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Late Abstracts

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892A

The role of Rap1 in regulation of actin dynamics during *Drosophila* border cell migration. Anna C. Jang, Zih-Min Liao, Yi-Shan Huang, Yi-Chi Hsieh, Tzu-han Huang. Institute of Biotechnology, National Cheng Kung University, Tainan, Taiwan.

Cell migration plays a critical role in normal embryo development and homeostasis. Abnormal cell migration is detrimental that causes not only growth defects but also dissemination of cancers. My lab applies a group of migratory cells called border cells in *Drosophila* oogenesis as a model to examine the molecular mechanism by which the collective cell migration is regulated *in vivo*. Here we report Rap1, a small GTPase that belongs to a member of Ras superfamily, which is involved in actin dynamics during border cell migration. When the mutant clones of $rap1^{1B}$ were generated in border cells, nearly 94% of border cells did not arrive at oocyte. Moreover, 75% of border cells fail to detach in overexpression of UAS-$Rap1^{V12}$, a constitutive-active form of Rap1, and overall displayed 100% migration defects. Interestingly, in overexpress $Rap1^{V12}$, we observed highly accumulated F-actin in border cells and 42% of cell clusters were scattered in fixed-sample analysis. To further elucidate the actin dynamics in overexpression of UAS-$Rap1^{V12}$, we performed live imaging analysis and recorded the dynamics distributions of Actin::GFP in border cells. In wild type border cells, Actin::GFP was asymmetrically enriched in the leading cells and especially at the extended protrusions. However, in border cell cluster with UAS-$Rap1^{V12}$ overexpression, Actin::GFP was overall elevated in whole cluster, which caused multiple protrusions projecting to different directions. By contrast, we observed reduced phalloidin staining in $rap1^{1B}$ clone generated in border cell. Taken together our observations suggest that Rap1 regulates directional protrusions during border cell migration by coordinating asymmetrical distributions of actin.

893B

Functional and expression analysis of a novel putative basement membrane degrader in *Drosophila melanogaster*. Christopher J Fields, Ajay Srivastava. Biology, Western Kentucky University, Bowling Green, KY.

We have been interested in identifying potential genes that promote the process of basement membrane degradation. In this study we present preliminary data with respect to characterization of one such candidate gene. This is a protein coding gene that has several domains associated with zinc ion binding. Only a handful of experiments have been performed on this gene and its biological functions remains largely unknown. Preliminary data from a previous study suggests that it plays an important role in maintenance of the stem cell niche. In an effort to improve our understanding of this gene we have attempted to study the consequence of overexpressing this gene and also inhibiting its function using the UAS-Gal4 system. Overexpression of this gene results in phenotypes at both 18°C and 25°C. Data from selective inhibition of this gene using RNA interference will be presented as well. We have constructed a clone to utilize RNA in-situ hybridization to show expression levels at various stages in the developing Drosophila melanogaster lifecycle.

894C

First X-ray crystal structure of a *Drosophila* muscle myosin. James Caldwell, Girish Melkani, Tom Huxford, Sanford Bernstein. Biology Dept, San Diego State University, San Diego, CA, 92182.

Drosophila melanogaster contains one gene encoding all striated muscle myosin II isoforms. The alternatively spliced mRNA exons impart unique biochemical and biophysical properties to the myosin molecule. The three-dimensional structure of the truncated head from an embryonic body wall myosin isoform (EMB) is reported at 2.2 Å resolution. The histidine-tagged recombinant protein was expressed in and purified from the indirect flight muscles of an engineered fly line. The EMB myosin subfragment 1 (S1) contains the myosin heavy chain motor domain (MD) and the essential light chain (ELC). Crystals of the complex belong to space group $P2_12_12_1$ with the unit cell parameters of a = 108.5 Å, b = 148.5 Å, and c = 148.7 Å. There were two independent copies of the molecule resolved in the asymmetric unit, each having slight conformational differences. The enzymatic conformational state of the myosin cross-bridge cycle was determined as post-rigor by comparisons with known myosin structures. Comparison of the crystal structure of the EMB myosin isoform to that of other isoforms and mutants will elucidate the basis for biochemical differences amongst the forms.

895A

***Wavy*, a gene affecting wing morphology, encodes an inositol 1,4,5-triphosphate kinase.** Derek M.

Dean[1], Eric Spana[2], Luana Maroja[1], Brent Bomkamp[1], David L. Deitcher[3]. 1) Biology, Williams College, Williamstown, MA; 2) Biology, Duke University, Durham, NC; 3) Neurobiology and Behavior, Cornell University, Ithaca, NY.
Mutations in *wavy* (*wy*) affect wing morphology in a distinct fashion: phenotypes range from a mild ripple at a specific location in the coxal vein to a more pronounced buckle at the same location, the latter accompanied by an upturn at the most distal margin of the wing. Three-point test crosses and complementation analysis mapped *wy* to the inositol 1,4,5-triphosphate kinase gene *IP3K2*. In support of these findings, a frameshift mutation was recovered in the *IP3K2* coding sequence of wy^2 flies. Further, RNAi of *IP3K2* phenocopies the *wy* phenotype, and misexpression of *IP3K2* in various compartments of the wing disc affect the morphology of the adult wing. Finally, we present evidence of genetic interactions between *wy* and other genes involved in inositol signaling. Taken together, our findings support an important role for *IP3K2*, and more broadly for inositol signaling, in wing development.

896B

Lolal is maternally required for proper Dpp responsiveness. Janine Quijano, Jacob Seemann, Stuart Newfeld. School of Life Sciences, Arizona State Univ, Tempe, AZ.
During dorsal/ventral patterning in Drosophila melanogaster, the Dpp pathway directs cells toward a dorsal fate with the dorsal-most cells receiving the highest level of Dpp signaling. We have found the gene lolal in a screen for dominant maternal enhancers of dpp. Analysis of cuticles from dpp maternal enhancement experiments with multiple lolal alleles confirm that cuticles are ventralized. However, complementation tests of lolal alleles does not reveal a reduction of amnioserosa cells, indicating that there is no zygotic requirement for lolal in Dpp dorsal/ventral signaling. We are currently performing experiments were we rescue dpp maternal enhancement with lolal alleles by ectopically expressing constitutively active components of the Dpp pathway. If there is no rescue, then lolal affects Dpp signaling the signal transduction pathway after these points. Also, we hope to present results from in-situ staining of embryos from lolal germ-line clones, which may reveal changes in dpp expression during Dorsal/Ventral patterning. If there is a change, then lolal is necessary for proper expression of dpp mRNA during dorsal/ventral patterning. Lolal has been shown to be a co-factor for chromatin remodelers. We believe that lolal is necessary to specifically target and open either the chromatin around dpp or downstream target genes. We hope that this series of experiments will determine the point of interaction of lolal in Dpp signaling.

897C

ROS regulate cardiac function in Drosophila via a novel paracrine mechanism. Hui-Ying Lim[1,2]. 1) Free Radical Biology and Aging, Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) Development, Aging and Regeneration Program, Sanford-Burnham Medical Research Institute, La Jolla, CA.
The consensus view of ROS as signaling molecules has been that ROS act in a cell-autonomous manner, in which intracellular production of ROS leads to physiological or pathophysiological responses in the ROS-generating cell. More recently, paracrine roles of ROS signaling have been reported to occur under pathological conditions. Here, we reveal a novel paracrine signaling mechanism of ROS that occurs naturally to regulate normal tissue function, and does not involve the diffusion of ROS. We found that the non-myocyte pericardial cells (PCs) of the Drosophila heart contain elevated levels of ROS compared to the neighboring cardiomyocytes (CMs) under physiological conditions. When we genetically alter ROS levels to sub- or supra-physiological levels in PCs, this adversely affects cardiac rhythm and morphology, suggesting that ROS in PCs act in a paracrine manner to regulate normal cardiac function. We show that genetic down- or up-regulation of ROS levels in the PCs do not alter the levels of ROS in CMs. Moreover, similar manipulations of ROS levels in the CMs do not have any effect on cardiac function. Therefore, these results indicate that ROS do not diffuse from PCs into CMs to exert their function, but rather, ROS control the production of downstream signals in PCs that act in a paracrine manner on CMs to regulate their proper function. We further identified that ROS activates downstream D-p38 signaling in PCs that in turn directs normal cardiac function. We also determined a major developmental role of pericardial ROS-D-p38 signaling in establishing normal adult heart function. Paracrine communication between myocytes and non-myocytes is critical for the proper development and homeostasis of the myocardium, but the underlying mechanisms are not well-understood. Our study in Drosophila provides insights into a novel, and likely conserved mechanism by which ROS mediate interactions between different cell types that is essential for normal heart function.

898A

Mucin-type O-glycosylation is required for polarized secretion in the Drosophila digestive system. Liping Zhang, Kelly Ten Hagen. Developmental Glycobiology Section, NIDCR/NIH, Bethesda, MD 20892.
Polarized secretion is an important cellular process that plays critical roles in development and disease. Here we found that mucin-type O-glycosylation functions as a novel regulator of polarized secretion in specialized secretory cells of the Drosophila digestive system. One member of the large enzyme family responsible for the initiation of mucin-type O-glycosylation is expressed specifically in a subset of Drosophila foregut cells (PR cells) that are responsible for secreting the peritrophic membrane of the digestive system. Mutations in or RNAi to pgant4 resulted in lethality and defective proventriculus formation, with larger and irregularly-shaped PR cells. Furthermore, we observed disrupted apical secretion of ECM proteins, abnormal secretory granule formation, and altered localization and structure of the secretory apparatus in PR cells. We propose that O-glycosylation is responsible for stabilizing certain proteins that play important roles in polarized secretion within the digestive system. This study demonstrates that mucin-type O-glycosylation is required for polarized secretion in vivo and provides insight into the relationship between abnormal O-glycosylation and diseases of the digestive tract that are seen in mammals.

