/or timepoints at which health metrics are collected. Second, it encourages focus on a single or small number of health metrics. Though some efforts have been made to improve throughput, these systems typically require worms to be in conditions that are somewhat unlike standard laboratory culture conditions.

We are completing a roboticized behavioral analysis system that provides a high throughput platform for collecting healthspan and lifespan data. Worms are housed on large-format trays in wells slightly larger than a standard 3.5 cm plate, and are grown on agar with OP50 as usual. A forklift delivers these trays to an imaging platform consisting of 18 high-speed high-resolution cameras that analyze behavior using the open-source Multi-Worm Tracker software. With this system, we can collect behavior from 700 distinct strains or conditions, with four biological replicates (in aggregate containing over 100 animals) per condition, from within one incubator.

In its current incarnation, the system can track not just average and maximum movement speed, but also track fraction of time spent in forward, reverse, and still states, and speeds within each; and can deliver tap stimuli and monitor response probability, distance, and reaction rates, among others; and can score habituation of these tap responses. This provides a rich behavioral repertoire from which to build metrics of health. The system can also easily be extended to deliver blue light stimuli and to automatically score lifespan (in process).

We anticipate that the capabilities of this system will greatly accelerate our understanding of the biology of the maintenance of health and its relationship to lifespan.

As a key link connecting regulatory and bioenergetics process, NAD⁺ has emerged as a central metabolic co-factor playing a critical role in regulating cellular metabolism and energy homeostasis. Successfully recycling NAM, released from NAD⁺-consuming reactions, back to NAD⁺ is a major challenge for maintaining NAD⁺ metabolism and homeostasis. The first step in C. elegans’ salvage NAD⁺ synthesis involves the nicotinamidase, pnc-1 (T L Vrablik, Huang, Lange, & Hanna-Rose, 2009). Via global metabolic profiling we previously linked loss of salvage NAD⁺ synthesis, via loss of pnc-1 activity, to disruptions in glycolysis and a subsequent reproductive developmental delay (Wang et al., 2015). However, the citric acid cycle was not perturbed and functions of the mitochondria were intact in pnc-1 mutants (Wang et al., 2015). This observation suggested that loss of salvage NAD⁺ biosynthesis affects the cytoplasm specifically. To investigate this model, we decided to directly test the effects of loss of pnc-1 on glycolytic and citric acid cycle flux via application of metabolic isotopic carbon tracing tools. After a short four-hour exposure with universally labeled glucose (¹³C₆-Glucose), we were able to detect an isotopically labeled glucose pool in both wild-type animals and pnc-1 mutants. Metabolic carbon tracing confirmed that glycolysis is impeded at the NAD⁺-dependent step in pnc-1 mutants. Although glycolysis is compromised, we observed no change in the isotopic flow of label from pyruvate to lactate in pnc-1 mutants. Additionally, we observed an increase of isotopic label from pyruvate entering the TCA cycle in these mutants. Although trehalose steady state levels are increased in pnc-1 mutants, we did not observe increased shunting of glucose towards trehalose. This data bolsters the model that loss of NAD⁺ salvage biosynthesis preferentially affects cytoplasmic functions. Moreover, our work supports stable isotopic labeling as a robust technique to track the passage of metabolites through metabolic pathways in C. elegans.

1216C  A Simple Culture System for Long-Term Imaging of Individual C. elegans.  W.E. Pittman¹,², W.B. Zhang¹, D.B. Sinha¹,², Z.S. Pincus¹,² 1) Department of Genetics, Washington University School of Medicine, St. Louis, Missouri 63110, USA; 2) Department of Biomedical Engineering, Washington University in St. Louis, St. Louis, Missouri 63110, USA.

Populations of isogenic C. elegans, reared in identical environments, nevertheless exhibit a broad distribution of developmental and adult phenotypes. In order to study such inter-individual variability, particularly variability in wild-type lifespan, it is necessary to culture each individual nematode in an isolated, homogenous, and controlled environment. We have built a device that cultures an array of individual C. elegans, each confined to a small “corral” of E. coli OP50 food on a gel surface. Unlike other possible approaches to individual culture, this solid-phase system closely mimics NGM-agar plates. High-resolution images captured via an automated microscopy system allow us to monitor growth, movement, feeding, and reporter fluorescence throughout the entire life of each individual in the culture.

Specifically, we produce a 1mm-thick hydrogel bed (either agarose-based, or from cross-linked PEGs) with a novel surface chemistry, supported by a standard microscope slide. We then place individual eggs on the surface of the gel in a droplet of OP50, and pour liquid PDMS silicone atop. This material polymerizes overnight into an optically transparent and gas-permeable membrane. In a novel cross-linking reaction, the PDMS also covalently binds to the gel beneath except where separated from the gel by the OP50 food. Thus, each individual animal is confined to only the region of its food pad. Self-reproduction is inhibited through the use of a temperature-sensitive sterile strain.

We conducted health assays to ensure the culture system does not have adverse effects on C. elegans physiology. We found no significant deviation between the time to adulthood, brood size, and mean lifespan in worms cultured in the device or NGM plates. In each assay, the variance around the mean is significantly smaller, suggesting the homogenous environment does reduce physiological variability.

Generally, we form the PEG gel under a glass coverslip to produce a smooth top surface. By substituting this coverslip with a surface-patterned material, we are able to transfer the patterning into the PEG gel. For our application, we have found it valuable to create a PEG gel containing an array of pre-formed indentations to precisely control the geometry of the bacterial food pads pipetted into each indentation. Controlling droplet geometry allows us to increase the density of the worms on the device and do developmental studies at higher magnifications.

1217A  Region specific irradiation of Caenorhabditis elegans with heavy ion microbeam.  Michiyo Suzuki, Yuichiro Yokota, Tomoo Funayama  Department of Radiation-Applied Biology Research, National Institutes for Quantum and Radiological Science and Technology (QST), Takasaki, Gunma, JP.

Animals including humans are exposed to radiation from natural and artificial sources, cosmic rays, and nuclear accidents. Radiation may affect vital functions such as locomotion, feeding, learning. To develop effective methods to investigate the effects of radiation exposure at tissue level as well as whole body is needed.

In the present study, we therefore aimed to establish a novel method for radiation-targeting of specific regions of Caenorhabditis elegans using a collimating microbeam system, thus allowing vital functions, especially locomotion, to be observed immediately after irradiation. We immobilized individual adult C. elegans in straight microfluidic channels (60 µm in width) in a polydimethylsiloxane chip and subjected them to irradiation targeted to the nerve ring, mid, or tail region, respectively, using carbon ions. The ions were delivered from the AVF cyclotron at TIARA of QST-Takasaki and then were collimated using a f20 µm beam exit (micro-aperture) of a collimating microbeam system. Furthermore, for wider area of the body, a f60 µm micro-aperture was used. In the ventral half-body irradiation, we targeted to the f60 µm semicircle area in order from head to tail and irradiated at fifteen or more times. For comparison, whole body was irradiated using a scan beam. Immediately after irradiation, we added a drop of buffer solution on a microfluidic chip and collected animals using a picker. Each animal was transferred to a NGM plate without food and the locomotion was evaluated.

The effects of whole-body irradiation tended to be more effective than those of region-specific microbeam irradiation at the same
was detected and is being introgressed. C. briggsae Santeuil virus. Using AF16xHK104 Advanced Intercrossed RILs (AIRILs) (Ross et al. 2011), two QTLs were detected on C. briggsae performing QTL analysis. Several Near Isogenic Lines (NILs) were created by introgressing the candidate regions. A specific manner. The RILs were phenotyped for the sensitivity to the relevant viruses using Fluorescent In Situ Hybridization (FISH). The genotype (SNP markers from pool sequencing) and phenotype (resistance/sensitivity from FISH) data were used to identify optimized C. elegans strains for chemical toxicity assessment assays. H. Xiong, C. Pears, A. Woollard Department of Biochemistry, University of Oxford, Oxford OX1 3QU, United Kingdom.

Newly developed chemical compounds to be released into the environment, including those used by agrochemical, cosmetic, petrochemical and pharmaceutical industries, must be subjected to stringent regulatory toxicity assessments. Currently, the potential of each chemical to induce developmental and reproductive toxicity (DART) is routinely assessed over at least two generations of rats or rabbits. Such assays require large numbers of animals, making these assessments expensive (in terms of both animals and chemicals) and time-consuming (representing an opportunity cost). We have established a simple and fast DART prediction assay using C. elegans to screen for high risk DART compounds early in the product development pipeline, which can then be eliminated from animal testing. An adaptation of the assay allows exposure to volatile compounds or those with low solubility. However, the worm’s robust cuticle presents a significant barrier to chemical uptake, increasing the amount of chemical required and reducing the value of the assay for regulatory assessment. We took advantage of available mutants with altered cuticle properties to identify sensitized strains more suitable for toxicity assays. We tested agmo-1, bus-5, bus-8, bus-16 and bus-17 for chemical permeability of their cuticle, setting this advantage in terms of chemical sensitivity against a possible reduction in physiological fitness. These criteria narrowed the candidate group down to three bus mutant strains, which were subjected to toxicity assays using a collection of hydrophilic and hydrophobic chemicals present in agrochemicals, cosmetic products, petrochemicals and pharmaceuticals, and known to cause DART in mammals. All three bus mutant strains are sensitized to chemical exposure compared to wildtype worms across a range of chemicals. With regard to our evaluation criteria and all toxicity assays, we identified bus-5 as the ideal sensitized strain for chemical toxicity assessments that is capable of detecting DART effects at lower chemical levels, including some masked in wildtype worms, thus minimizing the probability of a false-negative outcome.

Physiology - Pathogenesis


The discovery of RNA viruses that naturally infect C. elegans and C. briggsae serves as an ideal model system to study antiviral immunity and host-pathogen co-evolution. The Orsay virus only infects C. elegans whereas Santeuil and Le Blanc viruses only infect C. briggsae. Intraspecifically, within both species we found a wide variation in viral sensitivity, as well as a positive correlation among wild isolates in sensitivity to both viruses in C. briggsae. An exception to this correlation is the C. briggsae strain HK104, which is specifically resistant to Le Blanc virus but sensitive to Santeuil virus. Taking advantage of this natural variation in the host, we use a genetic approach from the host side and use Recombinant Inbred Lines (RILs) to first map the recombinant genomic regions participating to the resistance/sensitivity in a general and/or specific manner. The RILs were phenotyped for the sensitivity to the relevant viruses using Fluorescent In Situ Hybridization (FISH). The genotype (SNP markers from pool sequencing) and phenotype (resistance/sensitivity from FISH) data were used to perform QTL analysis. Several Near Isogenic Lines (NILs) were created by introgressing the candidate regions. C. briggsae AF16 is resistant to both Santeuil and Le Blanc viruses while C. briggsae HK104 is specifically sensitive to the Santeuil virus. Using AF16xHK104 Advanced Intercrossed RILs (AIRILs) (Ross et al. 2011), two QTLs were detected on chromosomes III and IV for Santeuil virus sensitivity. The NILs in the AF16 background confirm both candidate regions. C. briggsae JU1498 is sensitive to both Santeuil and Le Blanc viruses. Using JU1498xHK104 RILs, a QTL on chromosome II was detected and is being introgressed.
Once candidate polymorphisms associated with the virus sensitivity/resistance are identified, we will test them by RNAi knockdown, transformation rescue and/or CRISPR-mediated gene replacement.

1221B Worms and Ladders: Steps Towards a Cure for Alpha-1 Antitrypsin Deficiency. A.E. Brown, F.A. Partridge, D.B. Sattelle, D.A. Lomas Division of Medicine, University College London, London, GB.