899B

Centrosomes are key components of mitotic spindle assembly and orientation in the symmetric divisions of Drosophila epithelial cells. John Poulton, John Cuningham, Mark Peifer. Biology, Univ North Carolina, Chapel Hill, NC.
Centrosomes help form the bipolar mitotic spindle, enabling accurate chromosome segregation. Errors in this result in DNA damage and aneuploidy. Despite the role of centrosomes in spindle assembly, surprising in vivo data demonstrated that flies lacking centrosomes develop to adulthood, suggesting that centrosomes are not essential for somatic cell mitosis. To explore these perplexing findings, we used somatic cells of larval wing discs as a model to study the consequences of acentrosomal mitosis. We found that wing discs lacking centrosomes had significantly elevated apoptosis. Acentrosomal wing disc cells assembled the mitotic spindle around chromatin and relied heavily on the Augmin pathway. Time to anaphase onset was roughly twice as long in acentrosomal cells compared to wildtype, suggesting inefficient spindle assembly. Consistent with this, in cells lacking both centrosomes and the Spindle Assembly Checkpoint (SAC), cell death increased significantly. This suggests many acentrosomal cells experience spindle assembly errors, some of which are corrected through SAC activity. Consistent with defects in spindle assembly and chromosome segregation, acentrosomal cells have elevated DNA damage. Interestingly, apoptosis in these cells does not appear to involve a p53 DNA damage response. Instead, we found that JNK signaling mediates apoptosis in acentrosomal cells. Despite the increased cell death, tissue homeostasis was maintained by compensatory proliferation, also regulated by JNK. Lastly, we found that centrosomes play an important role in orienting spindles in wing disc cells, and perturbing the Pins-Mud pathway in acentrosomal cells dramatically increased the rates of apoptosis. Together our in vivo data indicate that although cellular checkpoints protect against some of the errors inherent in acentrosomal mitosis, many acentrosomal somatic cells die, demonstrating that centrosomes play a vital role in mitotic fidelity. However, robust developmental processes like compensatory proliferation are able to buffer the animal against the loss of cells.

900C

The role of Clu in germ cell mitochondrial function. Aditya Sen, Rachel Cox. Biochemistry and Mol. Biology, Uniformed Services Univ., Bethesda, MD.
Fully functional mitochondria are critical for cellular health, particularly in high energy tissues such as skeletal muscle, heart muscle and the brain. Germ cell mitochondria are particularly important for females because germ cells require energy to nurture the developing oocyte, and all of the newly developing embryo's mitochondria are inherited maternally. In order to understand what genes and processes ensure healthy oocyte mitochondria, we are characterizing the gene *clueless* (*clu*). *clu* mutant flies are short-lived, female and male sterile, suffer mitochondrial oxidative damage and have reduced levels of ATP. These phenotypes are shared by flies mutant for *parkin* and *PINK1*, two genes necessary for targeted destruction of mitochondria, a process known as mitophagy. *clu* mutant germ cells have swollen, clumped mitochondria, which we have shown also happens in *parkin* mutant germ cells and now show in *PINK1* mutant germ cells. In addition, we showed *clu* can genetically interact with *parkin*, suggesting Clu may play a role in mitophagy. We find Clu can physically associate with mitochondria, and are investigating what role it may play in the Parkin/PINK1

mitophagy pathway.

901A

Transcriptomic insights into extreme pH. Gayle Overend, Louise Henderson, Pawel Herzyk, Shireen A Davies, Julian AT Dow. Molecular, Cell & Systems Biology, University of Glasgow, Glasgow, Scotland, United Kingdom.

Harmful insects incur enormous health and economic costs; crop damage or insect-borne plant diseases, and animal diseases, cause the loss of 15-25% of GDP worldwide (with developing nations hit hardest). Very few new insecticides have come to market in the last decade, and there is regulatory pressure that any new insecticides should not harm pollinators such as honeybees. It is therefore vital to find new 'green' insecticide targets outwith the CNS, which are selective for harmful insects without targeting important pollinators. Many insect species considered 'pests', such as mosquitoes and caterpillars, have an extraordinary specialization that could provide just such a target. Whereas vertebrates and most insects run their digestive tracts at an acidic pH, many Lepidopteran and Dipteran larvae maintain alkaline gut regions with pH values as high as pH 12. Here we show that by mapping pH along the length of the insect midgut, and comparing the transcriptome of gut regions with different pH, we can identify genes putatively involved in both acid and alkaline pH generation. By using a comparative approach and repeating this work in fruit-fly, mosquito and caterpillar, it is possible to determine conserved motifs of genes responsible for pH generation in diverse species. By manipulating such genes using RNAi in Drosophila, or dsRNA in Aedes, we can verify those responsible for pH generation and maintenance. The molecules responsible are therefore prospective targets for novel insecticides that would be selective for two of the most destructive Orders of insects - Diptera and Lepidoptera.

902B

Diet-induced changes in *Drosophila* lifespan and metabolism. M. Irina Stefana, Timothy J. Ragan, Paul Driscoll, Alex P. Gould. Physiology and Metabolism, MRC National Institute for Medical Research, London, United Kingdom.

Drosophila is emerging as a powerful model system to study physiology and metabolism. Many studies of metabolism in *Drosophila* have focused on the larva, leaving adult stages underexplored. Here we investigate the effects of four diets - with varying concentrations of yeast and/or glucose - upon a series of physiological readouts in the adult, including lifespan, lipid accumulation, fatty acid profiles, haemolymph polar metabolites and other markers of age/ageing. Comparisons between high glucose-low yeast and high glucose-high yeast diets reveal that increasing glucose shortens median lifespan but this can largely reversed by concomitantly increasing dietary yeast. Despite lifespan differences, ^{1}H NMR analyses of sugars and other polar metabolites in the haemolymph of adults fed on these two diets are very similar. High dietary glucose-to-protein ratios have been proposed to be obesogenic but the relationship between this effect and ageing has not yet been clarified in *Drosophila*. We therefore used GC-MS fatty acid profiling to show that male adiposity levels are similar on all four diets at 2-weeks of age but differences emerge at 3.5 weeks and become marked by 5-weeks. There is a general trend for overall adult adiposity to decrease with age on all four diets but we find that the rate of decrease differs between diets. Thus, higher-than-normal adiposity values on some diets can be explained by differential rates of fat loss during ageing, rather than by diet-induced fat gain, as the term "obesogenic" would imply. To gain further insight into the mechanisms linking diet to adiposity, we are currently manipulating components of the IIS, TOR and lipid metabolic pathways in adults fed on diets associated with high versus low rates of fat loss.

903C

Dietary regulation of amylase and maltase expression in the adult *Drosophila* midgut. Wen-Bin Alfred Chng. Global Health Institute, School of Life sciences, Station 19, EPFL, 1015 Lausanne, Switzerland.

Digestion and absorption are two principal functions of the digestive tract. Since dietary intake in the environment is inherently variable, rebalancing an imbalanced diet requires post-ingestion mechanisms. Animals therefore need to actively assess their nutritional state and adapt their digestive capacity to the demands for various nutrients. While the effects of dietary sugar on amylases activity have long been recognized in Drosophila melanogaster, the mechanistic underpinning of such regulation remains unclear. Through an in vivo RNAi screen, we identified transcription factors required for this regulation.

904A

Target of Rapamycin Signalling Pathway as a Potential Mediator of the Lifespan-Extending Effects of Dietary Restriction by Essential Amino Acid Alteration. Sahar Emran, Mingyao Yang, Xiaoli He, Matthew Piper. University College London, London, United Kingdom.

Dietary restriction (DR), defined as a moderate reduction in food intake short of malnutrition, has been shown to extend healthy lifespan in a diverse range of organisms, from yeast to primates. Using *Drosophila melanogaster* as a model organism, we explore the physiological changes that occur under DR and speculate how these changes may lead to lifespan extension. Recently, the essential amino acids (EAAs) have been implicated as the dietary mediator of the lifespan extending effects of DR, and nutrient signalling pathways are thought to be important modulators. By characterising the physiological and metabolic parameters that define DR and EAA-supplemented flies, we identify candidate factors for causation of the lifespan response to DR. Moreover, we find that pharmacologically downregulating TORC1 signalling alters some of these phenotypic responses, suggesting a role for TORC1 in lifespan regulation under DR.

905B

The putative role of DCISD3 in *Drosophila*. KT Huang[1,2], JC Li[2], HD Wang[1], CH Chen[2]. 1) National Tsing Hua University, Hsinchu, Taiwan, Taiwan; 2) National Health Research Institutes,Miaoli,Taiwan, Taiwan.

Members of this protein family contain the CDGSH iron-sulfur domain, which consists of clusters of iron-sulfur (Fe-S) binding sites. In *Drosophila*, the CDGSH iron-sulfur domain-1 protein, dmitoNEET, is an integral membrane protein of the outer mitochondrial membrane. The dmitoNEET protein is involved in the transport of iron into the mitochondria. Iron is essential for the function of several mitochondrial enzymes. In *Drosophila*, only the dmitoNEET and CG3420 proteins contain the CDGSH domain. The dmitoNEET protein contains one CDGSH domain in the N-terminal region and another in the C-terminal region. The CG3420 protein also contains two CDGSH domains, but shares more amino-acid sequence similarity with the mammalian protein, CISD3. Hence, we refer to the CG3420 protein as DCISD3. In our current study, we found that the overexpression of DCISD3 caused mitochondria to have a rounded morphology with extensive fragmentation, suggesting that DCISD3 plays a role in dynamic mitochondrial remodeling. However, in contrast to dmitoNEET, we found that DCISD3 did not localize to mitochondria. Immunofluorescent staining showed that the DCISD3 protein was present throughout the indirect flight muscle of flies, perhaps localizing to an unidentified cellular organelle network. The post-translational modification of DCISD3 also suggests a unique biological function with regard to its role in dynamic mitochondrial remodeling.

906C

Molecular dissection of Vasa function in germ plasm localization and assembly. Szu-Chieh Wang[1,2], Hao-Jen Hsu[2], Gee-Way Lin[3], Ting-Fang Wang[1], Chun-Che Chang[3], Ming-Der Lin[1,2]. 1) Dept. of Mol. Biol. & Human Genetics, Tzu-Chi University, Hualien, Taiwan; 2) Dept. of Life Science, Tzu-Chi University, Hualien, Taiwan; 3) Department of Entomolog, National Taiwan University, Taipei, Taiwan.

Vasa is a highly conserved RNA helicase in metazoan and has been considered as an universal germ cell marker. In *Drosophila* and other species, the formation of primordial germ cells is dependent on proper assembly of germ plasm. Vasa is required for germ plasm assembly downstream of Oskar, and is required for maternal RNA translation including *gurken* during oogenesis. However, little is known about the functional motifs of Vasa required for its germ plasm localization and the assembly of germ plasm. Here, we demonstrated that the evolutionarily conserved HELICc domain is essential and sufficient to direct the localization of Vasa to the germ plasm in the oocyte and nuage in nurse cells. Our molecular dynamic simulation study indicated that the HELICc domain may preserve a bipartite interaction pocket required for the germ plasm localization of Vasa. Our results explored the functional domains of Vasa required for its localization in germ plasm and the formation of abdomen and germ cells.

907A

pineapple eye, a putative Drosophila E3 ligase, functions as an essential factor in germline and intestinal stem cell self-renewal. Yalan Xing, Hannele Ruohola-Baker. Department of Biochemistry, University of Washington, Seattle, WA.

Adult stem cells are key for regeneration. However, we need to understand the molecules that control this process to fully harness adult stem cell potential in future medicine. Drosophila serves as a good model for understanding adult stem cell regulation. Three excellent models for adult stem cells in Drosophila

melanogaster have been extensively investigated: female and male germline stem cells (GSCs), and intestinal stem cells (ISCs) in fly midguts. Previous studies have revealed that these adult stem cell types share similar molecular regulatory pathways, suggesting that common regulators for adult stem cells can be unraveled. One of these shared regulatory pathways is Insulin receptor (InR) pathway. Through a loss-of-function screen, we identified pineapple eye (pie), a homologue of human E3 ubiquitin ligase G2E3, as an essential factor in female GSC maintenance and division. We now show that pie is also required for male GSCs self-renewal and ISCs proliferation. These data suggest that pie has a conserved role in maintaining pluripotency. One of the negative downstream targets of InR signaling pathway is transcription factor FOXO. Phosphorylation by Akt inhibits the nuclear localization and consequently the transcriptional activity of FOXO. In this study we demonstrated that pie regulates stem cell self-renewal through mediating FOXO on protein level, but not on transcription level. FOXO protein is upregulated in pie mutant stem cells. Further, reduction of FOXO efficiently rescues loss-of-pie caused stem cell loss and division defects. Based on these data, we propose a mechanism for pie action: pie regulates FOXO levels in Drosophila GSCs and ISCs, possibly through ubiquitination-mediated protein degradation. FOXO protein level in stem cells is a rheostat that is responsible for regulating self-renewal.

908B

Roles of PINK1, mTORC2, and mitochondria in preserving brain tumor-forming stem cells in a noncanonical Notch signaling pathway. Kyu-Sun Lee[1,2], Zhihao Wu[1], Yan Song[3], Siddhartha S. Mitra[4], Abdullah H. Feroze[4,5], Samuel H. Cheshier[4,5], Bingwei Lu[1]. 1) Department of Patholgy, Stanford University, Stanford, CA 94305 USA; 2) BioNanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea; 3) Peking-Tsinghua Center for Life Sciences, Peking University, Beijing, China; 4) Institute of Stem Cell Biology and Regenerative Medicine, Stanford, CA 94305 USA; 5) Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA 94305 USA.

The self-renewal versus differentiation choice of *Drosophila* and mammalian neural stem cells (NSCs) requires Notch (N) signaling. How N regulates NSC behavior is not well understood. Here we show that canonical N signaling cooperates with a noncanonical N signaling pathway to mediate N-directed NSC regulation. In the noncanonical pathway, N interacts with PTEN-induced kinase 1 (PINK1) to influence mitochondrial function, activating mechanistic target of rapamycin complex 2 (mTORC2)/AKT signaling. Importantly, attenuating noncanonical N signaling preferentially impaired the maintenance of *Drosophila* and human cancer stem cell-like tumor-forming cells. Our results emphasize the importance of mitochondria to N and NSC biology, with important implications for diseases associated with aberrant N signaling.

909C

EGFR and Notch signaling respectively regulate proliferative activity and multiple cell lineage differentiation of *Drosophila* gastric stem cells. Chenhui Wang[1,2], Xingting Guo[1,2], Rongwen Xi[1]. 1) National Institute of Biological Sciences, No. 7 Science Park Road, Zhongguancun Life Science Park, Beijing 102206, China; 2) College of Life Sciences, Beijing Normal University, Beijing ,100875, China.

Quiescent, multipotent gastric stem cells (GSSCs) in the copper cell region of adult *Drosophila* midgut can produce all epithelial cell lineages found in the region, including acid-secreting copper cells, interstitial cells and enteroendocrine cells, but mechanisms controlling their quiescence and the ternary lineage differentiation are unknown. By using cell-ablation or damage-induced regeneration assays combined with cell lineage tracing and genetic analysis, here we demonstrate that Delta (Dl)-expressing cells in the copper cell region are the authentic GSSCs that can self-renew and continuously regenerate the gastric epithelium after sustained damage. Lineage tracing analysis reveals that the committed GSSC daughter with activated Notch will invariably differentiate into either a copper cell or an interstitial cell, but not the enteroendocrine cell lineage, and loss-of-functional and gain-of-functional studies revealed that Notch signaling is both necessary and sufficient for copper cell/ interstitial cell differentiation. We also demonstrate that elevated EGFR signaling, which is achieved by the activation of ligand Vein from the surrounding muscle cells and ligand Spitz from progenitor cells, mediates the regenerative proliferation of GSSCs following damage. Taken together, we demonstrate that Dl is a specific marker for *Drosophila* GSSCs, whose cell cycle status is dependent on the levels of EGFR signaling activity, and Notch signaling has a central role in controlling cell lineage differentiation from GSSCs by separating copper/interstitial cell lineage from enteroendocrine cell lineage.

910A

P53-Mediated Rapid Induction of Apoptosis Conveys Resistance to Viral Infection. Bo Liu[1], Susanta Behura[2], Rollie Clem[3], Anette Schneemann[4], James Becnel[5], David Severson[2], Lei Zhou[1]. 1) Dept Molec Genetics/Microbiol, Univ Florida Col Medicine, Gainesville, FL; 2) Eck Institute for Global Health, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN; 3) Division of Biology, Kansas State University, Manhattan, KS; 4) Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA; 5) Center for Medical, Agricultural and Veterinary Entomology, USDA/ARS, Gainesville, FL.

Arthropod-borne pathogens account for millions of deaths each year. Understanding the genetic mechanisms controlling vector susceptibility to pathogens has profound implications for developing novel strategies for controlling insect-transmitted infectious diseases. The fact that many viruses carry genes that have anti-apoptotic activity has long led to the hypothesis that induction of apoptosis could be a fundamental innate immune response. However, the cellular mechanisms mediating the induction of apoptosis following viral infection remained enigmatic, which has prevented experimental verification of the functional significance of apoptosis in limiting viral infection in insects. In addition, studies with cultured insect cells have shown that there is sometimes a lack of apoptosis, or the pro-apoptotic response happens relatively late, thus casting doubt on the functional significance of apoptosis as an innate immunity. Using *in vivo* mosquito models and the native route of infection, we found that there is a rapid induction of *reaper*-like pro-apoptotic genes within a few hours following exposure to DNA or RNA viruses. Recapitulating a similar response in Drosophila, we found that this rapid induction of apoptosis requires the function of P53 and is mediated by a stress-responsive regulatory region upstream of *reaper*. More importantly, we showed that the rapid induction of apoptosis is responsible for preventing the expression of viral genes and blocking the infection. Genetic changes influencing this rapid induction of reaper-like pro-apoptotic genes led to significant differences in susceptibility to viral infection.

911B

Molecular mechanisms underlying Neuroglian (L1 CAM) mediated axonal interactions essential for mushroom body development. Dominique Siegenthaler, Eva-Maria Enneking, Eliza Moreno, Jan Pielage. Friedrich Miescher Institute, Basel, Switzerland.

The function of the brain relies on precise connectivity of neurons, mediated by the guidance of axonal growth cones to appropriate targets. Cell adhesion molecules (CAM) have been implicated in a multitude of different functions including axon guidance as they can mediate direct cell-cell interactions through homo- or heterophilic interactions. A prominent example is the control of fasciculation of axons into major tracts that enable guidance along pioneer neurons. Here we dissect the differential contribution of the extra- and intracellular domains of the Drosophila L1 CAM-type Neuroglian (Nrg) during the wiring of the nervous system. We use the development of the complex architecture of the Drosophila mushroom bodies as a model system to identify potential regulatory and cell-specific requirements of different domains of Nrg. We find that Nrg mediates interaction between axons of different types of mushroom body neurons demonstrating a pioneering role of early born neurons. Using cell-specific rescue assays we then identify extra-cellular adhesion and intracellular clustering mediated by Ankyrin2 as a mechanism to control these axonal interactions. Importantly, these functions are evolutionary conserved as corresponding mutations in human L1-CAM lead to severe neurological disorders partially caused by axon pathfinding defects.

912C

The Conserved MicroRNA miR-8 Regulates Synapse Morphogenesis. Cecilia S. Lu[1,2], Bo Zhai[2], Alex Mauss[3,4], Matthias Landgraf[3], Stephen Gygi[2], David Van Vactor[1,2]. 1) Okinawa Institute of Science and Technology, Onna-son, Okinawa, Japan; 2) Department of Cell Biology, Harvard Medical School, Boston, MA, USA; 3) Department of Zoology, University of Cambridge, Cambridge, UK; 4) Max Planck Institute of Neurobiology, Martinstried, Germany.

Synapse morphogenesis is a finely orchestrated process that requires spatiotemporal coordination between both pre- and post-synaptic cells to initiate and maintain precise contacts throughout development. Cell surface receptors and adhesion molecules play critical roles in synapse formation and stabilization but the mechanism by which such molecules are regulated at post-transcriptional level is not clear. Here we show that the conserved microRNA miR-8 promotes the refinement of embryonic motor neuron targeting to muscles in *Drosophila*. Using quantitative proteomics and imaging, we find the expression of two synaptic adhesion receptors, Neuroglian (Nrg)/L1-CAM and Fasciclin III (FasIII), which act synergistically towards

refining the earliest neuromuscular connections to be dependent on miR-8. Since microRNAs are short (~22 nt) non-protein coding RNA with pervasive functions in biology through post-transcriptional regulation of gene expression, our study presents miR8 as a regulator in synapse morphogenesis and postulates a potential mechanism to investigate the coordinated assembly of pre- and post-synaptic compartments during the development of nervous system.

913A

Aplip1/JIP1 is a transport adaptor for axonal transport of active zone proteins. Husam Babikir[1,2], Matthias Siebert[1,2], Matthias Böhme[1,2], Nicole Holton[3], Stephan Sigrist[1,2]. 1) Institute of Biology & Genetics -FU Berlin, Berlin, Germany; 2) NeuroCure Cluster of Excellence, Charité Berlin, Berlin, Germany; 3) Institut für Chemie und Biochemie, Abteilung Strukturbiochemie, Freie Universität Berlin,.