Alpha-1 antitrypsin deficiency is an autosomal recessive genetic condition affecting approximately 1:1600 individuals of Northern European descent. It arises due to point mutations in the SERPINA1 gene. These mutations result in the expression of polymerogenic alpha-1 antitrypsin and its consequent retention within the endoplasmic reticulum of hepatocytes. Accumulation of polymer in the liver gives rise to cirrhosis and predisposes individuals to hepatocellular carcinoma. The ensuing decrease in circulating functional monomeric alpha-1 antitrypsin results in the dysregulation of neutrophil elastase in the lung, leading to uncontrolled tissue degradation, COPD and early-onset emphysema. Currently, there are no therapeutic options to treat liver disease in alpha-1 antitrypsin deficiency, with transplantation being the only effective treatment. There is therefore a need to develop new therapeutic strategies.

A novel transgenic C. elegans model of alpha-1 antitrypsin deficiency has been generated and characterised in order to be used in a small molecule screen to search for novel compounds which ameliorate the disorder. The C. elegans model expresses and accumulates mutant alpha-1 antitrypsin within the muscle resulting in an impaired motility phenotype which is scorable using an in-house high-throughput imaging and analysis platform. The motility impairment correlated with protein accumulation and was found to be temperature-sensitive. Culture of the mutant alpha-1 antitrypsin-expressing worms at higher temperatures (25°C) gave rise to a significant movement deficit when compared with mutant worms cultured at a lower temperature (20°C) and with a non-transgenic control. The motility phenotype will form the basis of a small molecule screen to search for novel compounds which improve movement in the mutant worms. Any hit compounds could then potentially be considered as new therapeutic agents for alpha-1 antitrypsin deficiency.

1222C Regulation of the innate immune response by a dopaminergic neural circuit in C. elegans. Xiou Cao, Alejandro Abalay Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC.

Increasing evidence from studies in neurobiology and immunology implies an extensive and universal interaction between the nervous and immune systems, which is responsible for organismal control of immune homeostasis. In contrast to the complexity of mammalian systems, the model organism C. elegans has been validated as a powerful tool to study host-pathogen interactions and elucidate the principles of neural regulation of immunity.

The study mainly focuses on studying the immune regulatory function of dopamine receptor DOP-4 and the underlying neural circuit that controls the immune response upon pathogen infections. We found that some dopamine antagonists are putative activators of the PMK-1/p38 MAPK pathway. One of the dopamine antagonists, chlorpromazine, as well as dopamine, could modulate the immune response of C. elegans to P. aeruginosa infection. We found that the mutants with deletions in the dopamine receptor gene dop-4 showed an enhanced survival rate and reduced bacterial burden upon P. aeruginosa infection. Further experiments showed that PMK-1-dependent genes were upregulated in dop-4 mutants either at basal level or upon pathogen infections. To explore the role of dopamine signaling in immune modulation, we performed targeted ablation of dopaminergic neurons CEP and found that animals lacking CEP neurons are more resistant to P. aeruginosa infection. Animals treated with dopamine are more susceptible to infections, and dopamine is able to rescue the phenotype of the CEP-ablated strain. Additionally, single-neuron expression of dop-4 in ASG, the neurons having direct synaptic connection with CEP neurons, could suppress the enhanced resistance of the dop-4 mutant, validating the model that dopaminergic CEP neurons control immunity through dopamine receptor DOP-4 expressed in ASG neurons.

1223A HLH-30 regulates epithelium intrinsic cellular defenses in intestinal cells against bacterial pore-forming toxin in C. elegans. Huan-Da Chen1,2, Cheng-Yuan Kao3, Bang-Yu Liu4, Shin-Whei Huang5, Cheng-Ju Kuo4,5, Jen-Wei Ruan6, Yen-Hung Lin7, Cheng-Rung Huang1,2, Yu-Hung Chen1,2, Hong-Dar Wang8, Raffi Aroian9, Chang-Shi Chen1,2 1) Department of Biochemistry and Molecular Biology, College of Medicine, National Cheng Kung University, Tainan, Taiwan; 2) Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan; 3) Immunology Research Center, National Health Research Institute, Miaoli, Taiwan; 4) Institute of Biotechnology, National Tsing Hua University, Hsinchu, Taiwan; 5) School of Medicine, College of Medicine, National Cheng Kung University, Tainan, Taiwan; 6) Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, Massachusetts, USA.

Autophagy is an evolutionarily conserved self-clearance system that maintains cellular homeostasis by degrading and recycling various superfluous or damaged cellular components. The transcription factor HLH-30 (TFEB in mammals) has been reported to modulate bacterial infection by regulating autophagy, but less is known about the bona fide bacterial effector that activates HLH-30 and autophagy. Here, we unveil that bacterial membrane pore-forming toxin (PFT) induces cellular autophagy via an HLH-30-dependent manner in Caenorhabditis elegans. The PFT-activated autophagy controls the tolerance of host to PFT intoxication through xenophagic degradation of PFT and repair of membrane-pore cell-autonomously within the PFT-targeted intestinal cells. All together, our data demonstrate a new connection between HLH-30 and epithelial intrinsic cellular defense (INCED) against the single most common mode of bacterial attack in vivo.


Excretory-secretory products (ESP) are a wide range of biomolecules, including proteins and chemicals, that are continuously
excreted or secreted into the niche during the whole life cycle of parasitic and free-living nematodes. ESPs act directly at the interface parasite-host or worm-environment interface and have pivotal roles in nematode survival, reproduction and host immune system regulation. Therefore, ESPs have gained more and more interest as promising drug targets and anti-parasitic vaccine candidates. Although there are a few articles reporting parasitic nematode ESP, the structure of nematode excretome/secretome is not complete due to several limitations. First, not many parasitic nematodes genomes are fully sequenced and limits number of ESPs identified in some species. Second, most genome-editing tools are not available or immature in parasites, which limits the verification of ESPs. Not much is known about the mechanisms and related pathways given that complex processes occur throughout different life stages. Here we used liquid chromatography (LC)/MS-based proteomics to profile excreted/secreted proteins with Caenorhabditis elegans. We have characterized more than 500 proteins with mixed-staged worms with 4 replicates. GFP tagged expression assays and signal peptide prediction indicate that some representative proteins get secreted from the intestine and the hypodermis. Gene Ontology annotation of identified proteins showed that many are metalloproteases, cysteine proteases and lysozymes. Many proteins are implicated to have roles in regulating the molting cycle and growth rate, defense against bacterial infection and pathogen susceptibility. Whether the parasitic proteinases inherited and evolved whole or partly from free-living ancestral worms in response to parasitism has not been addressed. Thus, we compared C. elegans ESP with reported parasitic nematodes. Surprisingly, protein family analysis confirmed that nematode’s ESP composition are very similar between species even with drastically different life styles. In summary, in depth C. elegans ESP analysis provides a platform to predict ESP composition and promote study in parasites.

1225C Role of TGF-beta signaling and pathway interactions during innate immune response in the nematode Caenorhabditis elegans.  E.J. Ciccarelli, C. Savage-Dunn  Queens College and the Graduate Center, CUNY.

Innate immunity provides plants and animals with an immediate defense against infection. In humans, this immune response involves the activity of phagocytic cells as well as secretion of antimicrobial molecules. When exposed to infection, the nematode C. elegans mounts an innate immune response through secretion of molecules like lectins and lysozymes that serve antimicrobial functions. Different signaling pathways in the worm regulate release of these antimicrobial factors. Our research aims to determine the way in which the C. elegans TGF-b family pathway - regulated by the ligand DBL-1 - interacts with other signaling pathways in order to confer immunity in C. elegans. One effector of this pathway in C. elegans is SMA-3, which is encoded by a gene endogenously expressed in the hypodermis, intestine and pharynx. Preliminary research conducted in our laboratory on Serratia marcescens bacteria demonstrates that sma-3 expression in either the hypodermis or the pharynx is capable of increasing survival of worms as compared to sma-3 mutants. These results indicate possible cross talk between these two tissues and the intestine – the site of the immune response. Future directions for this research include demonstrating a connection between the DBL-1 pathway and the C. elegans immune response through qRT-PCR of target immune related genes after exposure to bacteria. We will also look to establish how DBL-1 signaling interacts with other pathways to contribute to innate immunity in C. elegans.


We have employed glp-1(ar202), a temperature-sensitive gain-of-function (gf) C. elegans NOTCH mutant to delineate NOTCH-driven stem tumor responses to DNA damage. We found that after ionizing radiation all tumor cells traffic rapidly to G2/M post-irradiation, attempt to repair DNA strand breaks exclusively via homology-driven repair, and when this fails die by mitotic death. One explanation for this phenomenon might be that the Fanconi Anemia (FA) complex of proteins disables non-homologous end joining (NHEJ)-mediated DNA repair and promotes homology-directed repair (HDR). A conserved C. elegans fcd-2 has been identified, and loss-of-function fcd-2 in worms results in exquisite sensitivity to ICL agents, measured as embryonic lethality and germ cell apoptosis. However, in a glp-1(ar202);fcd-2(tm1298) double mutant sensitivity of germline tumor cells to DNA damage by ICL agents as well as ionizing radiation were distinct from more differentiated progeny. MMC- and CDDP-treated germline tumor cells in glp-1(ar202) display dose-dependent reduction of germline nuclei. Loss-of-function of fcd-2 had no impact on either MMC or CDDP sensitivity in glp-1(ar202) germline. In contrast to the effect of ICL inducing agents on embryonic survival in fcd-2 deficient worms, elimination of fcd-2 in gld-1(ar202) rendered germline tumor highly resistant to ionizing radiation. Additionally, radioreistance of fcd-2-deficient mitotic germline was suppressible by NHEJ blockade. These studies define a novel DNA damage response phenotype expressed specifically by C. elegans germline stem cells.

1227B Targeted mutagenesis in human-parasitic nematodes.  S. Gang1, M. Castelletto2, A. Bryant2, E. Yang2, J. Lopez2, E. Hallem1,2  1) Molecular Biology Institute, University of California, Los Angeles, Los Angeles, CA; 2) Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, Los Angeles, CA.

Parasitic nematodes infect over 1.5 billion people worldwide and cause some of the most common neglected tropical diseases. Despite their prevalence, the development of novel strategies to prevent nematode infections has been limited by the genetic intractability of these parasites. Here, we report the use of CRISPR-Cas9 for generating targeted gene disruptions in the human-parasitic threadworm Strongyloides stercoralis. We used CRISPR-Cas9 to disrupt the S. stercoralis ortholog of the C. elegans unc-22 gene, which encodes twitchin, a large intracellular muscle protein homologous to mammalian connectin. In C. elegans, unc-22 mutants have an uncoordinated (unc) phenotype characterized by decreased motility and body twitching; the twitching phenotype is enhanced upon exposure to acetylcholine receptor agonists such as nicotine. We generated Strongyloides-specific CRISPR-Cas9 constructs targeting Ss-unc-22 and microinjected the gonads of S. stercoralis free-living adults. A subset of F1 progeny displayed characteristic unc phenotypes, including abnormal swimming and crawling, and severe twitching when exposed to nicotine. We exploited the Ss-unc-22 twitching phenotype in nicotine to optimize CRISPR-Cas9 conditions in Strongyloides. We tested plasmid-based and ribonucleoprotein complex delivery of CRISPR constructs at three
different Ss-unc-22 target sites. Both plasmid and RNP delivery methods were effective and all three target sites tested produced Ss-unc-22 F1 mutant progeny. CRISPR-Cas9-induced double-strand breaks were robustly resolved by homology-directed repair (HDR) when an appropriate repair template was provided. The presence of an HDR template significantly improved targeting efficiency and occasionally produced Ss-unc-22 homozygous knockouts in the F1 generation. Interestingly, in the absence of HDR, we found no evidence for indels at Ss-unc-22 targets tested, but instead observed large deletions (>1 kb) surrounding the target locus. We are now investigating the mechanism of double-strand break repair in the absence of an HDR template. Finally, we demonstrated that Ss-unc-22 mutations are heritable by passing mutant F1 progeny through a laboratory host and collecting F2/F3 nematodes with Ss-unc-22 phenotypes. Our results pave the way for mechanistic studies of gene function in parasitic nematodes, and may enable the development of new strategies for nematode control.

**1228C** A novel model of mtDNA disease uncovers multiple biological pathways that control disease progression. S. Haroon1, A Li2, C Fritsch3, N Ericson4, C Haynes4, J Bielas4, T Gidalevitz5, M Vermulst1,2 1) Children's Hospital of Philadelphia, Philadelphia, PA; 2) University of Pennsylvania, Philadelphia, PA; 3) University of Washington, Seattle, WA; 4) University of Massachusetts Medical School, Worcester, MA 01605; 5) Biology Department, Drexel University, Philadelphia, PA.

Mutations in the mitochondrial genome (mtDNA) are detrimental to human health since disrupting mitochondrial function disables high-energy consuming cells like neurons and muscle fibers. Mutations in mtDNA accumulated during natural aging or inherited by children lead to diseases characterized by neuromuscular dysfunction. Currently, there are no treatments or cures for mtDNA disease. We want to identify novel therapeutic targets by discovering biological pathways that can be exploited to ameliorate mtDNA disease. In order to accomplish this goal, we are using a new worm model for mtDNA disease that carries an error-prone allele of polg-1, the polymerase that replicates the mitochondrial genome. Remarkably, this model exhibits hallmark features of mtDNA disease in humans, which include mtDNA instability, mitochondrial dysfunction, loss of neuromuscular function and a shortened lifespan. The mimicry of mammalian disease progression in the polg-1 mutant worms make them ideal for discovery experiments. With a small, targeted RNAi screen of 130 genes, the knockdown of 22 genes were identified to produce directed repair (HDR) when an appropriate repair template was provided. The presence of an HDR template significantly increased targeting efficiency and occasionally produced Ss-unc-22 homozygous knockouts in the F1 generation. Currently, we are testing whether the expression level and cellular localization of LIN-53, as well as histone modifications, are affected in the patient-derived lamin mutants in order to assess the crosstalk between Lamin and LIN-53 in vivo.

**1229A** Implication of LIN-53 in laminopathies. M. He1,2; S. Seelk1, B. Tursun1 1) Max Delbrück Centre for Molecular Medicine, Berlin, DE; 2) China Scholarship Council, Beijing, CN.

Lamin proteins assemble a complex protein meshwork known as the nuclear lamina of the inner nuclear membrane. Besides their structural role, lamins are essential in multiple cellular functions, such as DNA replication, chromatin organization, gene regulation, and cell differentiation. Mutations in the human LMNA gene encoding for Lamin A/C can result in a diverse set of genetic disorders known as laminopathies. Examples comprise Emery-Dreifuss muscular dystrophy (EDMD) characterized by muscle wasting or Hutchinson-Gilford progeria syndrome (HGPS) causing a severe form of premature ageing.

To genetically dissect the pathogenic mechanisms of human laminopathies, we introduced the corresponding mutations of patients from our clinic into the lamin gene lmn-1 of C. elegans. To recapitulate the muscle disorders observed in the patients, the mutated lmn-1 forms were specifically expressed in the body wall muscle cells. The previously described EDMD-causing Y59C (Mattout et al., 2012) was employed as a positive control. Motility assays based on swimming revealed that lamin mutations impair the motility of transgenic worms. Immunostaining for muscle proteins detected structural defects in the internal Myosin and Actin filaments as well as in the attachment structures based on Myotactin and Perlecan proteins. Currently, we are also examining worms with expression of lamin mutations in other tissues such as the intestine.

The NuRD (Nucleosome Remodeling and Deacetylase) complex has been implicated in HGPS (Pegoraro et al., 2009). Particularly, the histone chaperone RBBP4 (LIN-53 in C. elegans), a core subunit of NuRD, was identified to be an interactor of Lamin A. Moreover, loss of RBBP4 correlates with global ageing-related chromatin defects in both HGPS and normally aged cells. Notably, depletion of the RBBP4 homolog LIN-53 by RNAi or mutation in worms leads to muscle dysfunction and lifespan shortening. Currently, we are testing whether the expression level and cellular localization of LIN-53, as well as histone modifications, are affected in the patient-derived lamin mutants in order to assess the crosstalk between Lamin and LIN-53 in the development of laminopathies.

**1230B** Functional assay identifies drugs that improve muscle strength in dystrophin-deficient Caenorhabditis elegans. J.E. Hewitt1, A.K. Pollard2, N.J. Szewczyk2, S.A. Vanapalli1 1) Department of Chemical Engineering, Texas Tech University, Lubbock, TX; 2) MRC/Arthritis Research UK Centre for Musculoskeletal Ageing Research, University of Nottingham, UK.

The most common type of muscular dystrophy, Duchenne muscular dystrophy (DMD), affects 1 of every 3500 live male births. Currently, the disease is poorly understood and there is no cure—only treatments such as prednisone to control symptoms. Previous research with DMD has utilized a number of model organisms, including mice, dogs, and nematodes. Each organism has its challenges; for mice and dogs, challenges include limited screening ability and ethical issues. While C. elegans

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1284A Functional assay identifies drugs that improve muscle strength in dystrophin-deficient Caenorhabditis elegans. J.E. Hewitt1, A.K. Pollard2, N.J. Szewczyk2, S.A. Vanapalli1 1) Department of Chemical Engineering, Texas Tech University, Lubbock, TX; 2) MRC/Arthritis Research UK Centre for Musculoskeletal Ageing Research, University of Nottingham, UK.

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seems like an ideal platform for testing compounds and improving understanding of mechanisms relating to dystrophin, researchers have not been able to obtain a DMD-like phenotype in dystrophin mutants cultured in standard environments unless using a strain with a secondary mutation. This background mutation raises some questions about the similarity of the muscle degeneration mechanism to that of humans with DMD. Thus, there is a great need to establish an assay that can detect muscular degradation and measure the effects of drugs in dystrophin-deficient mutants. For this reason we have developed nematode strength as a phenotype of interest in strains with muscular mutations.

For this study, we utilized two dystrophin deficient strains: dys-1(cx18), the most commonly utilized mutant in C. elegans DMD studies, and dys-1(eg33). Both strains are reported as having nonsense mutations, although the stability and interactions of the resulting proteins are unknown. Using our NemaFlex platform, we measured the strength of the nematodes on days 3, 5, and 7 of life. While the cx18 allele did not confer discernible differences from the wild-type control at any time point, the eg33 allele was associated with weakness on days 5 and 7 when post-developmental muscle degeneration becomes more apparent. We then checked if prednisone or melatonin had any effect on the strengths of the worms. As expected, treated wild-type animals and the cx18 animals were not discernible from their non-treated counterpart, whereas the eg33 mutants improved their strength significantly under all treatments. Furthermore, some treatments returned the strength of the eg33 mutants to wild-type levels. These results suggest that there is an interesting difference between the two mutants that requires further investigation.

The ability to screen drugs for the effect on nematode strength offers a couple of promising outcomes. Using this assay, we may be able to discover new pharmacological compounds that improve strength, offering more options for treating DMD in humans. Moreover, combining our assays with analyses of cellular components in the nematode could offer additional insight on the genetic mechanisms behind muscular dystrophy.

1231C The GT92 candidate glycosyltransferase SUBS-4 is widely expressed and required for viability, osmotic integrity and susceptibility to pathogens. Maria J Gravato-Nobre, Delia O’Rourke, Dave Stroud, Angelica Lindsey-Clark, Jonathan Hodgkin Biochemistry, University of Oxford, Oxford, UK.

The GT92 (predicted galactosyltransferase) gene family, previously known as the DUF23 family, has about 60 representatives in the C. elegans genome, few of which have any known function. Mutants in one GT92 gene, subs-4 (Y47D3B.1) were recovered as Suppressors of Bacterial Sensitivity, exploiting the observation that srf-2 and srf-5 mutants are efficiently killed by the surface pathogen Leucobacter Verde1, unlike wildtype worms (Hodgkin et al. 2013). In srf-4 backgrounds, however, the subs-4 mutants exhibited embryonic lethality. The lethal mutant phenotype was rescued by a 7 kb genomic transgene, indicating that a 3 kb upstream region is sufficient for normal subs-4 expression. Some but not all multicopy subs-4(+) transgene arrays were found to provide partial maternal rescue to subs-4 mutant progeny, so that these survived as arrested larvae rather than dying as late embryos. These arrested larvae exhibited massive vacuolation and greatly increased permeability to dyes, indicating that subs-4 is essential for osmotic integrity and permeability barriers. Reporter transgene constructs using subs-4::GFP showed that the gene is expressed in all somatic tissues. Surprisingly, the GFP expressed in these transgenic lines was strongly localized to mitochondria. Inspection of the predicted 3’UTR for Y47D3B.1 revealed an unpredicted additional in-frame exon, which may be mitochondrially targeted.

Other GT92 proteins in C. elegans include GALT-1, which has known galactosyltransferase activity and confers sensitivity to a fungal toxin (Titiz et al. 2009), BAH-1 (Drace et al. 2009), BAH-2, BAH-4 and SRF-2. We found that mutations in all of the corresponding genes affected adhesion to worms by Leucobacter Verde1 and to biofilm formation on worms by Yersinia pseudotuberculosis (the Bah, Biofilm Absent on Head, phenotype), implying that all of these proteins modify surface coat properties. Interactions between different GT92 mutants were investigated, because subs-4 lethality is suppressed in subs-4; srf-2 double mutants. The other GT92 mutants (galt-1, bah-1, bah-2, bah-4) did not suppress subs-4 embryonic lethality but other double mutant effects were discovered, indicating complex interactions among GT family members.

1232A Sphingomyelin abundance is likely a key determinant in regulating drug accumulation in C. elegans. M. Kamal1,2, H. Moshiri1,2, L. Magomedova3, M. Yeo1,2, K. Szlapa1,2, R. Baga1,2, H. Zheng1,2, A. Fraser1,2, D. Hall5, C. Cummins3, P. Roy1,2,4 1) Department of Molecular Genetics, University of Toronto; 2) The Donnelly Centre for Cellular and Biomolecular Research, University of Toronto; 3) Department of Pharmaceutical Sciences, University of Toronto; 4) Department of Pharmacology and Toxicology, University of Toronto; 5) Albert Einstein College of Medicine, New York.

One major barrier to a drug’s access to target is the endothelium’s plasma membrane (PM). In metazoans, the four major classes of phospholipids include phosphatidylcholine (PC) and sphingomyelin (SM), which are enriched in the outer leaflet of the PM, and phosphatidylserine and phosphatidylethanolamine, which are enriched in the inner leaflet. How different phospholipids impact the PM’s permeability to drugs is not fully understood.

We previously identified 275 synthetic small molecules that kill C. elegans (PMID 26108372). We discovered that a subset of these, including a molecule called wact-190, accumulate as crystals within the marginal cells of the anterior pharynx and perforate the luminal PM. Dose-response analyses indicate a tight correlation (r=0.99) between crystal formation and death.

Towards understanding crystal formation, we screened for mutants that resist wact-190’s lethality and identified 46 resistant mutants. We found that reduction-of-function of a presumptive sphingomyelin (SM) synthesis pathway and a p-glycoprotein pump called PGP-14 are each sufficient to confer resistance to both the lethality and crystal formation that results from wact-190 and other crystal-forming compounds. Mutants in the SM pathway and ppg-14 not only resist the lethality induced by crystal-forming compounds, but are hypersensitive to relatively smaller molecules. Mass-spectrometry revealed that ‘resistant’ molecules accumulate less in the mutants, while ‘hypersensitive’ molecules accumulate more in the mutants relative to wild type. We found that the SM synthase, which is the terminal enzyme in the SM synthesis pathway, is expressed exclusively in the pharynx and the spermatheca, while ppg-14 is expressed exclusively in the marginal cells of the anterior pharynx. Both proteins function cell-autonomously in the marginal cells with respect to wact-190 resistance.
A human homolog of PGP-14 is ABCB4, which translocates PCs from the inner to the outer leaflet of the PM. SM synthases use the choline head from PCs to make SM. Hence our current model is that PGP-14 provides SM synthase with PCs to generate SM in the PM. Indeed, preliminary analyses indicate that both pgp-14 mutants and an SM synthase mutant are deficient in SM in anterior pharynx.

Together, our work suggests that SM abundance in the PM is a key determinant of small molecule accumulation within animal cells. Further implications will be discussed.