Presynaptic active zones (AZs) are the sites of synaptic vesicle release and AZ proteins are crucial determinants of the functional characteristics of this release process. How AZ proteins are transported to sites of initial synapse assembly is only poorly understood. One of the presenaptic active zone proteins is ELKS family protein Bruchpilot (BRP) which acts as the building block of the electron-dense AZ cytomatrix (T-bar) in Drosophila. In addition to BRP, Drosophila Rim-binding protein (DRBP) is essential for the integrity of the AZ scaffold and for exocytotic neurotransmitter release. Here we investigated AZ protein transport for the first time in intact animals, third instar Drosophila larvae. We found that the AZ master organizer protein Bruchpilot is transported actively along the axon and accumulates together with (DRBP) in several previously described mutants with transport defects. Moreover, our findings suggest that Aplip1/JIP1 works as a transport adaptor for both of BRP and DRBP. We confirmed the physical interaction of Aplip1-BRP and Aplip1-DRBP using Yeast-2-Hybrid, co-immunoprecipitation and Isothermal Titration Microcalorimetry (ITC). We observed live co-transport of fluorescently-tagged Bruchpilot/DRBP and Aplip1 constructs in axons of intact animals. Moreover, Bruchpilot and DRBP accumulated in axons of aplip1 mutants. Live imaging confirmed that Bruchpilot transport was severely impaired in aplip1 mutants. Re-expression of a wild-type Aplip1 cDNA in an aplip1 null background rescued axonal accumulation of Bruchpilot and DRBP whereas a cDNA with point mutations in the proline-rich interaction motif of Aplip1 did not. Together we here for the first time identified a transport adaptor specific for AZ protein transport and show that it is required in vivo for their transport.

914B

Ecdysis Triggering Hormone: Metamorphosis of a Developmental Signal into a Regulator of Reproduction in the Fruit Fly Drosophila melanogaster. Matthew R. Meiselman[1], Hongjiu Dai[1], Sang Soo Lee[1], Crisalejandra Rivera-Perez[2], Fernando Noriega[2], Thilini Wijesekera[3], Brigitte Dauwalder[3], Adams Michael E.[1]. 1) Department of Cell, Molecular, and Developmental Biology, University of California, Riverside, Riverside, CA 92521; 2) Department of Biological Sciences, Florida International University, Miami, FL 33199; 3) Department of Biology and Biochemistry, University of Houston, 369 SR2, Houston, TX 77204.

Ecdysis triggering hormone (ETH) is an established command peptide responsible for molt termination through initiation and scheduling of the ecdysis behavioral sequence. Nevertheless Inka cells, which are the sole source of ETH, persist through metamorphosis into adulthood, when ecdysis behaviors are absent. This raises interesting questions regarding possible functional roles for Inka cells and ETH in adult life. We have found that ETH plays a significant role in reproductive control in Drosophla melanogaster, both as an allatotropic factor in determining juvenile hormone (JH) levels and by positively regulating sex-peptide receptor expressing proprioceptor cells in the uterus. Silencing of ETH receptors (ETHR) in the corpora allata causes reduced juvenile hormone levels, leading to reduction in progeny. This phenotype is rescued by juvenile hormone replacement through topical application of methoprene. We also found that silencing of ETHR in ovarian proprioceptor cells causes elevation in number of eggs laid by virgin females. We conclude that, in addition to its developmental functions during immature stages, ETH plays essential roles in reproductive physiology. Supported by NIH Grant GM067310.

915C

Functional Consequences of Amyloid-like Oligomerization of Drosophila Orb2. Mohammed R Khan. Stowers Institute For Medical Research, Kansas City, KS.

Drosophila neuronal CPEB (Orb2), a putative translation regulator, is required for the persistence of memory over time. Orb2 protein exists in two physical states in adult fly brain; a monomer and an amyloid-like

oligomer. Oligomerization of this protein is important for long-term memory. Orb2 mutant flies deficient in oligomerization show no defect in learning and short term memory but long term memory is severely affected after 24hours. However, what is the role of Orb2 in neuronal protein synthesis and what is the functional consequence of conversion to the oligomeric state is largely unknown. We addressed these questions using Orb2-dependent translation reporter and in vitro cell-free translation system made from Drosophila embryo. We have also used UAS-Gal4 system to express the translation reporters in the fly brain to assess Orb2-dependent protein synthesis in vivo. Both in-vitro and in-vivo studies indicate that monomeric form of Orb2 acts a translation repressor and the amyloid-like oligomeric Orb2 acts a translation activator. We also uncovered the molecular basis of Orb2-dependent translational regulation using cell free extract. Most proteins in their amyloid state are either biologically inactive or detrimental/harmful for the host cells. Our findings suggest that Orb2 amyloids are distinct from pathological amyloid and in the Orb2 gains new activity in the amyloid-like state. These results are consistent with the idea that Orb2 amyloid serves as a stable substrate for long-term memory.

916A

Ecdysis Triggering Hormone Mediates Courtship Memory via Regulation of Juvenile Hormone Levels.
Sang Soo Lee[1], Natalie Karapetians[2], Crisalejandra Rivera-Perez[3], Fernando Noriega[3], Thilini Wijesekera[4], Brigitte Dauwalder[4], Michael Adams[1,2]. 1) Neuroscience Graduate Program; 2) Department of Entomology and Cell Biology & Neuroscience, University of California, Riverside, Riverside, CA 92521; 3) Department of Biological Sciences, Florida International University, Miami, FL 33199; 4) Department of Biology and Biochemistry, University of Houston, 369 SR2, Houston, TX 77204.
Memory formation is an important factor in the survival and reproductive success of animals. In *Drosophila*, male memories of rejection by the female have a well-known negative influence on subsequent courtship behavior. We have found that formation of such "courtship memory" depends on hormonal state. Ecdysis Triggering Hormone (ETH) regulates production of Juvenile Hormone (JH) by endocrine glands called corpora allata (CA). RNA knockdown of ETH receptors (ETHR) specifically in the CA reduces JH levels and abolishes courtship memory. Stage-specific knockdown of ETH receptors in CA suggest ETH-mediated JH signaling in the adult stage is crucial to memory formation. Ablation of Inka cells, the sole source of ETH, also resulted in memory deficiency. Memory loss is rescued by treatment with the JH analog methoprene. Dissociation experiments confirmed that ETHR knockdown males are dysfunctional with respect to memory formation. Our findings suggest that ETH and JH are essential for courtship memory formation.

917B

The BK Channel Slowpoke and Cardiac Function. Santiago Pineda[1], Karen Ocorr[1], Rolf Bodmer[1], Diane Fatkin[2]. 1) Biomedical Sciences, Sanford Burnham Medical Research Institute, La Jolla, CA; 2) Victor Chang Cardiac Research Institute,405 Liverpool Street, Darlinghurst NSW 2010.
Abnormalities in ion channels that modify conduction in cardiomyocytes are a common cause of cardiac arrhythmias. One channel that may have an important role in heart function is the Big Potassium (BK) channel, a Ca2+ and voltage gated K+ channel encoded by the slowpoke gene. While the channel has been studied in neural tissue and the vasculature, only recently has it been studied with respect to cardiac function. We have discovered an association between non-synonymous variants in this channel gene and human cardiac arrhythmias. Using Semi-Automated Optical Heartbeat Analysis, we have examined the effects of BK channel loss of function in the denervated, semi-intact Drosophila heart prep. Cardiac-specific BK channel RNAi knockdown as well as genomic null mutants exhibited a slower heartbeat due to increased diastolic and systolic intervals. Additionally, intracellular recording from hearts of genomic nulls exhibited arrhythmias in the form of early after depolarizations. Both of these suggest an effect on aspects of cardiac repolarization. Conversely pharmacological activation of the channel resulted in increased heart rate. We also expressed both the mutated form and wild type human BK channel genes in a fly heart lacking Drosophila BK channel expression. While fly hearts expressing the wild type human channel had a somewhat negative effect on function, due perhaps to dosage effects, flies expressing the mutant human channel had much poorer heart function. This negative outcome was manifest as a slower heart rate and increased systolic interval. Our results show that modification of BK channel activity affects heart function in the fly suggesting that the human BK mutation is responsible for the arrhythmia seen in the human cohort. Using the fly heart model we hope to elucidate the specific component of BK channel function that contributes to these patients' heart disease.

918C

Identification of Modifiers of Amyotrophic Lateral Sclerosis in *Drosophila*. Mark W Kankel[1], Anindya Sen[1], Douglas Dimlich[2], Marianthi Kiparaki[2], Marina Theodorou[2], Nicole Sakellari[2], Basel Tarab[2], Spyros Artavanis-Tsakonas[1,2]. 1) Molecular Discovery, BiogenIdec, Cambridge, MA; 2) Department of Cell Biology, Harvard Medical School, Boston, MA.

Amyotrophic Lateral Sclerosis (ALS) is a devastating motor neuron disease that leads to loss of motor function, where life expectancy for patients from the time of diagnosis averages about two to five years. Here, we describe two ongoing screens for genes that modify degenerative eyes phenotypes resulting from GAL4 directed expression of disease causing alleles of human TDP-43 and FUS. Given the conservation of gene function across species, our assumption is that such a screen will potentially define novel targets for therapeutic development, as well as help dissect and define the genetic circuitry in which the ALS phenotypes are embedded. One third of ALS remains unexplained at the genetic level and it is believed that a significant fraction of sporadic ALS is likely to have a genetic basis. Thus, genetic screens in Drosophila that identify modifiers of phenotypes resulting from prevalent ALS disease causing genes may provide a more comprehensive perspective on the disease.

919A

Identification of modifiers of Parkinson's disease in *Drosophila*. Anindya K. Sen[1], Mark Kankel[1], Doug Dimlich[2], Harsha Kuthethur Gururaj[1], Basel Tarab[2], Christina Wong[2], Nicole Sakellari[2], Samia Aly[2], Chapman Beekman[2], Spyros Artavanis-Tsakonas[1,2]. 1) Molecular Discovery, Biogen-Idec, Cambridge, MA 02142; 2) Department of Cell Biology, 240 Longwood Avenue, LHRRB 410, Boston, MA 02115.

To uncover the biological processes that may impact neurodegeneration (ND), we use genetic screens to identify modifiers of disease-relevant phenotypes in *Drosophila*. Parkinson's Disease (PD) is a devastating neurodegenerative disease affecting dopaminergic (DA) neurons causing several patient symptoms, ranging from tremors to rigidity, posture instability and dementia. To uncover the biological processes that may affect PD, we undertook a genetic screen to identify modifiers of PD-related Pink1 and parkin phenotypes in *Drosophila*. Here we present a summary of our screens and describe the array of follow up assays we will employ to prioritize genes of interest. Our methodology for probing the functionalities of potential genes of interest includes functional, biochemical, bioinformatics as well as cell biological assays. Integrating the results from our screens using these assays, we will define an unique interactome for PD-related genes that provides a knowledge base for better understanding Parkinson's Disease.

920B

Astrocyte-specific regulation of human MeCP2 expression in *Drosophila*. David Hess-Homeier[1], Chia-Yu Fan[3], Tarun Gupta[2], Ann-Shyn Chiang[3,4], Sarah Certel[1,2]. 1) Department of Biological Sciences, University of Montana, Missoula, MT; 2) Neuroscience Graduate Program, University of Montana, Missoula, MT; 3) Brain Research Center, National Tsing Hua University, Taiwan; 4) Institute of Biotechnology, National Tsing Hua University, Taiwan.