1233B Siderophore-mediated mitochondrial damage and host immune response. Donghoon Kang, Daniel Kirienko, Natalia Kirienko  Department of Biosciences, Rice University, Houston, TX.

*Pseudomonas aeruginosa* is a re-emerging, multi-drug resistant pathogen responsible for a large number of hospital-acquired infections each year. Virulence in this organism largely requires siderophore pyoverdine, which plays a central role in growth and regulation of a number of toxins and other pathogenic determinants. Here, we show that pyoverdine also directly damages host tissues by removing iron, disrupting normal metabolic functions.

We demonstrate that pyoverdine can translocate into *C. elegans* cells, even in the absence of live pathogen. Once inside, pyoverdine binds intracellular iron, making it unavailable to the host. Within a short period of time, iron-bound pyoverdine exits the cell, significantly depleting host iron. Since mitochondria are a rich source of iron within the host, we assayed mitochondrial health in *C. elegans* exposed to pyoverdine. Using mitochondrionally-targeted GFP, we demonstrate that pyoverdine causes extensive mitochondrial disruption. Such damage is sufficient to severely compromise host ATP production. Finally, a PINK-1-GFP reporter demonstrated that pyoverdine-mediated damage activates mitophagy.

Using transcriptional profiling, we investigated the host immune response to pyoverdine. Microarray data indicates that pyoverdine exposure triggers a SKN-1 independent detoxification response that resembles a response to hypoxia. In contrast, *C. elegans* genes upregulated by pyoverdine bear little resemblance to those activated by *P. aeruginosa* in slow kill assays or infection with *Yersinia pestis* and *Candida albicans*.

Cystic fibrosis (CF) patients are particularly sensitive to *P. aeruginosa* infections, and infection strongly correlates with pronounced downturn in patient prognosis. Despite the general importance of pyoverdine in *P. aeruginosa* virulence, its role in CF pathology is controversial. We used a pair of humanized pgp-3/CFTR alleles (one intact, one missing F508, which recapitulates the most common genetic lesion causing CF) to assess the impact of this allele on pyoverdine sensitivity. Surprisingly, the CF allele of pgp-3/CFTR upregulated by pyoverdine bear little resemblance to those activated by *P. aeruginosa* in slow kill assays or infection with *Yersinia pestis* and *Candida albicans*.

Our study demonstrates a mechanism for pyoverdine toxicity for the first time. We show that pyoverdine causes substantial mitochondrial damage, and that host activates a specific immune response to this damage. Finally, our data suggest that CF patients may have a previously unappreciated hypersensitivity to pyoverdine damage.

1234C Natural variation in *C. elegans* resistance and xenophagy against a natural intracellular parasite. Cheng-Ju Kuo1, Keir Balla2, Emily Troemel2  1) Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan; 2) Division of Biological Sciences, Section of Cell and Developmental Biology, University of California, San Diego, La Jolla, California, United States of America.

Microsporidia are obligate intracellular parasites that infect animals ranging from single-celled ciliates to humans. *Nematocida ironsii* (ERTm5), is a natural microsporidian parasite of *Caenorhabditis elegans* isolated from Hawaii (Balla et al PLoS Pathogens 2015), and we previously found that the Hawaiian *C. elegans* strain CB4856 can clear infection by *N. ironsii*, in contrast to the standard N2 laboratory strain from England that always succumbs to infection. Quantitative trait locus mapping indicated this enhanced immunity in CB4856 is a complex trait, and maps to four separate loci in the genome. We constructed and tested near isogenic lines to confirm that two of these loci (on chromosomes II and V) confer resistance against infection. Interestingly these two loci contain some of the most rapidly evolving genes in the *C. elegans* genome (Thomas, Genome Research 2006), including F-box-containing genes, which are predicted to be substrate-binding subunits in multi-subunit ubiquitin ligase complexes. These findings led the hypothesis that the CB4856 genome encodes for ubiquitin ligase adaptors that are able to target microsporidia parasite cells with ubiquitin, in order to recruit the autophagy machinery and facilitate so-called ‘xenophagy’ to clear these pathogens from intestinal cells.

Here we present unpublished data that support our hypothesis that CB4856 animals are better able to target the natural parasite *N. ironsii* for xenophagocytic clearance. We found that when we compared ubiquitin localization to *N. ironsii* parasite cells in N2 and CB4856 animals, the increased resistance to *N. ironsii* infection by CB4856 animals correlates with increased localization of ubiquitin to parasite cells in these animals. Surprisingly, when we found that the frequency of localization of the key autophagy marker LGG-1 (GABARAP orthologs) to parasite cells was NOT higher in CB4856 animals compared to N2 animals. Instead, we found that the less well-studied autophagy protein LGG-2* (LC3 ortholog), like ubiquitin, was localized to a higher percentage of parasite cells in CB4856 animals compared to N2 animals. We are currently investigating a functional role for LGG-2 in the ability of CB4856 Hawaiian animals to clear this Hawaiian *Nematocida* species. Our findings may shed new light onto the mechanisms underlying xenophagy and intracellular parasite clearance, as well as natural host/pathogen co-evolution.

* Thanks to Renaud Legouis' lab for GFP::LGG-2 plasmid used to generate these strains.

1235A Wnt signaling mediates acetylcholine-triggered intestinal host defense against infection. s. LABED1, J. Irazoqui1, H. Rahamathullah2, S. jagadeesan2  1) Department of Microbiology and Physiological Systems, University of Massachusetts Medical School, quincy, MA; 2) Massachusetts General Hospital.

Regulated antimicrobial peptide expression in the intestinal epithelium is key to defense against infection and to microbiota homeostasis. Understanding the mechanisms that regulate such expression is necessary for understanding
immune homeostasis and inflammatory disease, and for developing safe and effective therapies. We used Caenorhabditis elegans in a preclinical approach to discover mechanisms of antimicrobial gene expression control in the intestinal epithelium. We found an unexpected role for the cholinergic nervous system. Infection-induced acetylcholine release stimulated muscarinic signaling in the epithelium, driving downstream induction of Wnt expression in the same tissue. Wnt induction activated the epithelial canonical Wnt pathway, resulting in the expression of C-type lectin and lysozyme genes that enhance host defense. We discovered that the muscarinic and Wnt pathways are linked by conserved transcription factors. These results reveal a tight connection between the nervous system and the intestinal epithelium, with important implications for host defense, immune homeostasis, and cancer.

1236B  Genetic screens identified loss-of-function mutations in genes required for sensory cillum formation confer resistance to the nematicidal mushroom Pleurotus ostreatus.  C. Lee, Y. Hsueh  Institute of Molecular Biology, Academia Sinica, Taipei, TW.

The oyster mushroom Pleurotus ostreatus is a nematicphagous basidiomycete which is known to produce toxin and paralyze nematodes within minutes. To understand the molecular mechanisms of the mushroom caused paralysis, we conducted forward genetic screens to isolate mutants resistant to P. ostreatus. Approximately 30 mutants were isolated after 3 rounds of screens with the coverage of ~200,000 genomes. Genetic mapping and whole-genome sequencing identified 10 mutants have independent loss-of-function alleles in osm-1, osm-6, daf-6 and dyf-7. Complementation test with fosmid clones containing these genes rescue the phenotype. Dye filling experiments showed that all mutants isolated from the screens were unable to uptake the dye (Dil). These results demonstrated that mutants defected in the sensory cillum formation confers resistance to the mushroom, suggesting that the toxin uptake likely occurs at the sensory cillum which then caused muscle hyper contraction in the body wall muscle of C. elegans via a still unclear mechanism.

1237C  Investigating the “toxic pore” model for beta-amyloid peptide toxicity.  Carl Julien, Christine Roberts, Joshua Morgenstern, Cole Tomberlin, Chris Link  Integrative Physiology, Univ Colorado, Boulder, CO.

Many lines of evidence support the view that accumulation of the betaamyloid peptide (Abeta) is central to Alzheimer's disease (AD) pathology, but the relevant molecular mechanisms remain unresolved. Abeta can convincingly make ion-conducting pores in synthetic membranes, but whether this occurs in vivo is not known. We have developed a novel assay for Abeta toxicity, based on feeding worms. E. coli engineered to secrete human Abeta. We find that feeding worms E. coli secreting wild type Abeta leads to the induction of intestinal endocytosis previously observed for worms fed E. coli producing the known pore-forming toxin CRY5B. Endosome induction did not occur in parallel experiments with E. coli producing Abeta Gly37Leu, a single residue substitution that blocks pore formation in synthetic membranes. Super resolution microscopy revealed that Abeta-induced endosomes contain membrane-associated Abeta, supporting the view that endosome induction reflects a membrane repair process that removes plasma membrane-associated Abeta pores. To investigate the relevance of this phenomenon to AD, we assayed the role of worm orthologs of human genes associated with increased AD risk by genome-wide association studies (“GWAS genes”) in endosome induction. We find that loss of function of either unc-11 (ortholog of human PICALM) or amph-1 (ortholog of human BIN1) significantly increase Abeta-induced endosome induction, as well as sensitize worms to CRY5B toxicity. We are currently assaying the effects of Abeta-responsive genes (identified in our gene expression analysis of Abeta transgenic worms) on endosome induction in this model.

1238A  Mechanisms of immune activation after cuticle damage.  J.-C. Lone, J. Belougne, J.J. Ewbank, N. Pujol Aix Marseille Univ, CNRS, INSERM, CILM, Marselle, France.

We are studying the innate immune response of C. elegans to infection by a natural fungal pathogen. Through genetic and RNAi screens, we have defined several signaling pathways that act in the adult epidermis to regulate the expression of defense genes, including genes encoding antimicrobial peptides (AMP). We have shown that infection provokes the conversion of tyrosine to hydroxyphenyllactic acid (HPLA) and this in turn activates the G-protein coupled receptor DCAR-1 that acts upstream of a PKC-p38 MAPK pathway and the STAT-like transcription factor STA-2. Not only are dcar-1 mutants deficient in their response to infection, but they also fail to switch on AMP expression following physical injury. Thus HPLA appears to be a damage-associated molecular pattern (DAMP), and DCAR-1 to be the first DAMP receptor identified in C. elegans [1-3]. Just how infection or injury leads to an increase in HPLA is, however, unknown. We are currently dissecting the mechanism leading to its production through a forward genetic screen. Interestingly, we found that mutants lacking annuli, the structure on the cuticle that delineates the furrows, exhibit a high constitutive expression of AMP genes. We know for one of them, dpv-10, that the level of HPLA is elevated, and for all of them that the high AMP gene expression is dependent on dcar-1. Thus, we designed a genetic screen using a furrow mutant (dpy-7) background in which an infection-inducible AMP reporter gene is constitutively turned on, from the early larval stage. To avoid isolating mutants for components downstream of the GPCR, the strain used also expresses a constitutively active Ga protein (GPA-12) specifically in the adult epidermis. Screening for mutants that block reporter gene expression in larvae but not in adults will identify genes acting upstream of GPA-12 and hence potentially reveal the initial steps leading to the production of HPLA.

We have screened over 60,000 genomes and obtained 7 candidate mutants. We are now outcrossing them and further phenotypically characterising them before using whole-genome sequencing to identify the underlying molecular lesion(s). We hope that this will give insights into how infection and injury is recognised in the worm.

1239B Exploring the link between CL-5, a potential anthelmintic, and oxidative stress.  J.A. Miskowski, K. Kennedy, J. Lindstrand, A. McKeever, C. McKeever Bio Dept, Univ Wisconsin, La Crosse, La Crosse, WI.

C. elegans has long been a model system for parasitic worms, or helminths. Anthelmintic treatments affect C. elegans and helminths similarly, and the mechanisms of action for commercially available anthelmintics were elucidated in C. elegans. Previously, we reported the identification of CL-5, a chemical with anthelmintic activity, from our library of natural product derivatives. CL-5 affects worm motility and survival in two microscale, liquid-based assays. When added to NGM plates, CL-5 shortens lifespan at low concentrations and causes death within minutes or hours at higher concentrations. CL-5 is effective against mutant strains that are resistant to the major anthelmintics on the market, including ivermectin, benomyl, and levamisole, suggesting a novel mode of action.

CL-5 is structurally related to resveratrol, and high levels of resveratrol attenuate the motility and killing effects of CL-5. Resveratrol is reported to lower oxidative stress in C. elegans, thereby increasing longevity. Treatment with CL-5 leads to nuclear localization of DAF-16::GFP and increased expression of its target gene sod-3, both of which are indicators of oxidative stress. Using the Amplex Red assay, elevated levels of hydrogen peroxide were detected in wildtype animals treated with CL-5 compared to controls. gas-1 mutants, which are defective in a subunit of Complex I in the mitochondrial electron transport chain (ETC) and exhibit increased ROS, are hypersensitive to CL-5. Likewise, clk-1 mutants that prevent biosynthesis of the electron carrier ubiquinone show increased sensitivity to CL-5. When wildtype animals were exposed to both CL-5 and ubiquinone in our liquid assay, rates of paralysis decreased compared to worms treated with only CL-5. Furthermore, ubiquinone supplementation in solid plates containing CL-5 dramatically reduced the percentage of animals that die. Additional experiments are underway to explore whether CL-5 elicits its effects by perturbing normal mitochondrial function.


Multiple disease-associated proteins (Abeta, alpha-synuclein, amylin) have been proposed to form membrane-damaging oligomers, but this has not been directly observed in vivo. Following the elegant studies of the Aroian group on the toxicity of the CRYSB pore-forming toxin in C. elegans, we have generated E. coli strains that secrete disease-associated proteins, including wild type and variant Abeta. Feeding E. coli expressing wild type Abeta (but not the non-toxic single residue variant Abeta G37L) to worms results in an induction of intestinal endocytosis. This is analogous to the endocytosis induced as a membrane repair process in mammalian cells exposed to streptolysin O, another pore-forming toxin. This endocytosis can be visualized using a fluorescent reporter localized to the intestinal lumenal membrane (vha-6::mCherry) or by uptake of Texas Red-dextran. Importantly, induction of endocytosis can be suppressed by mutations in genes encoding acid sphingomyelinase, a key component of the membrane repair process in mammalian cells. Induction of endocytosis can also be modulated by mutations in worm genes orthologous to human associated with increased Alzheimer's disease risk (e.g., BIN1 and PICALM) or by genes we have previously identified as upregulated in response to transgenic Abeta expression (e.g., clp-4). Currently we are investigating the mechanistic roles of these interacting genes and developing methods to follow the fate of endocytosed toxic proteins.

1241A Effects of the anti-microbial agent triclosan on C. elegans survival and behavior.  Noël Khamis, Amanda Favetta, William Hoffmann, Edith Myers Biological and Allied Health Sciences, Fairleigh Dickinson University, Florham Campus, Madison, NJ.

Triclosan is an antimicrobial agent currently found in products such as soaps and toothpastes. Triclosan has been found to alter endocrine function, development, and lifespan in animals ranging from mollusks to mammals. We are using C. elegans as a system to understand how triclosan may be acting. We have found that triclosan affects C. elegans survival, killing them in a dose-dependent manner. Sublethal concentrations of triclosan have a significant effect on C. elegans movement and egg-laying behavior. Using egg in worm assays, staged egg assays and brood size assays, we have found that triclosan affects egg-laying by decreasing egg production, while at the same time increasing the time eggs are retained before they are laid. Triclosan also appears to affect locomotion in an unc-68 dependent manner. unc-68 encodes a worm ryanodine receptor ortholog. Ryanodine receptors have been implicated in the mode of triclosan action in mouse cardiac and skeletal muscle (Cherednichenko et al. 2012). We are interested in determining the pathways involved in the effect of triclosan on survival and egg development.


1242B Identification of new anthelmintic chemotypes by screening both parasitic nematodes and C. elegans models.  Frederick E. Partridge, Anwen. E. Brown, David B. Sattelle UCL Respiratory, Division of Medicine, University College London, London, GB.

Parasitic nematodes infect around one billion people, with ascariasis, hookworm infection and trichuriasis each afflicting hundreds of millions of people. These diseases cause massive morbidity and are closely linked to poverty in the developing
world. Parasitic nematodes are also of considerable cost due to their infection of domesticated animals, both livestock and companion animals; and their damage to crop species. We have developed automated high-throughput approaches to measuring nematode motility and growth, and used these methods to screen compound libraries for new anthelmintics. We utilise both parasite nematodes themselves, and C. elegans as a model nematode as it is particularly suited to high-throughput screening.

Here we report on our progress with these studies, including the discovery of a new anthelmintic chemotype, the dihydrobenz[e][1,4]oxazepin-2(3H)-ones, which are active in both in vitro and in vivo studies against the model whipworm Trichuris muris. We are carrying out further iterative synthesis and improvement of this chemotype to gain an understanding of the structure-activity relationship. A new agent for Trichuris is particularly needed as current benzimidazole drugs have notably limited single-dose efficacy against this parasite, which limits the effectiveness of ongoing mass drug administration programs.

1243C FLCN and AMPK confer resistance to bacterial pathogens via TFEB/TFE3 mediated innate immune response. E. Possik1,2, T Vijayaraghavan1,2, M Paquette1,2, L El-Houjeiri1,2, A Kapelanski-Lamoureux1,2, A Pause1,2 1) Department of biochemistry, McGill University, Montreal, Quebec, CA; 2) Goodman cancer research center, McGill University, Montreal, Quebec, Canada.

Humans and animals are constantly exposed to pathogenic microorganisms that can enter the body and cause disease. Mechanisms of defense against invading pathogens are essential for all living organisms to propagate and survive. The transcription factor EB (TFEB), a master transcriptional regulator of lysosomal and autophagic pathways, has been recently shown to play a critical role in innate immunity both in Caenorhabditis elegans (C. elegans) and mammals. Despite the important role(s) of TFEB in physiological and pathological states including neurodegenerative diseases, immunity, and cancer, its upstream regulation is not fully elucidated. Here, we show that FLCN-1 and its binding partner SAMP-activated protein kinase (AMPK) regulate HLH-30, the TFEB worm homolog. FLCN-1 is the nematode ortholog of the tumor suppressor FLCN, responsible for the Birt-Hogg-Dubé (BHD) tumor syndrome. Using C. elegans, we show that fcn-1 mutant animals exhibit an increased resistance to bacterial pathogens in an hhl-30-dependent manner. Loss of fcn-1 induces the nuclear translocation of HLH-30 upon infection with Staphylococcus aureus, which increases the transcription of antimicrobial genes. We further show that this phenotype is independent of mTOR. Using RNA-Seq technology, we demonstrate that AMPK regulates a subset of the hhl-30-dependent antimicrobial peptide genes. Accordingly, loss of AMPK decreases the HLH-30 nuclear translocation and suppresses the increased resistance of fcn-1 mutant animals to Staphylococcus aureus and Pseudomonas aeruginosa. Importantly, our findings highlight that this pathway is evolutionarily conserved. Specifically, we show that loss of Fcn in mouse embryonic fibroblasts also induces the nuclear translocation of TFEB/TFE3 and the upregulation of inflammatory cytokines in an mTOR independent manner. Overall, our findings support an important role for FLCN and AMPK in the regulation of TFEB, which is a central mechanism of resistance to pathogenic agents.

1244A Identification of Caenorhabditis elegans genes involved in the response to pathogenic Stenotrophomonas maltophilia bacteria. L. Radeke, C. White, M. Herman Division of Biology, Kansas State University, Manhattan, KS.

Stenotrophomonas maltophilia is an emerging nosocomial pathogen that is associated with hospital-acquired pneumonia, cystic fibrosis, and cancer. Despite the prevalence of S. maltophilia infections, little is known about its virulence mechanisms. S. maltophilia is ubiquitous in the environment where it is encountered by many organisms, including Caenorhabditis elegans. Members of the Stenotrophomonas genus are part of the native C. elegans microbiome and were found in greater relative abundance within the worm than in the environment sampled, suggesting that these bacteria accumulate within C. elegans (Dirksen, et al., 2016). We found a local S. maltophilia isolate (JCMS) in association with grassland soil nematodes to be more virulent to C. elegans than other S. maltophilia isolates (R551-3 and K279a) tested. We are using these strains as well as another virulent S. maltophilia strain, JV3, to study host-pathogen interactions. We previously found that C. elegans employs several innate immune pathways in response to JCMS, but the DAF 2/16 pathway, a major C. elegans defense pathway, was ineffective (White et al., 2016). To determine genes that might be directly involved in the response to JCMS, a microarray experiment was performed to identify differentially expressed genes between JCMS, K279a and the standard C. elegans food E. coli OP50. Wormnet, a probabilistic gene network model, was used to prioritize the most connected differentially expressed candidate genes. To more directly characterize the role of the DAF 2/16 pathway in response to JCMS, we performed an RNA sequencing experiment on wild-type and daf-2 mutants exposed to JCMS, K279a, JV3, and OP50. We have identified 145 C. elegans genes that are differentially expressed in response to pathogenic and nonpathogenic strains in wild-type worms. Gene ontology (GO) analysis revealed that terms related to the innate immune response and response to bacterium are significantly enriched among these 145 genes. Furthermore, 164 of the 257 genes differentially expressed in response to JCMS are DAF-2 dependent. Almost all (92%) of these genes are downregulated in response to JCMS, suggesting that JCMS inhibits expression of many immune response genes. Wormnet was used to prioritize the DAF-2 dependent genes for functional validation by examining the genes that are most connected within this network. These findings along with further functional validation of individual genes will provide an in-depth understanding of the C. elegans innate immune response to S. maltophilia JCMS, and perhaps reveal how S. maltophilia JCMS defeats the DAF 2/16 pathway defenses.

References:
White et al., 2016. Infect. Immun. 84: 524-536

1245B Nickel, a common environmental pollutant, is an animal genotoxin and mutagen. D. Rudel, E. Hvaslavs, J. Atkinson, N. Schaal, H. Campbell, J. Lockley Department of Chemistry, East Carolina University, Greenville, NC.
Natural nickel (Ni) exposure occurs through volcanism and weathering of rocks. However, Ni’s anthropogenic exposure has increased and overwhelmed natural exposure. Ni is a component in metal alloys and car exhaust. Ni is also an essential metal in plants and a component of cigarette smoke. Ni is highly allergenic and genotoxic, inducing carcinomas in industrial workers. Despite known Ni-induced DNA damage in prokaryotes, Ni-induced DNA damage has not been demonstrated in animals. Our data from genetic screens using Ni treatments on N2 animals and programmed cell death (PCD) mutant animals indicate Ni induces the apoptosis of germ cells in Caenorhabditis elegans. We have quantified Ni-induced germ cell PCDs using an apoptotic gfp expression marker and dyes. Ni-induced PCDs rely upon genome surveillance as removal of p53 reduces these Ni-induced PCDs. In a PCD mutant background, animals treated with Ni acquire mutations as demonstrated through the generation of heritable morphological phenotypes, i.e. Dpy, Unc, Bl, Rol, Muv, and Vul. Previously Ni’s mutagenicity may have been masked through apoptosis of affected cells. Two models are proposed for Ni action. Direct-action implies Ni or Ni-generated reactive oxygen species (ROS) damages the DNA in an autonomous fashion. Indirect-action implies that Ni interacts with DNA packaging and repair proteins making DNA more susceptible to endogenous direct-acting mutagens. Genetic screens involving Ni-alone, ethyl methanesulfonate (EMS)-alone, and joint treatments were performed. Ni does not substantially enhance the mutagenicity of the direct-acting mutagen EMS. The joint treatment’s effect appears additive in terms of number of mutations generated. Thus Ni putatively acts directly. Using an electrochemical assay, DNA harvested from Ni-treated worms shows damage in comparison to DNA from un-treated controls. Chemical reconstitution experiments using Ni, hydrogen peroxide and genomic DNA suggests Ni generates ROS as a part of its mechanism. We are identifying chemical changes to DNA upon Ni exposure. Preliminary mass spectrometry (MS) analysis of hydrolyzed Ni-damaged DNA in comparison to untreated DNA from the N2 strain shows accentuated molecular species and the generation of new m/z species corresponding to chemically modified DNA bases. Lastly we have compared Ni-induced DNA damage from strains isolated from highly volcanic regions (high Ni) to common laboratory stocks isolated from non-volcanic regions (low Ni) for both C. elegans and P. pacificus. Volcanic strains do not accrue as much damage as non-volcanic upon Ni exposure. This indicates compensatory genomic differences between strains based upon ecology and life history.