Methyl-CpG-binding protein 2 (MeCP2) is expressed in nearly every cell in the human body and is one of the most dosage-sensitive genes involved in neuron function. Common features of Rett Syndrome (RTT), MeCP2 duplication disorder, and neuropsychiatric disorders indicate that moderate changes in MeCP2 protein levels result in functional and structural cell abnormalities. Tightly controlled levels of MeCP2 are essential for maintaining homeostasis in individual cells and neural networks. We addressed two areas of MeCP2 pathophysiology; the cell-type specific effects of MeCP2 on microcircuits controlling sleep, and identification of cell-specific regulators of MeCP2 expression for therapeutic strategies. We expressed human MeCP2 (hMeCP2) in astrocytes and distinct subsets of amine neurons. Our results indicate hMeCP2 expression can reduce sleep levels, alter day/night sleep patterns, and disrupt initiation and maintenance of sleep. Each parameter is uniquely affected depending on the cell-type expressing hMeCP2. Additionally, we found a temporally and spatially regulated reduction or absence of full length and RTT R106W hMeCP2 protein in astrocytes and other glial cells five days post-eclosion. In contrast, expression of the deletion Δ166 MeCP2 protein is unchanged in the astrocyte population. Glial counts and verification of Gal4 driver functionality show that astrocytes are not dying. qPCR experiments showed a reduction in full-length *hMeCP2* transcript levels compared to *hMeCP2*$^{\Delta 166}$ levels suggesting transgenic hMeCP2 expression is regulated at the transcriptional level. By examining hMeCP2 properties at the level of specific amine neuron and astrocyte

subsets, our results indicate that an endogenous glial factor may regulate levels of transgenic *hMeCP2* expression and amine-specific hMeCP2 expression may alter sleep circuitry output.

921C

Altered Glycosylated Synaptomatrix Composition and Synaptic Architecture in a Drosophila Classic Galactosemia Disease Model. Patricia P. Jumbo-Lucioni, Kendal S. Broadie. Department of Biological Sciences, Vanderbilt University, Nashville, TN.

Classic galactosemia (CG) is an autosomal recessive disorder resulting from loss of galactose-1-phosphate uridyltransferase (GALT), which catalyzes conversion of galactose 1-phosphate + UDP-glucose to glucose 1-phosphate + UDP-galactose. UDP-galactose 4'-epimerase interconverts UDP-galactose to UDP-glucose, and is responsible for the biosynthesis of UDP-N-acetylgalactosamine and UDP-N-acetylglucosamine. All four UDP-sugars are essential donors for the synthesis of the glycoproteins and glycolipids that heavily decorate cell surfaces and extracellular spaces. In addition to acute, potentially lethal neonatal symptoms, mature patients develop substantial motor and cognitive impairments. Previous studies suggest an association of neurological symptoms to glycosylation defects, with CG described as a Congenital Disorder of Glycosylation (CDG) showing combined defects in assembly and processing of N-glycans. Our goal was to test for impacts on behavioral traits, synaptic development and glycosylated synaptomatrix formation using a GALT-deficient Drosophila CG model. We tested larval coordinated movement and found that loss of dGALT impairs this trait. We characterized larval neuromuscular junction (NMJ) structure, and found that mutant larvae exhibit structural overelaboration. Dietary galactose and co-removal of either dGALK or sugarless genes are important modifiers of these behavioral and neurological outcomes. We assayed the extracellular synaptomatrix with a panel of lectin labels and found profound alterations in glycan composition in the absence of dGALT, including significant reductions in galactosyl and N-acetyl galactosamine residues, and fucosylated HRP epitopes. Synaptogenesis relies on bidirectional trans-synaptic signals modulated by this carbohydrate environment, and dGALT NMJs display striking changes in heparan sulfate proteoglycan (HSPG) co-receptor and Wnt ligand levels. These results are the first to reveal synaptomatrix glycosylation losses, synaptic architecture defects and impaired trans-synaptic signaling during synaptogenesis in a CG disease model.

922A

Systems biology and metabolomics approaches: towards the core metabolic map of *Drosophila melanogaster*. Dominika Korzekwa[1], Dan Erben[1], Shireen A. Davies[1], David G. Watson[2], Julian A. T. Dow[1]. 1) University of Glasgow, Glasgow, United Kingdom; 2) University of Strathclyde, Glasgow, United Kingdom.

A computer-annotated metabolic map of Drosophila has been predicted by Kyoto Encyclopedia of Genes and Genomes (KEGG). However, 'gaps' still remain with no identified orthologues for metabolic enzymes. In order to provide a holistic understanding of organismal function and validate Drosophila as an in vivo model of human metabolism we generated a computational model of core metabolism of the fruit fly. The model was developed using BioCyc database. Pathway Tools was used to generate the list of metabolic gaps. The ability to produce biomass precursors was checked using COBRA toolbox and Matlab. Present model contains 2484 metabolites, 2759 reactions and 133 metabolic gaps. Subsequently, a list of most-fillable gaps was generated. One of these gaps is Drosophila gene CG30016. Based on sequence homology it is hypothesised to be a homologue of 5-hydroxyisourate hydrolase (5-HIUH) involved in purine metabolism. Enzyme defects within purine metabolism in humans result in inborn errors of metabolism including hyperuricemia. Here, we propose a method for identifying metabolic 'gaps' using metabolomics approaches. Liquid chromatography-mass spectrometry (LC-MS) was performed for CG30016 knockout flies and a control. The comparison confirmed that CG30016 is involved in purine metabolism and specifically urate degradation. Interestingly, our results suggested that CG30016 is either catalysing a reaction downstream from 5-HIUH (OHCU decarboxylation), or is a bifunctional enzyme, playing a role as both 5-HIUH and OHCU decarboxylase. Moreover, an apparent tubule phenotype was observed in CG30016 knockout line. Flies were observed to have an inflated ureter and evidence for luminal occlusion. This is consistent with a blockade of purine metabolism and might reflect what is observed in hyperuricemia and subsequent kidney damage. Filling in the 'gaps' is essential for completion of the core metabolome of Drosophila and its validation as a model of metabolic disorders.

923B

Heterochromatin Dynamics in Early Embryogenesis Might Contribute to a Sexual Dimorphism for Gene Expression Noise. Carlos Diaz-Castillo. Independent Researcher, Irvine, CA.
Here I propose that a sexual dimorphism in gene expression noise importantly contributes to the difference in sex-biased gene expression response to conditional cues and divergence widely found in metazoan. This hypothesis is based in, (i) the potential different contribution of sexes to genome evolution, (ii) the potential important contribution of gene expression noise for gene expression conditional response/divergence, and, (iii) the fact that gene expression conditional response/divergence is faster for heterogamety-biased than for homogamety-biased or unbiased gene expression. To test the existence of the hypothetical sexual dimorphism in gene expression noise, I proceed to reanalyze a Drosophila transcriptomic dataset in which the variation in transcript abundance might only be of sexual and/or stochastic nature. As hypothesized, the dataset in question shows that gene expression is generally noisier in heterogametic males than in homogametic females. Also, the study of the noise sex bias in the dataset under consideration with regards to heterochromatin dynamics in early embryogenesis in the presence/absence of Y chromosomes suggests that the stochastic variation in the content of repetitive DNA in sex-specific heterochromatic chromosomes might be the main contributor for the metazoan sexual dimorphism in gene expression noise.

924C
Evolution and Function of Positionally Relocated Genes in Drosophila Genomes. Richard P. Meisel. Biology and Biochemistry, University of Houston, TX.
Genomes can evolve through the substitution, addition, deletion, duplication, or rearrangement of genetic material. Non-tandem gene duplication can be followed by the loss of the gene at the ancestral locus, creating the appearance that the gene was relocated in the genome. This form of "gene relocation" is as common as canonical non-tandem duplication in *Drosophila* genomes, and, just like canonical duplication, gene relocation has an X-to-autosome bias. Despite the frequency with which gene relocation occurs and the interesting genomic patterns of relocation, little is known about the evolutionary processes underlying the fixation of relocated genes and the biological consequences of gene relocation. For example, there is debate in the literature as to whether the X-to-autosome relocation bias is driven by sexually antagonistic selection, spermatogenic silencing of the X chromosome, or mutational pressures. To address these shortcomings, we have performed an evolutionary and functional analysis of relocated genes in *Drosophila* genomes.

925A
Courtship songs in the *Drosophila montium* species-subgroup. Chuancheng Chen[1], Xiaoshen Lu[1], Masayoshi Watada[2], Michael G. Ritchie[3], Shuoyang Wen[1]. 1) Department of Entomology, South China Agricultural Univ. 483 Wushan Road, Guangzhou, Guangdong, China; 2) Graduate School of Science and Engineering, Ehime University, 3 Bunkyo-Cho, Matsuyama, Ehime 790-8577, Japan; 3) School of Biology, University of St Andrews, St Andrews, Fife KY16 9TH, UK.
Courtship behavior and courtship song are extremely variable in *Drosophila* species. Typical precopulatory courtship has been lost in species of the *montium* species-subgroup. Sine song is species-specific and plays an important role for mate recognition in the *D. lini* clade of the *montium* subgroup. To investigate the courtship songs of the *montium* subgroup, we recorded and analyzed courtship songs of 22 species. 14 species produced only sine song, five species produced mainly sine song with some irregular pulse song, one species produced long pulses and some sine song, and two species have only pulse songs. The most basal species, *D. parvula*, has only typical pulse song and precopulatory courtship. The sine song frequency is different in each species within a species-complex, as in the *lini* clade. These results suggest that pulse song might be associated with precopulatory courtship, whereas sine song becomes more important to copulatory courtship. The pulse song has subsequently been lost in the *montium* species-subgroup as has precopulatory courtship. The sine song might be used for mate recognition during copulatory courtship as in the *lini* clade.

926B
Allele-specific splicing in panel of genotype-specific transcriptomes of Drosophila melanogaster. Yerbol Kurmangaliyev[1,2], Kjong Lehmann[3], Daniel Campo[1], Peter Chang[1], Alexander Favorov[4], John Tower[1], Mikhail Gelfand[2], Sergey Nuzhdin[1]. 1) Molecular and Computational Biology, University of Southern California, Los Angeles, CA; 2) Institute for Information Transmission Problems, Moscow, Russia; 3) Memorial Sloan-Kettering Cancer Center, New York, NY; 4) Johns Hopkins University School of Medicine, Baltimore, MD.
Majority of protein-coding genes in higher eukaryotes consist of multiple exons. Splicing patterns of these

genes are determined by multiple cis-regulatory signals, which are involved in proper recognition of exon-intron boundaries by trans-splicing factors. Genetic variation in splicing signals may lead to serious differences in splicing patterns between different genotypes (allele-specific splicing). We analyzed large panel of genotype specific transcriptomic data from F1-crosses of 84 Drosophila melanogaster inbred lines with known genotypes and common tester line (common reference design). Such experimental design allows to partition cis and trans effects. In total, we were able to detect several hundred cases of allele-specific splicing patterns associated with single-nucleotide polymorphisms (SNPs). Identified putative cis splicing quantitative trait loci (cis-sQTLs) were strongly enriched in close proximity to regulated exon/intron boundaries. Previous studies on allele-specific splicing were primarily focused on changes in ratios of known annotated isoforms. Here we applied annotation-free approach to detect and quantify splicing events which allows to identify qualitative changes in exon-intron structure of genes, including de novo creation and/or activation of cryptic splicing signals. Mapping of transcriptomes to genotype-specific reference genomes and analysis of allelic imbalance revealed that majority of detected allele-specific splicing events represents cis effects, providing additional evidence that associated cis-sQTLs may represent causative regulatory variants.

927C

Cytoplasmic incompatibility and infection frequency of *Wolbachia* in a Michigan population of *D. melanogaster*. Allison McClish, Roger Albertson. Albion College Biology Department, Albion, MI.

In some species of *Drosophila*, *Wolbachia* infection results in an effect known as cytoplasmic incompatibility (CI). This effect inhibits the viability of offspring produced from the mating between an uninfected female fly and an infected male. Because *Wolbachia* is transferred through the mother to the offspring, this effect gives a reproductive advantage to those females that are infected, thus raising the overall infection frequencies of the population. In *D. melanogaster*, this effect has been found to be minor or non-existent, and in general this species has a lower infection frequency than *D. simulans*, which has been found to evidence a very strong CI effect. In a study of a Michigan population of *D. melanogaster* in 2012, an abnormally high infection frequency was found. In order to explain this high frequency, this population was tested for CI. Three different sets of flies were tested, including originally wild-caught stocks that had been in the lab for several months, freshly caught flies, and the first-generation offspring of wild-caught flies. In these tests, infected males were crossed to uninfected females and the percentage of eggs hatched calculated. Though initial crosses showed a potential CI effect, further crosses evidenced very little or no CI effect for this population of *D. melanogaster*.

928A

Genotype-by-environment interactions of demographic values in fluctuating thermal environments using *Drosophila melanogaster*. Alison Egge, Olivia Eller, Theodore Morgan. Kansas State University, Manhattan, KS.

Statement of Purpose: Organisms often experience a wide range of temperatures in nature, brought on by daily and seasonal fluctuations. Ectotherms are particularly susceptible to these fluctuations and must alter their physiology in order to survive and reproduce in potentially stressful conditions. *Drosophila melanogaster* have adapted to a range of thermal regimes and inhabit much of the world. Assessment of egg laying and survivorship at different temperature regimes provides significant information on how different genotypes are affected by thermal fluctuations. In addition, comparisons of overall fitness to survival following acute, extreme cold stresses may provide an understanding the evolution of a "thermal profile" of a specific genotypic form. Methods Used: Using the *Drosophila melanogaster* Genetic Reference Panel (DGRP) we chose 40 genotypes to assess absolute lifetime fitness measures at two different fluctuating environments: 18° ± 6° C and 25°± 6° C (average 16.8° C and 23.8° C, respectively). Eggs were counted daily for 20 females per genotype, and daily survivorship was recorded. We also assessed survival following a one hour cold stress for flies reared in these two different environments. Summary of Results: The 40 genotypes we tested exhibit significant correlations between the two rearing temperatures for average age and average eggs laid, demonstrating a strong genetic component to the capacity of fitness parameters, influenced heavily by the rearing environment. Significant differences in demographic parameters such as lambda (λ), net reproductive rate, and generation time are also present among these 40 genotypes, and further association mapping will provide candidate genes for these fitness parameters. There were no significant correlations between acute cold tolerance and fitness in these 40 genotypes, suggesting that life-time fitness and acute cold tolerance are largely genetically and physiologically independent of one another.

929B

Protein evolution through the lens of the sperm proteome. Timothy Karr. Biodesign Inst, PO Box 875001, Arizona State Univ, Tempe, AZ.

Although it is well known that eukaryotic proteomes (≈500 aa) are, on average approximately two-thirds longer compared to prokaryotes (≈300 aa), there is as yet no consensus regarding the evolutionary mechanisms responsible for these differences. The length of a given protein is determined in part by the cellular context in which it functions. Selective pressures for small efficient proteins present in high concentration (e.g., metabolic enzymes) in fast growing environments are understandable. However, the complexity of eukaryotic cells compared to prokaryotes might necessitate longer proteins carrying out specialized functions (e.g., cytoskeletal and membrane proteins). Knowledge of cell-type specific proteomes could allow deeper understanding of proteome length variation at the cell level. Unfortunately, high throughput MS techniques and data analyses has yet to achieve deep coverage of the proteomes of complex diploid cells (which may contain upwards to 10,000 proteins). However, MS has proven useful for defining sperm proteomes, a cell type of lower proteome complexity. I analyzed the sperm proteome lengths from a variety of species including Drosophila, mouse, rat, human and macaque and compared them to the average whole proteome lengths of these species. Remarkably, and without exception, the average sperm proteome length of all species analyzed were significantly longer than the whole proteome. The Dmel sperm proteome length was greater than twice the whole proteome length (1335 aa vs. 580 aa). These datasets have also provided insights into the variation of individual cellular proteomes within a complex metazoan and possibly provides an index for the degree in which evolutionary pressures (selection) have shaped protein length. Thus, protein length evolution may have been driven by traits related to sperm functionality (i.e., motility, high axial ratios). This represents the first analysis of whole cell proteomes and provides a foundation for future functional, bioinformatic and evolutionary analyses of cellular proteome evolution in an organismal context.

930C

Comprehensive Analysis of Genes Involved in the Dark Adaptation of a *Drosophila* Line. Minako Izutsu[1], Osamu Nishimura[2], Kiyokazu Agata[1], Naoyuki Fuse[1,2]. 1) Laboratory for Molecular Developmental Biology, Graduate School of Science, Kyoto University; 2) RIKEN Center for Developmental Biology, Japan.

The ability to evolve and adapt to diverse environments is one of the fascinating features of organisms. The molecular mechanism of environmental adaptation is still poorly understood. Experimental evolution studies provide information about the history of organisms evolved in a laboratory environment, and could be a powerful approach for revealing the relationships between adaptation, traits and genes. A laboratory at Kyoto University has maintained a *Drosophila melanogaster* line in a constant dark condition for 59 years, 1400 generations. Our group has utilized this "Dark-fly" to investigate molecular mechanisms underlying environmental adaptation. Although previous studies showed some traits of Dark-fly, it is still unclear whether Dark-fly is really adapted to dark conditions. Here, we examined the fitness of Dark-fly under competition against the wild-type fly, and found reproductive dominance of Dark-fly in dark conditions. To address the molecular basis of the adaptive traits, we determined the whole-genome sequence of Dark-fly using second-generation sequencing, and identified about 220,000 single nucleotide polymorphisms (SNPs) in the Dark-fly genome. Since most of these SNPs would be expected to be functionally neutral, we took a population genetics approach to identify adaptive SNPs: we reared large mixed populations of Dark-fly and the wild-type fly (about 1,000 flies) under dark and light conditions. We analyzed the population genome at 0, 22nd and 49th generations, and successfully identified some chromosomal loci that were selected in the mixed populations. We also observed the trajectory of SNPs frequencies during the selection, and discovered positive and negative selections toward different loci of the genome. Our results identified potential candidate genes involved in the environmental adaptation of Dark-fly, and provided an approach to connect the adaptive traits and the adaptive genes.

931A

A Structure-Function analysis of *Drosophila* Tolloid. Jennifer Winstanley, Clair Baldock, Hilary Ashe. Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom.

Drosophila Tolloid metalloproteinase is a key regulator of the Bone Morphogenetic Protein (BMP) signalling gradient that specifies cell fates during dorsal-ventral (DV) axis formation. This regulatory role is conserved across vertebrate and invertebrate systems. In *Drosophila*, the DV gradient is formed as BMP ligands are

transported dorsally in a complex with the BMP antagonist Sog. Yet, in order to signal, the ligands must be released by Tolloid-mediated cleavage of Sog. Despite its importance, the mechanism and regulation of Tolloid activity is unclear, in particular with respect to the role of its non-catalytic CUB and EGF domains. Here we show the low resolution structure of *Drosophila* Tolloid and define the role of specific CUB domains. *In vitro* activity and binding assays of mutated Tolloid proteins identify the domains that are most important for function, providing the basis for substrate interaction. We interpret this data in light of the SAXS and TEM structural models and assess the relative importance of the different domains. Our results give mechanistic insight into the metalloproteinase family, providing a platform to further understand cell signalling control and tissue formation.

932B

Dynamic Regulation of Eve Stripe 2 Expression in Living Embryos. Emilia Esposito[1,3], Jacques Bothma[1,3], Gavin Shlissel[1,3], Hernan Garcia[2], Thomas Gregor[2], Michael Levine[1]. 1) Dept. of MCB, UC Berkeley, Berkeley, CA; 2) Dept of Physics, Princeton University, Princeton, NY; 3) These authors contributed equally to the work.
Recent improvements in imaging and computational methods permit the visualization of gene expression in living embryos (Garcia et al. 2013; Lucas et al. 2013). Here, we have applied these methods for examining the dynamics of eve stripe 2 regulation in the precellular Drosophila embryo. The eve 5' flanking region was attached to an MS2/yellow reporter gene and nascent transcripts were visualized with an MCP::GFP fusion protein. As expected, in situ hybridization assays using fixed preparations of staged embryos reveal a tight stripe of reporter gene expression. However, live imaging reveals a highly dynamic pattern of de novo transcription, beginning with a broad domain of expression during cc12, and progressive refinement during cc13 and cc14. The classical stripe 2 transcription pattern is dynamic and ephemeral, present for just ~10-15 min of the ~90 min period of expression. This study provides evidence for "mitosis-assisted" repression, whereby the stripe 2 transcription pattern becomes spatially restricted upon cell division. In addition, the eve transgene exhibits transcriptional bursts, which might render the stripe 2 enhancer "poised for repression" by gap repressors forming the the anterior and posterior borders of the stripe.