1246C Context-dependent olfactory behaviors of the parasitic nematode Heligmosomoides polygyrus. F. Ruiz, M. L. Castelletto, S. S. Gang, E. A. Hallem Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, Los Angeles, CA.

Passively ingested parasitic nematodes cause extensive disease and economic loss worldwide, with some species infecting humans and others infecting livestock. While skin-penetrating parasitic nematodes have been shown to actively search for hosts using human skin and sweat odorants, the extent to which passively ingested parasitic nematodes engage in odor-driven host seeking was unclear. Here, we examined the olfactory behaviors of the passively ingested murine parasite Heligmosomoides polygyrus. We found that H. polygyrus infective juveniles (IJs) are highly motile and are robustly attracted to host fecal odor, consistent with their ability to infect mice from feces when the mice consume feces containing IJs. However, despite their attraction to feces, many of the IJs on a fecal source migrate away from the feces and engage in environmental navigation. H. polygyrus IJs are also attracted to a diverse array of mammalian odorants, suggesting that they use olfactory cues to actively navigate toward hosts as well as host feces. Surprisingly, the olfactory preferences of H. polygyrus IJs are highly context-dependent: some odorants are repulsive for IJs cultured on feces but attractive for IJs cultured off feces. The context-dependent modulation of olfactory behavior may be a mechanism that enables IJs to switch from dispersal behavior to host-seeking behavior. Our results demonstrate that passively ingested nematodes do not remain inactive waiting to be swallowed, but instead engage in complex odor-driven behaviors to position themselves for host ingestion. A better understanding of these behaviors may lead to new strategies for nematode control.

1247A The molecular mechanism regulating quick avoidance behavior to pathogenic bacteria P. aeruginosa. R. Saito1,2, Y. Shinkai1, M. Doi1,2 1) Biomedical RI, AIST; 2) Life Sci & Bioeng., Grad. Sch. of Life & Env. sci., Univ. Tsukuba.

Avoidance from unfavorable environments is one of important survival strategies in many animals. As for Caenorhabditis elegans, avoidance from pathogenic bacteria is required to select its better living environment. Worms show this avoidance behavior to Pseudomonas aeruginosa which is a common Gram-negative pathogenic bacterium and is known to affect health condition in many animals including humans. For this reason, C. elegans which fed P. aeruginosa shows an associative learning behavior between odors (secondary metabolites from the bacteria) and several uncomfort in the body. The components of secondary metabolites, which are perceived by single class of sensory neuron, have been identified. However, it is not clear what kind of uncomfort signals is detected in the body, and how those signals are integrated into the nervous system to regulate learning behavior. Therefore, in this study, we are trying to elucidate the molecular and cellular mechanism which acts to detect an uncomfort signal from P. aeruginosa in the body.

To identify the genes involved in the detection of bacteria as an unfavorable signal, we searched mutants which show altered response to P. aeruginosa (PA14). The immune response systems in C. elegans is known to be activated from 4 hours after worms fed PA14. We found that spending more than 6 hours on the PA14 lawn was required to retain an associative learning in the bacteria choice assay. We also examined how quickly worms respond to PA14, and found that more than 80% of wild-type animals showed avoidance behavior to PA14 within 1 hour. Therefore, we screened mutants which did not show quick avoidance behavior to PA14 in this period. We successfully isolated a candidate mutant allele (ta218) which show a significantly lower quick avoidance behavior to PA14 (only 20% avoidance in 1 hour). We mapped the ta218 mutation at the central region of the 4th chromosome.

Simultaneously, we focused on the immune response pathway in C. elegans whether some genes are responsible for quick avoidance behavior or not. Using avoidance assay, we found that some mutants such as age-1 and sek-1, both are known to function for the worm immune response, did not show quick avoidance behavior to PA14, suggesting that not all but some
genes may be acting to detect pathogenic bacteria for quick avoidance. We are now trying to identify the corresponding gene for the ta218 mutant by using both SNP mapping and next-generation sequence analysis. We hope that our analyses will identify the molecular mechanism for the pathogenic bacteria detection, separated from existing immune response pathway in C. elegans.


Mitochondria play a key role in muscle cells because strength and function of muscle tissue is highly dependent on the energy they produce. Muscles can be altered by two types of muscle degeneration: the physiological muscle degeneration caused by aging and the pathological muscle degeneration caused by diseases like muscular dystrophies. Mitochondria shape changes continuously due to fission and fusion events. In C. elegans, muscle cells display a tubular mitochondrial network, which fragments during physiopathological muscle degeneration.

Here we investigated the molecular mechanism of muscular mitochondrial dynamics by screening for genes which decrease muscle degeneration after RNAi knock-down in a model of dystrophin-dependant muscle degeneration, in order to identify genes with an action through mitochondria morphology. In a predefined list of 70 genes that reduce dystrophin-dependant muscle degeneration, we identified 29 candidate genes that participate in mitochondria biology in the literature.

Among them, we looked for RNAI that can increase tubular mitochondria in muscle cells during dystrophin-dependent muscle degeneration.

The screen revealed the first evidence that WAH-1 (AIF homolog) play a role in mitochondria morphology in muscle cells because mitochondria tubular forms increase with WAH-1 down-regulation. Moreover, we confirm that WAH-1 plays a role in dystrophin-dependant muscle degeneration. In the same way, DRP-1, the mitochondria pro-fission protein, is well known to control mitochondria fragmentation. A precedent study show also that DRP-1 diminution can reduce dystrophin-dependant muscle degeneration. We found in this study that DRP-1 can also reduce aging-dependant muscle degeneration. So DRP-1 is an actor that can decrease at least two types of muscle degeneration: physiological and pathological. Additionally, DRP-1 and WAH-1 are already known in the literature to be implicated also in cell death. There are links between mitochondria dynamics and cell death but currently not completely understood. We found that pro-apoptotic role of DRP-1 and WAH-1 seems to be implicated in muscle degeneration. Interestingly, reducing WAH-1 in absence of DRP-1 can decrease dystrophin-dependant muscle degeneration further than wah-1 RNAi or drp-1 null mutation alone suggesting two distinct molecular pathways.

Our findings point toward novels mechanisms involving DRP-1 and WAH-1 in mitochondria morphology to impact at large muscle degeneration.

1249 C Opposite strain-specific defence against pathogens mediated by the GATA transcription factor ELT-2. A. Zarate-Potes, W. Yang, H. Schulenburg, K. Dierking Evolutionary Ecology and Genetics, University of Kiel, Kiel, DE.

There is increasing evidence that invertebrates can produce strain-specific defence responses against pathogens. We explored such strain-specific responses by studying the interaction between C. elegans and different strains of the Gram-positive pathogen Bacillus thuringiensis. A transcriptomic analysis identified distinct inducible expression responses to the two tested pathogen strains with different virulence levels (BT18247 and BT18679). Transcription factor analysis further revealed a key involvement of the GATA transcription factor gene elt-2. elt-2 RNAi knockdown caused high susceptibility to the strain BT18679, yet surprisingly high tolerance to strain BT18247 (i.e., almost no mortality in spite of high bacterial load). Through epistasis analysis, we found that the p38-MAPK pathway acts either in parallel to or directly interacts with elt-2 in response to the pathogen BT18679, but is not required for the resistance to BT18247. The DAF-2/ILR pathway however is involved in the response to BT18247, likely acting downstream of elt-2. elt-2 mediated defence against BT18679 seems to depend on expression of putative immune effectors. In contrast, the increased tolerance to BT18247 after elt-2 knockdown is likely due to changes in lipid metabolism and differential regulation of detoxification genes. Moreover, we identified additional transcription factors, such as sbp-1 and nhr-193, which seem to regulate the specific response to BT18247 infection in interaction with elt-2. In conclusion, our study demonstrates an unusual contrasting effect of elt-2 on pathogen defence and uncovers the involved processes leading to either elt-2 mediated defence or pathogen tolerance after elt-2 knockdown. More generally, our findings highlight to what extent and how invertebrates can produce highly fine-tuned, pathogen strain-specific defence responses.

1250A Role of UDP-glucuronosyltransferase (UGT) enzymes in benzimidazole drug biotransformation by nematodes. N. Sharma1, S. Stasik1, PE. Mains2, JS. Gillette1 1) Department of Comparative Biology and Experimental Medicine, Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta, Canada; 2) Departments of Biochemistry & Molecular Biology, Medical Genetics, University of Calgary, Calgary, Alberta, Canada.

The benzimidazoles (BZ), which targets microtubules, are a family of broad-spectrum anthelmintics used in livestock, and are one of the few drug classes available for the control of human helminths. Resistance of parasitic nematodes to commonly used anthelmintics is currently a major problem for the livestock industries and an emerging concern for human health in the developing world. Xenobiotic/drug metabolism has been subject to intensive research in mammals because of its importance in modulating drug potency. Drug metabolism is an important modulator of drug efficacy in number of systems including insects and mammals. In contrast, there has been much less research in nematodes. Our previous work has shown that the free-living model nematode, C. elegans and the small ruminant parasite Haemonchus contortus detoxify/efflux several BZ anthelmintics by
metabolizing them by conjugation with a glucose residue (Stasiuk, S et al, manuscript in preparation). This suggests the potential involvement of phase II xenobiotic metabolizing enzymes (XMEs), specifically the UDP-glucosyltransferase (UGT) enzyme family. Although rare in mammals, conjugation with a glucose residue is common in plants and bacteria, and likely nematodes. This biochemical difference from mammalian hosts could potentially represent a "druggable" target to improve the potency of BZ's and UGTs could be involved in BZ resistance. We have recently performed RNAi screen on 15 C. elegans UGTs prioritized from transcriptomics analysis from worms exposed to BZ drugs. Through RNAi screen we have found that knockdown of UGT-9, UGT-11 and UGT-22 make worms more sensitive to BZ. We are currently characterizing these enzymes further to determine how they modulate BZ potency using loss-of-function mutant strains and by transgenic overexpression. We will also examine changes in metabolite production using HPLC-MS/MS. The investigation of BZ drug metabolism in C. elegans will provide a platform upon which we can investigate and extrapolate information to other nematodes initially for H. contortus and then for other parasites.

1251B The role of the decapping enzyme EOL-1 in intracellular infection response. Jessica Sowa, Emily Troemel Division of Biological Sciences, University of California at San Diego, La Jolla.

Microsporida comprise a phylum of obligate intracellular parasites most closely related to fungi. Although they infect a wide range of animal species and can cause lethal infections in immunocompromised patients, relatively little is known about the mechanisms of host defense against microsporida. Nematocida parisii is a species of microsporida that is a natural pathogen of C. elegans. N. parisii infects the C. elegans intestine, causing fusion of intestinal cells and ultimately lethality (Troemel et al, PLoS Biology 2008; Balla et al, Nature Microbiology 2016). Our lab has profiled the C. elegans transcriptional response to N. parisii (Bakowski et al, PLoS Pathogens 2014), with the goal of identifying features of the host response to microsporida infection.

One gene found to be induced by N. parisii infection was eol-1, a de-capping and exonuclease enzyme that has been reported by Yun Zhang's lab to be involved in regulating pathogen-induced aversive olfactory learning (Shen et al, J. Neuroscience 2014). Our RNAseq data shows that eol-1 is strongly up-regulated during N. parisii infection, beginning early at 8 hours post infection. In uninfected worms, we find that EOL-1 is expressed primarily in the spermatheca and URX neurons based on analysis of EOL-1 transcriptional and translational fusions*. Interestingly we find that when worms are exposed to N. parisii, eol-1 expression is induced in the intestine, which is the tissue targeted by N. parisii. Our preliminary results from analysis of eol-1 mutants suggest that eol-1 plays a role in host response to N. parisii infection.