933C

Understanding the mechanism of pigment rim formation at the periphery of the fly eye. Sudha R. Kumar, Andrew Tomlinson. Genetics & Development, Columbia University, New York, NY.
The Drosophila eye periphery undergoes peripheral patterning in response to a graded Wingless (Wg) signal emanating from the surrounding head capsule. High levels of Wg signaling lead to the formation of the Pigment Rim (PR). The PR is a thick band of pigment cells that serves to optically insulate the eye from extraneous light rays. The PR is composed mainly of the pigment cells that surrounded the outermost row of ommatidia in the developing pupal eye. These peripheral ommatidia undergo timed developmental apoptosis, leaving the remaining pigment cells to coalesce and form the PR. Earlier work showed that high levels of Wg signaling induced the expression of Escargot, Wg and Notum in a subset of the cells of the peripheral ommatidia, namely the cone cells. But the mechanism of apoptosis of the entire ommatidia remained unclear. Our aim is to understand the mechanism by which Wg leads to the apoptosis of the different cell types of the ommatidia in a concerted manner. The peripheral apoptosis follows a precisely timed sequence of events, with the cone cells of the ommatidia collapsing first, followed by apoptosis of the entire ommatidium. Ectopic expression of Wg at high levels causes the entire eye to respond in a manner similar to the peripheral ommatidia. In order to elucidate the mechanism of Wg induced apoptosis, we analyzed the effects of manipulations of the Wg signaling pathway in the subsets of the cells of the ommatidia. We found that the expression of Escargot in the cone cells is required for their collapse, while the Wg expression appears to be a booster signal for the apoptosis of the remaining cells of the ommatidia. We also show that the activation of Wg signaling in the cone cells alone is insufficient for the apoptosis of the ommatidia, thereby suggesting a combinatorial response of all the cell types. We are currently investigating the role of photoreceptors and the surrounding pigment cells in the apoptotic cascade, and the possible model of the concerted response of the cells of the ommatidia to high levels of Wingless signaling.

934A

***E(spl)^{D}*-mediated repression of R8 cell-fate occurs independently of *N^{spl}*.** Adam Majot, Ashok Bidwai. Biology, West Virginia University, Morgantown, WV.
Notch signaling plays a dichotomous role in Drosophila retinogenesis, concurrently driving photoreceptor

differentiation and neurorepression. The repressive pathway is punctuated by activation of HES proteins, as revealed by genetic interaction between the hypermorphic allele N^{spl} and $E(spl)^{D}$. $E(spl)^{D}$ encodes a truncated variant of E(spl)M8 that is an active repressor despite its inability to interact with the corepressor Groucho. Further analysis of the E(spl)M8 C-terminus reveals a cluster of phosphorylation consensus sites that putatively regulate activity and expression. Genetic interactions between $E(spl)^{D}$, protein kinase CK1 and E3-ubiquitin ligase slmb indicate that E(spl)M8 may be subject to targeted degradation during retinogenesis. Interestingly, $E(spl)^{D}$ elicits retinal patterning defects in proneural-deficient backgrounds that are otherwise N^{+};, suggesting that hypersensitivity of N^{spl} to $E(spl)^{D}$ likely results from pathway defects upstream of E(spl) expression. In the absence of a direct effect on E(spl) expression, N^{spl} may compromise a subset of components within both the proneural and neurorepressive pathways to elicit a cumulative patterning defect.

935B

Investigation of dmyc Promoter and Regulatory Regions. Jasmine Kharazmi[1], Cameron Moshfegh[2]. 1) Department of Neuroanatomy, UZH, Zurich, Switzerland; 2) Department of Health Sciences, ETHZ, Zurich, Switzerland.

Products of the myc gene family integrate extracellular signals by modulating a wide range of their targets involved in cellular biogenesis and metabolism to regulate cell death, proliferation, and differentiation. However, understanding the regulation of myc at the level of transcription is still a challenge. Here we performed rapid amplification of dmyc cDNA ends (5' RACE), and mapped the transcription start site at P1 promoter 18 base pairs upstream of the start of the known EST GM01143 within the 5' UTR. Our data show that the first TATA box, previously computationally predicted, is utilized to generate dmyc full length mRNA. The largest transcript contains all three exons generated after the removal of the introns by splice events. Further investigation of Downstream Promoter Element (DPE) by studying lacZ reporter activity revealed that this element is only active in conjunction with its upstream cluster of binding sites. These findings may provide valuable tools for further analysis of dmyc cis-elements.

936C

Zelda functions in larval disc and brain development. Hsiao-Yun Liu, Kevin O'Brien, Christine Rushlow. Biology, New York University, New York, NY.

The Drosophila Zelda (Zld) gene plays an important role in activating the zygotic genome in blastoderm embryos during the maternal-to-zygotic transition (Liang et al., 2008). In older embryos and larvae, zld is expressed in the head, CNS, and imaginal discs (Staudt N. et al., 2006, Pearson J. et al., 2012). However, little is known about the function of Zelda at these later time points. We used the UAS/GAL4 system to express zld or zldRNAi in different imaginal discs and the larval brain. We observed several phenotypes that are similar to developmental pathway mutants. For example, loss of zld activity during early larval stages in the eye disc leads to eyes 40~60% smaller than wild type that display an abnormal structure found in os1 mutant flies. In contrast, the absence of zld in the leg disc caused abnormal tumor-like growth similar to that observed in mutants of fat and/or other tumor suppressor genes in the hippo pathway (Buratovich M. et al., 1997). In the larval brain, loss of zld affects the neuroepithelial-to-neuroblast (NE-to-NB) transition; the NE cells lose identity and become NB cells prematurely, reminiscent of the Notch loss of function phenotype (Yasugi T. et al,.2010, Egger B. et al., 2010). These results indicate that Zelda is required for post-embryonic growth, and we propose that Zelda may coordinate networks that are interacting with signaling pathways in the larval brain and imaginal discs.

937A

Dynamic regulation of the Dpp signalling-responsive transcriptional network in the *Drosophila* embryo. Lisa Deignan, Abbie Saunders, Catherine Sutcliffe, Tim Burgis, Leo Zeef, Ian Donaldson, Hilary L. Ashe. Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom.

The Bone Morphogenetic Protein (BMP) signalling pathway has a conserved role in dorsal-ventral axis patterning during embryonic development. In *Drosophila*, graded BMP signalling is transduced by the Mothers against Dpp (Mad) and Medea transcription factors, and opposed by the Brinker (Brk) repressor. Using the *Drosophila* embryo as a model, we have combined RNA-seq with Mad and Brk ChIP-seq to decipher the BMP-responsive transcriptional network underpinning differentiation of the dorsal ectoderm. Data assessing recruitment of Mad and Brk to enhancers, including competition between these factors, will be

presented. A number of the Dpp target genes identified are consistent with multiple tiers of feedback regulation, and we present data in relation to signalling pathway cross-talk based on analysis of mutant embryos. Overall, our data provide a framework for understanding how BMP signalling regulates entire gene expression programmes in other systems, including humans where it has important roles in development and disease.

938B

Analysis of transvection using fluorescent reporters. Jack R. Bateman, Amanda J. Blick, Ilana Mayer-Hirshfeld, Beatriz Malibiran, Justine E. Johnson. Biology Department, Bowdoin College, Brunswick, ME.

In Drosophila, maternal and paternal homologs are intimately paired in virtually all somatic cells. Pairing of homologs can permit *trans*-interactions between an enhancer on one homolog and a promoter on another, a phenomenon known as transvection. We recently established a transgenic system that uses fluorescent reporters to assess the capacity of an enhancer to act in *trans* on a paired homolog. Using this system, we have shown that the eye enhancer *GMR* is capable of activating expression of *GFP* via the *hsp70* promoter in *trans*, and does so in a variegated pattern, suggesting stochastic interactions between the enhancer and promoter when they are carried on separate chromosomes. Furthermore, we quantitatively assessed the impact of two concurrent promoter targets, one in *cis* and one in *trans* to *GMR*, and demonstrated that each promoter is capable of competing for the enhancer's activity, with the presence of one negatively impacting expression from the other. Our current experiments address whether the capacity to act in *trans* is common to diverse enhancers using this transgenic system.

939C

Analysis of the D. melanogaster genome organization. Qingjiao Li, Harianto Tjong, Xianghong Jasmine Zhou, Frank Alber. CMB, University of Southern California , Los Angeles, CA.

Genomes structures are non-randomly organized in the nucleus of higher eukaryotes. Drosophila melanogaster (DM) as a model organism is widely used to study the functional relevance of the genome structural organization, including gene regulation, DNA replication and other cellular processes. However, the global 3D genome structure of DM is yet unknown in its entirety. Here we combine Hi-C interaction frequency of DM with chromatin-nuclear envelope (NE) association information from FISH experiments to model the 3D genome structure, defined by the positions of 1169 physical chromatin domains. To allow structural heterogeneity due to the expected plasticity in genome structures in different cells, we model a population of structures by optimizing a scoring function so that the genome structures satisfy all the experimentally derived restraints. Our population structures explain the experimental hallmarks of DM genome organization in a statistical way: (1) the probability of a domain to be located at the NE, determined from the structure population, correlates well with the lamin-binding signal frequency of the corresponding loci in DamID experiment, (2) the tethering of centromere to heterochromatin cluster (HET) causes a dramatical shift of the centromere-HET cluster towards the interior of nucleus, (3) HP1 domains, which are far from the centromeres, tend to be more interior than surrounding domains.

940A

Role of MicroRNA Turnover in the Maternal to Zygotic Transition in *Drosophila*. YC Lin[1,2], JC Li[2], HD Wang[1], CH Chen[2]. 1) National Tsing Hua University, Hsinchu, Taiwan; 2) National Health Research Institutes, Miaoli, Taiwan.

The turnover and steady-state levels of microRNAs (miRNAs) are regulated by post-transcriptional processes. In a wide range of taxa, 3'-terminal adenylation and uridylation are involved in miRNA maturation and degradation, respectively. In *Arabidopsis*, 3'-terminal methylation protects miRNA and siRNA from degradation via 3' polyuridylation. In mammals, the 3' terminus of miR-122, a mature liver-specific microRNA, can be uridylated by a putative poly(U) polymerase or adenylated by the cytoplasmic poly(A) polymerase, GLD-2, and 3' adenylation by GLD-2 is required for cellular stability of miR-122. The role of miRNAs in promoting the turnover of maternal mRNAs during the maternal to zygotic transition is highly conserved phenomenon between *Drosophila* and *Danio rerio*. However, the relationship between the degradation of maternal mRNA and the steady-state levels of miRNAs remains unclear. We used tissue-specific maternal-gal4 drivers to knock down the expression of the drosophila homolog of GLD-2. The steady-state levels of miRNAs in the ovary were affected, and the female flies became sterile. The sterile phenotype could be rescued by knocking down the expression of an exoribonuclease. Our results suggest that the

activities of poly(A) polymerase and exoribonuclease in microRNA turnover influence the maternal to zygotic transition.