*Thanks to Yun Zhang's lab for sending eol-1 strains.

1252C Comparative genomics analysis unveils genetic factors governing the pathogenicity of P. aeruginosa towards C. elegans. Isana Vekser-Lublinsky1, Alejandro Vasquez-Rifo1, Zhenyu Cheng2, Fred Ausubel2, Victor Ambros1 1) Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA 01605; 2) Department of Microbiology & Immunology, Dalhousie University, Halifax, Nova Scotia, Canada; 3) Department of Genetics, Harvard Medical School, Boston, MA 02115.

The pathogenic potential of bacteria against animal host, such as Caenorhabditis elegans (C. elegans), can vary drastically between distinct strains of the same bacterial species. The remarkable fluidity of bacterial genomes, shaped by horizontal gene transfer (HGT) amongst populations of bacteria in their natural habitats, is thought to contribute to inter-strain variation in pathogenic behavior towards natural hosts, including nematodes. Pseudomonas aeruginosa (P. aeruginosa) is a free-living bacterium that is a pathogen of a broad range of eukaryotic hosts, including nematodes, insects, mammals and plants. Wild isolates of P. aeruginosa can lethally infect adult C. elegans worms, yet the pathogenicity (virulence) of P. aeruginosa strains against wild type C. elegans varies widely among distinct strains. Using a comparative genomics and phylogenetic approach, we investigated the genetic determinants that drive evolutionary changes in P. aeruginosa pathogenicity towards C. elegans. Our results suggest an evolutionary model that links the presence/absence of CRISPR with gain/loss of known virulence genes and potential anti-virulence genes, and accounts for differences in pathogenicity amongst P. aeruginosa strains against C. elegans.

1253A FSHR-1 and SKN-1 link Innate Immunity and Oxidative Stress. Z.C. Yeoh, R.R. Callaghan, J.R. Powell Department of Biology, Gettysburg College, Gettysburg, PA.

The innate immune system is a broadly conserved defense mechanism inherent to nearly all multicellular organisms. Production of Reactive Oxidative Species, or ROS, is a facet of the immune system that can damage both pathogen and host cells in response to infection. To protect itself from the oxidative stress resulting from infection-dependent ROS, host cells must coordinate a detoxification response; however, the mechanisms for this type of innate immune response are not well understood. In C. elegans, the Nrf transcription factor SKN-1 is a master regulator of the infection-independent oxidative stress response, activating a collection of genes that can detoxify ROS and repair damage to cells. We know of several proteins that regulate SKN-1 activity, including WDR-23, which is part of a ubiquitin-ligase complex that negatively regulates SKN-1. Even though SKN-1 is also known to play a role in infection-induced oxidative stress, we do not yet have a clear understanding of how this response is regulated. One possible candidate for regulation is FSHR-1, a G-protein coupled receptor that is known to play multiple roles in pathogenic defense and induces at least one SKN-1 target detoxification gene. However, the relationship between FSHR-1, SKN-1, and WDR-23 has not yet been elucidated. Using infection survival, pathogen avoidance, and oxidative stress reporter assays, we will determine how these proteins interact in response to infection-dependent oxidative stress.
Base excision repair (BER) is a repair pathway for relatively small damaged bases in DNA, and consists of various kinds of enzymes, such as DNA glycosylases and AP endonucleases. The molecular mechanism of BER has been elucidated so well, but its physiological roles in multicellular organisms are not fully understood. In this study, we investigated the relationship between DNA glycosylases and AP endonucleases and animal development and morphogenesis, using Caenorhabditis elegans (C. elegans). In C. elegans, two DNA glycosylases, nth-1 and ung-1, which remove oxidative pyrimidines and uracil respectively, and two AP endonucleases, apn-1 and exo-3, are conserved. We found that the exo-3 mutant showed developmental delay, and increased number of protruding vulva (pvl) under dut-1 RNAi, while the apn-1 mutant did not show either of them. The developmental delay was dependent on not ung-1 but nth-1 under normal conditions. However, when worms were treated with sodium bisulfite (NaHSO3), which causes excess uracil in DNA, the phenotype depended on ung-1. On the other hand, the pvl relied on ung-1, and was enhanced under additional oxidative stress conditions, such as ndx-1 RNAi, ndx-2 RNAi, or methyl viologen treatment. The enhancement of the pvl did not depend on nth-1. Moreover, both of these two phenotypes in the exo-3 mutant, developmental delay and increased number of pvl, were rescued by the lack of chk-2. These results suggest that deficient repair of AP sites has an adverse effect on development and morphogenesis through removing base damages such as oxidative base damages and uracils from genome and subsequently checkpoint activation.

Education

1255C Student attitudes of research-based experiences in first year lab courses. J.P. Chan, T.A. Staab Biology, Juniata College, Huntingdon, PA.

First year experiences are becoming increasingly assessed and analyzed as critical to student retention and development. Furthermore, STEM departments have increased their emphasis on implementing first year research experiences into their curriculum. Accordingly, high impact such as research experiences increase retention because they promote inquiry and discovery in students by providing opportunities to discover and contribute to projects. However, research experiences come in many formats, from short term projects to whole semester experiences and within first year to upper level courses. Many primarily undergraduate institutes are challenged to introduce research in first year biology labs that are based on short, modular timeframes and large enrollments. At Juniata College, our department is constricted by 3-week modular labs to facilitate our large incoming biology class. Here, we describe a C. elegans lab module aimed at introducing first year biology students to research, including data collection, quantification and scientific communication. We describe a procedure that allows students to analyze the slowing response of wild type animals in response to ethanol, in comparison to responses of slo-1/BK channel mutants. We will report data from surveys taken from two years of students regarding their attitudes on the importance of research experiences. We hope this information will inform how we can better implement and teach science in the biology curriculum.


Various model organisms allow researchers to draw valuable insights into larger complexities of the natural world. With a relatively simple nervous system consisting of only 302 neurons, stereotyped motor behavior, and method of cultivation, the nematode Caenorhabditis elegans is an ideal model organism to study neuroscience and biophysics. The Elegant Mind Club at UCLA seeks to study this simple yet behaviorally insightful organism to provide undergraduate students across STEM disciplines the unique opportunity to design their own research methods and carry out experiments in a laboratory setting, allowing them to explore the nature of scientific research. Students are tasked with ensuring specimen propagation, as well as with assembling their own experimental hardware. Additionally, undergraduates gain hands-on experience working with and helping design a collection of state-of-the-art in-house microscopes. Sole responsibility for biological sample quality teaches students the discipline associated with handling chemicals and maintaining sterility. Peer-reviewed publications and online resources associated with the prolific C. elegans community, such as WormBook, WormAtlas, and the Caenorhabditis Genetics Center, provide students with method reference. Today, systems for thermotaxis, electrotaxis, phototaxis, magnetotaxis and free motion have been reproduced from literature and embellished by our members to conduct novel research on the behavioral and neuronal level. Our lab was founded by a few core members and has now expanded to host over 50 students from universities across the world. We hope to instill students with a fervor for pursuing scientific discovery.

1257B A collaborative laboratory exercise between students in cancer biology and bioinformatics courses. Garrett Dancik, Amy Groth. 1) Department of Biology, Eastern Connecticut State University, Willimantic, CT; 2) Department of Computer Science, Eastern Connecticut State University, Willimantic, CT.

Although science is becoming increasingly interdisciplinary, undergraduate courses are frequently discipline specific, and laboratory exercises often do not reflect the real-life collaboration that occurs between scientists. We have developed a multi-week, collaborative laboratory exercise utilizing the model organism, C. elegans, for biology majors enrolled in a biology of cancer course and computer science majors enrolled in a bioinformatics course. This exercise is based on the potential targets of p53 in worms and was designed to mimic the collaboration between biological researchers and bioinformaticists. The cancer biology students present the bioinformatics students with a summary of cancer and the role of p53, and request an analysis to
identify candidate p53 targets. In the experiment summary, the consensus binding site for p53 is provided, as well as the parameters of the bioinformatics analysis (how to define a promoter, how many binding sites each promoter should contain, the format of the data they would like to receive, etc.) The bioinformatics students then download the appropriate C. elegans sequence data for all genes, using the UCSC Genome Browser, and write python scripts that identify all C. elegans genes containing the desired number of consensus binding sites. The students next annotate this list of genes, by identifying human orthologs using BioMart, and write a Biopython script to retrieve descriptions of the human orthologs from the Entrez Gene database. This list is then given to the cancer biology students. Based on what they have learned in cancer biology, the students pick several genes that they would like to test, and primers to amplify part of those cDNAs are ordered. P53 mutant worms (strain TJ1, cep-1(gk138)) and wildtype N2 worms are exposed to UV radiation, and then students prepare cDNA and perform semi-quantitative PCR to detect differences in expression. The cancer biology students report back to the bioinformatics students about their experiment and their findings. The groups meet several times throughout the course of the experiment, so that both groups of students have the opportunity to learn from their “collaborators.” The bioinformatics students learn about the basics of cancer, transcription factors, etc., while the cancer biology students learn about what databases are available and what types of data can be obtained through bioinformatics.

1258C Iterative inquiry increases incorporation of scientific skills in an undergraduate laboratory class.  E. Wiseman1, D. Carroll1, S. Fowler2, E. Guisbert1 1) Biological Sciences, Florida Institute of Technology, Melbourne, FL; 2) Education and Interdisciplinary Studies, Florida Institute of Technology, Melbourne, FL.

The advantages of active learning approaches for student retention and engagement have prompted recommendations for inquiry-based laboratories to replace traditional laboratory classes. We redesigned a traditional upper-level undergraduate developmental biology laboratory to include two iterative rounds of inquiry-based investigations and measured student performance after each round. Assessment, using a rubric designed to measure student incorporation of scientific skills, revealed substantial improvements between the first and second rounds of inquiry in five out of seven categories. Paradoxically, student questionnaires revealed a slight decrease in student perception of learning from the traditional laboratory class, which likely reflects a reduction in fact-based learning. In contrast, there was a substantial increase in student interest and engagement in the course as evidenced by numerous positive comments on the same surveys. Overall, these results indicate that two iterative rounds of inquiry is a promising strategy for enhancing student engagement and learning.

1259A TWU Active Engagement Academy: Improving active engagement in academic courses.  T.L. Gumieny, S. Westmoreland  Biology, Texas Woman's University, Denton, TX.

High enrollment classes (>50 students) have historically been associated with lower student engagement and higher failure rates than lower enrollment classes. Active learning has been demonstrated to increase student engagement and, indirectly, student persistence. In addition, faculty who use high engagement in their classrooms become pedagogical role models for the future teachers who are enrolled in their courses. By initiating an Active Engagement Academy at Texas Woman's University, faculty who teach large lecture classes have been recruited and trained to use active learning and teaching methods to foster student engagement, academic success, and retention. The Active Engagement Academy model will be self-perpetuating as participating faculty will become role models within their academic departments for other faculty and for the teacher candidates enrolled in their courses. This presentation will provide a model and framework for other instructors who wish to promote active engagement in their institution's classrooms, including the rationale, recruiting information, workshop plans, and designs for active engagement implementations and measurements for intervention success.

1260B Making your own narrated animated videos to supplement lectures and flip the classroom.  Melissa Henderson  DeBusk College of Osteopathic Medicine - Lincoln Memorial University, Harrogate, TN.

With the amount of videos available online describing concepts and techniques in genetics and molecular biology, both students and faculty spend a substantial amount of time finding supplemental videos describing the concepts in appropriate detail. Teaching in the professional school setting requires the delivery of relevant material and teaching clinical-based concepts in a time efficient manner. In order to supplement lectures and active learning activities I have developed short narrated animated videos (NAVs) covering the concepts that fit my curriculum and level of detail. Each video is less than 15 minutes in length and is produced using a Wacom tablet, screen capturing software, and video editing software. With the assignment of watching the videos before class, more in-class time is devoted to active learning activities increasing student engagement and learning. Narrated animated videos have also been used to address questions from students with detailed explanations and posted to the course LMS site. The production of the short narrated videos is a time efficient method to deliver specific information outside of designated class time while supporting the allotment of time for active learning activities.

1261C Seeing the forest AND the trees: concept mapping improved student performance in an upper-level Developmental Biology course.  J.A. Miskowski  Bio Dept, Univ Wisconsin, La Crosse, La Crosse, WI.

The field of Developmental Biology focuses on the events from fertilization through embryogenesis of multicellular organisms, and by its nature, is highly interdisciplinary and detail-oriented. After teaching an upper-level Developmental Biology course several times, it was apparent that students often struggled with “putting it all together”, and specifically, fell short on more challenging exam questions. In an attempt to facilitate better learning, I incorporated concept mapping throughout the lecture portion of the course as a formative assessment measure. To determine if this tool impacted student performance, scores on 10 exam questions that required higher order cognitive skills were compared between pre-concept mapping and post-concept mapping student populations. The data revealed a statistically significant increase in scores when concept mapping was used,
and qualitative feedback provided on student surveys corroborated the usefulness of this technique to student learning. In addition, concept mapping was revealed to be particularly well-suited to the study of development, where broadly conserved mechanisms of pattern formation can get lost amid molecular details and the chronological organization of content is imperative for understanding.

**1262A Worms in space for outreach on Earth.** A.K. Pollard1, C.J. Gaffney2, T. Etheridge2, N.J. Szewczyk1, B.E. Phillips1 1) School of Medical Sciences and Graduate Entry Medicine, University of Nottingham, Derby, United Kingdom; 2) Department of Sport and Health Sciences, University of Exeter, United Kingdom.

Muscle atrophy is one of the main (mal)adaptations associated with prolonged spaceflight. We have previously established C. elegans as a model for studying spaceflight induced alterations in muscle with past experiments demonstrating reproducible expression changes in cytoskeletal and metabolic proteins and their encoding mRNAs. We are currently preparing a European Space Agency sponsored spaceflight experiment that aims to manipulate the stability of integrin based muscle attachment complexes and insulin-like signalling to determine if either or both of these predicted molecular mechanisms causally regulate the gene and protein expression changes observed in response to spaceflight.

As spaceflight, particularly launches, captures the public’s imagination, we have planned outreach activities to capitalize on this existing interest in spaceflight and to raise the profile of space-based life science research in the United Kingdom (UK). We have divided our outreach messages into three main themes. Firstly, we will describe the advantages of using C. elegans as a model to study biology and highlight some of the impacts on human health that have arisen from studies of the worm. Secondly, we will discuss the use of C. elegans as a model to study space physiology with a focus on how our past and current experiments were physically conducted. Thirdly, we will raise awareness of how the findings from our studies are being translated into new knowledge of the regulation of human muscle both on Earth and in space. We will present examples of how each message will be presented in a poster format for use at our outreach activities and provide electronic versions of these posters for other educators who may wish to use them.

Three distinct dissemination routes will be used to maximise the impact of our outreach. Firstly, we will engage with the UK media to produce a video documentary of our experiment to provide the public with deeper understanding of how space experiments are actually conducted. Secondly, outreach days will be run at museums in each of the capital cities in the UK. Thirdly, we will run a number of hands-on workshops at schools in the middle and South West of England. For the outreach days we will employ the three posters described above and an extended version of our school workshop activities. The workshop activities will cover all three of our outreach messages, using wild-type worms and those with mutations relevant to our spaceflight studies (e.g., unc-112 and cha-1), and giving students the opportunity to handle spaceflight experimental hardware. We will discuss the details of our workshop activities for other educators who wish to run similar activities.

**1263B A course-based undergraduate research experience to identify novel regulators of innate immune signaling in C. elegans.** J.R. Powell Biology, Gettysburg College, Gettysburg, PA.

The positive impact of participation in undergraduate research has been well established. Apprentice-style research experience rewards students with a close mentoring experience; however, these positions are limited by faculty time and resources. Additionally, although students underrepresented in the sciences show even greater benefits from research, majority students often disproportionately seek out and receive apprentice positions. We have converted the laboratory component of Bio 211 Genetics at Gettysburg College into a semester-long intensive research experience. Because Genetics is a required course in three majors, this course-based research experience creates a more equitable entry point into research that extends the benefits of research to a broader group of students in a cost-effective manner. The students in Bio 211 Genetics perform an RNAi screen to look for regulators of immune signaling in C. elegans. With about 32 students enrolled in the course, the class can collectively screen approximately 500 RNAI clones in one semester, with built-in redundancy to ensure high data quality. After a primary screen, the students repeat the experiment on a subset of hits, image and quantify target gene expression using a compound epifluorescence microscope, and sequence the RNAI clones to confirm their identity. Through a series of progressive experiences over the semester, we encourage students to develop their scientific communication abilities by reading and discussing the primary literature, presenting their projects orally in group meetings and formal symposia, and maintaining a lab notebook. Assessment via the Grinnell CURE survey indicated that this course had a dramatically positive impact on student learning. For example, in the first semester, student intent to pursue graduate degrees in the life sciences rose from 26% at the beginning of the semester to 52% by the end. Genetics student rated their learning gains in this course equivalent or even higher than students who took a parallel survey after a summer apprentice-style research experience (the SURE survey) on elements such as ability to read and understand primary literature, tolerance for obstacles faced in the research process, understanding of the research process, and readiness for more demanding research.

**1264C No lectures here: How an active and problem-based learning classroom in genomics transformed the confidence, creativity and communication skills of all students.** Ahna Skop, Benjamin Minkoff, Sarah Neumann UW-Madison.

Effective use of active learning techniques remains a challenge in STEM disciplines. We will present our success with Genetics 564, a 100% active learning-based undergraduate capstone course on Genomics and Bioinformatics. Genetics 564 is devoid of ‘traditional’ lecture-based teaching. Students learn about bioinformatic techniques through the reading and presentation of primary literature, and apply this knowledge to their research project as they analyze a human disease gene. The culmination of students’ research is the writing of a set of specific aims and the publication of a website detailing their
results. Students also engage in an iterative process of peer review throughout the semester. This course uses active learning to provide students with presentation, research, and peer review experiences similar to those used by scientists. In Genetics 564, we have observed that, with direction, undergraduate students can utilize both primary literature and bioinformatic databases to design experiments and test hypotheses. Additionally, students constantly peer- and self-review their presentations and scientific writing, a second layer of active learning that challenges them to not only produce high-quality research but also to critique their work, an important aspect of a truly active classroom. Tasks such as these are usually reserved for graduate level students and higher. The success of the students in our course has demonstrated its utility for teaching students scientific presentation, writing, research, and review skills, and student responses to the course have been overwhelmingly positive. Altogether, this suggests that the active and project-based learning we have employed contributes to both students’ success and motivation within our course setting. Given this, we propose that multiple courses across disciplines would benefit from incorporating aspects of the Genetics 564 structure into their undergraduate classroom. All course materials are freely available on the Genetics 564 website (http://genetics564.weebly.com), where one can also find an archive of past student final projects.

1265A  A Semester-Long Course-based Undergraduate Research Experience using RNAi in *C. elegans*.  Erika B. Sorensen  Department of Biology, Wabash College, Crawfordsville, IN.

For students enrolled in an upper division Molecular Genetics course, I developed a semester-long Course-based Undergraduate Research Experience (CURE) utilizing the model organism *C. elegans*. The research goal of the CURE laboratory is to investigate the function of a gene in *C. elegans* using RNA interference (RNAi). The learning goals of the CURE are: 1) student engagement in scientific practices (asking questions, developing hypotheses designing experiments, and gathering / analyzing data); 2) novel scientific discovery (addressing scientific questions where the outcome of an investigation is unknown to both the students and the instructor); 3) broad relevance (working as part of a broader scientific endeavor that extends beyond the course); 4) collaboration (during wet-lab techniques, experimental design, and peer-review of drafts); 5) iteration (designing experiments that build on existing knowledge found bioinformatically and that may be repeated by other students in the future) (1).

The general research overview of the CURE lab sequence is to clone a feeding RNAi vector encoding the student’s gene of interest for eventual feeding to *C. elegans*. The lab sequence can be broken down into two main parts. In Part 1, the student clones a gene from *C. elegans* into a plasmid designed for RNAi. In Part 2, the plasmids created in Part 1 are used for RNAi and students perform phenotypic assays over a two-week period. Specific details about the experimental timeline and techniques for this CURE will be reported. In our CURE project, each student chose a different gene. Although each student’s CURE involved cloning a different gene and performing different phenotypic assays, the class as a whole performed the same techniques in parallel. This fact provided an element of collaboration among peers that would be difficult to achieve in an independent research internship. Additionally, the benefit to the instructor is a continuation/integration of scholarly work (research) during the semester when faculty time can otherwise be overwhelmed by teaching responsibilities. Overall, this approach has been well received by the students and I would recommend trying a CURE as an approach to engage more students in an authentic research project.


1266B  Biological GPS: Helping freshmen find their path in science with a CURE investigating the genes required for distal tip cell migration in *C. elegans*.  Heather Thieringer, Jaclyn Schwalm 1,2  1) Molecular Biology Department, Princeton University, Princeton, NJ; 2) Council on Science and Technology, Princeton University, Princeton, NJ.

The Freshman Scholars Institute (FSI) program at Princeton University allows first generation and low-income students an opportunity to take two courses for credit during the summer preceding their freshman year. As part of this bridge program we developed a Course-Based Undergraduate Research Experience (CURE) that allows students to gain conceptual knowledge and technical skills while also promoting a sense of belonging, identity and self-efficacy in research. The course runs for 7 weeks and students are in lab 12 hours per week. The research project uses RNAi to investigate the molecular mechanisms that regulate cell migration of the distal tip cell in *C. elegans*. The course is designed with a parallel project structure that allows the students to develop project ownership. Target genes were selected from an RNAi screen (Cram et al, 2006) that identified 99 genes required for distal tip cell migration. The selected genes are involved in G-protein signaling (*unc-73, rab-8, arf-3, gex-3 and trya-3*), cell architecture (*erm-1, ina-1, zen-4, unc-112 and ifc-1*) or function as transcription factors (*icd-1, cbp-1, and hlh-12*). Students investigated novel combinations of these genes and analyzed the resulting gonad migration defects. Based on the results of their first experiment, students were able to plan two follow up experiments during the course. Integrated into the course were opportunities for guided reading of primary literature related to the project, as well as coverage of topics needed to understand the project (for example, transcription and translation). Students wrote lab reports and delivered oral presentations to report their results and the course culminated with the publication of a Princeton version of Worm Breeder’s Gazette.

1267C  *C. elegans* as a model for age-related phenotyping, compound screening and toxicological assessment.  Elena Vayndorf, Joshua Hincks 1, Joshua Hincks 3, Barbara Taylor 4, Michael Harris 5  1) Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK; 2) Department of Biology & Wildlife, University of Alaska Fairbanks, Fairbanks, AK; 3) Department of Chemistry, University of Alaska Fairbanks, Fairbanks, AK; 4) College of Natural Science and Mathematics, California State University, Long Beach, CA; 5) Integrative Physiology, California State University, Long Beach, CA.

The *C. elegans* pharynx is a tubular, bi-lobed, muscular pump encased in a basement membrane. The pharynx muscle
contracts and relaxes as the animal feeds, generating a characteristic electrical signal that can be recorded as an "electropharyngeogram". The frequency of pharyngeal pumping decreases with age and is considered a reliable index of overall health. Recently, NemaMetrix has made available a device that easily monitors and quantifies the C. elegans electropharyngeogram. We have designed a two-week teaching module using the NemaMetrix platform in guided undergraduate research. Projects assay a locally relevant environmental toxin, a natural product, and the effect of age in C. elegans. We provide examples of research projects that can be done in a two-week research module as part of a biomedically-related undergraduate course.