941B

A new swing for flamenco, transcriptionnal analysis of a master piRNA cluster. Coline Goriaux, Sophie Desset, Yoan Renaud, Chantal Vaury, Emilie Brasset. GReD, Clermont Ferrand, France.

The past few years it has become clear from many transcriptomic studies that most of the eukaryotic genome is pervasively transcribed. This complex network of transcripts include several types of small RNAs classified as non-coding RNAs. The vast majority of small RNA act as transcriptional, postranscriptionnal and translational regulators, controlling specific target genes involved in various cellular functions. They are classified based on their biogenesis and mode of action.

A subclass of small non-coding RNA, the Piwi-interacting RNAs (piRNAs), ensures genomic stability by silencing endogenous transposable elements in both germline and somatic gonadal tissues. piRNAs are produced through two mechanisms, 1) The primary processing pathway from long single-stranded precursors produced by some specific loci in the genome, the piRNAs clusters or 2) The secondary pathway by the amplification loop called the ping-pong.

piRNAs clusters are composed of fragment of active mobile elements and are located in heterochromatic region. Little is known about the expression first steps of those heterochromatic piRNAs clusters like transcription regulation and transcripts maturation. We try to better understand the mechanism involved in their transcription by studying the flamenco locus, the major soma-specific piRNA clusters, where only the primary pathway is active. Here, we demonstrate that flamenco transcription is initiated from an RNA Polymerase II promoter containing Inr and DPE elements, and requires the transcription factor, Cubitus interruptus. We show that the flamenco precursor transcript undergoes differential alternative splicing to generate diverse RNA precursors that are processed to piRNAs. Our data reveal the dynamic nature of the processing steps giving rise to piRNA cluster precursors.

942C

Lobe-less RNA is essential for mushroom body morphogenesis in *Drosophila*. Sachi Inagaki[1], Masanao Sato[2,3], Tomoyuki Miyashita[4], Natsuki Nakamura[5], Satoru Kobayashi[2,3], Minoru Saitoe[4], Yuji Kageyama[1,5]. 1) Research Center for Environmental GenomicsKobe University, Kobe, Japan; 2) Okazaki Institute for Integrative Bioscience, Japan; 3) National Institute for Basic Biology, National Institutes of Natural Sciences, Japan; 4) Tokyo Metropolitan Institute of Medical Science, Japan; 5) Department of Biology, Graduate School of Sciences, Kobe University, Japan.

Although it has been shown that long noncoding RNAs (ncRNAs) are involved in a variety of biological phenomena, physiological roles of long ncRNAs in the central nervous sytem are still largely unknown. In previous studies, we have performed screening for long ncRNAs in *Drosophila* (Inagaki *et al*., 2005) and found one of the candidates Lobe-less (LOL) RNA is specifically expressed in the nervous system during embryogenesis. We found that more than 80% of *lol* mutant flies showed malformation of the vertical lobes of the mushroom body, an essential for insect behavior and memories. Consistently, odor preference assays showed that *lol* mutant flies show defective odor response to 3-octanol and 4-methylcyclohexanol. Additionally, *lol* mutants showed disorderd rhythm and low activity during locomotor activity assay. During development, *lol* mutant animals showed aberrant vertical extension of mushroom body neurons, even at the earliest stage in the larval period. On the other hand, specification of mushroom body neuroblasts was not affected by the *lol* mutation in late embryonic stages. Microarray analysis using adult head revealed that expression levels of more than 2,000 genes were dramatically changed in lol mutant flies. Analysis of *lol Pc* double mutants suggest that LOL RNA is involved in chromatin-besed transcriptional regulation. Current study provides the first evidence that long noncoding RNA contributes in establishment of integrative neural circuit in the brain.

943A

Characterisation of a broadly expressed long non-coding RNA, lnc703, in *Drosophila melanogaster*. Jenna Schwarz, Andrew Bassett, Robert Young, Chris Ponting, Jilong Liu. MRC Functional Genomics Unit, University of Oxford, Oxford, United Kingdom.

We have previously identified a set of 1,119 putative long intergenic non-coding RNAs in *Drosophila melanogaster* using modENCODE whole transcriptome (RNA-seq) data (Young et al., 2012). One of these,

lnc703, is a broadly expressed 1.3kb, multi-exonic, polyadenylated non-coding RNA that is conserved amongst *Drosophilids*. Here we have shown that reduced levels of lnc703 caused by a transposon insertion in the first exon leads to reduced fitness. Transcriptome profiling of this mutant identified 256 differentially expressed genes, which are significantly enriched for immune and metabolic processes. Further analysis using tissue specific shRNA mediated knock-down has been able to reproduce the changes in a subset of these genes. Analysis of GAL4 reporter lines and using quantitative RT-PCR has shown that lnc703 is expressed in immune relevant tissues including barrier tissues and the fat body as well as a subset of repo-positive glial cells. Future work includes infection experiments, to analyse the role of lnc703 in the immune response and developing a technique to isolate lnc703 expressing cells, in both wild type and mutant situations to investigate their function in more detail.

944B

Mutagenesis and homologous recombination in *Drosophila* cell lines using CRISPR/Cas9. Andrew Roger Bassett. MRC Functional Genomics Unit, University of Oxford, Oxford, Oxfordshire, United Kingdom.
We have applied the CRISPR/Cas9 system to *Drosophila* S2 cells to generate targeted genetic mutations in more than 85% of alleles. By targeting a constitutive exon of the AGO1 gene, we demonstrate homozygous mutation in up to 82% of cells, thereby allowing the study of genetic knockouts in a *Drosophila* cell line for the first time. We have shown that homologous gene targeting is possible at 1-4% efficiency using this system, allowing for the construction of defined insertions and deletions. We demonstrate that a 1 kb homology arm length is optimal for integration by homologous gene targeting, and demonstrate its efficacy by tagging the endogenous AGO1 protein. This technology enables controlled genetic manipulation in *Drosophila* cell lines, and its simplicity offers the opportunity to study cellular phenotypes genome-wide.

945C

De novo Assemblies of Drosophila melanogaster using third-generation PacBio sequencing. Jane Landolin[2], Kristi Kim[2], Sergey Koren[3], Chen-Shan Jason Chin[2], Charles Yu[1], Bill Fisher[1], Roger Hoskins[1], Casey Bergman[4], Adam M. Phillippy[3], Susan E. Celniker[1]. 1) Berkeley Dros Genome Ctr, Lawrence Berkeley National Lab, Berkeley, CA; 2) Pacific Biosciences, 1380 Willow Road, Menlo Park, CA 94025; 3) 3125 Biomolecular Sciences Bldg #296, University of Maryland, College Park, MD 20742; 4) Michael Smith Building, Oxford Road, University of Manchester, M13 9PT.
We sequenced and assembled the genome of adult males from a subline of the ISO1 (y; cn, bw, sp) strain of D. melanogaster. We used the latest P5-C3 chemistry with the PacBio RS II system to generate 15,208,567,933 nt using 42 SMRT Cells. Half of the bases were contained in reads greater than 14,214 nt, and the longest read is 44,766 nt long. We attempted both a haploid- and a diploid-assembly, and the longest contigs in both assemblies (25 Mb and 21 Mb respectively) span almost the entire length of chromosome arm 3L. PacBio long reads uniquely localized repetitive transposable elements up to ~10 kb in size, and can fill at least one of the rare but persistent gaps remaining in the euchromatic portion of the reference genome. By sequencing sex-selected male flies, we increased the coverage of reads from chromosome Y. In particular, we assembled a 650 kb contig of the heterochromatic region of chromosome Y containing a very complex nested repeat. The gene Pp1-Y2 (a testis-specific phosphatase), which spans several gaps of unknown size in the Release 5 sequence, is now entirely contained in this single contig. This level of completeness in a de novo assembly is unprecedented in a metazoan genome. The reference genome required an effort that has spanned two decades, cost millions of dollars, and involved laborious BAC sequencing and manual finishing. Comparatively, the current project required a total of 6 weeks from initial fly collection and sorting to final analysis and assembly, and the actual sequencing time was 6 days. The data has been released and development versions of the software are freely available from http://blog.pacificbiosciences.com/2014/01/data-release-preliminary-de-novo.html.

946C

Dispersal at the Chromosomal Level of the NK gene family in Drosophila willistoni. Moises S Paramo, Carolus Chan, Jose Ranz. University of California, Irvine, Irvine, CA.
The study of the NK gene family in D. melanogaster suggested a clustered organization within a relatively small range of kilobases, a phenomena that has been attributed as an inherent property of the NK family as well as other homeobox gene families. Here we report on the organization of the NK cluster in the genetically dynamic invertebrate D. willistoni. We physically mapped the location of the genes using polytene

chromosome in situ hybridization. Contrary to what is seen in D. melanogaster, the NK family is broken in D. willistoni in a quarternary fashion; the division observed was Dr on its own, lbl, lbe, and C15 forming the first subcluster, bap and tin forming the second subcluster, and slou and Hmx forming the third subcluster. This result contests the consensus that homeobox gene families require cluster organization. It also suggest that there is no selective pressure to maintain these genes together, or at least to the degree that has been postulated, stressing the importance of focusing on dynamic genomes to properly interpret genomic patterns. genomic patterns.

947A

***Drosophila melanogaster* and the role of genetic background in eggshell phenotype.** Laura Youngblood, Lisa Goering. St. Edward's University, Austin, TX.

Genetic variation between individuals can lead to differences in the expression of traits. For example, although 70% of all Europeans diagnosed with cystic fibrosis have the same genetic mutation, these individuals can display a wide range of disease severity, even when environmental conditions are held constant. This suggests that understanding the effects of genetic background can be important in trying to diagnose and properly treat human disease, as different patients may present with variations in disease severity and may also respond differently to a particular treatment regimen. Here, we used the fruit fly, *Drosophila melanogaster*, as a model system to explore the effects of genetic background on the expression of mutations in the Epidermal Growth Factor Receptor (EGFR) pathway. The EGFR pathway is critical for the patterning of the *Drosophila* egg chamber and the formation of the dorsal respiratory appendages. To examine the effects of genetic background in the EGFR pathway, flies of two different genetic backgrounds, Oregon-R and Samarkand, were used. The mutations *blistered, spitz, star*, and *argos* were examined in the two backgrounds for their effects on dorsal appendage placement along the anterior posterior axis of the eggshell. Although the various mutations did change the anterior-posterior positioning of the appendages, these phenotypes were not sensitive to genetic background, with the mutations showing the same effects in both Ore-R and Samarkand. This type of investigation helps to shed light on how naturally occurring genetic variants may contribute to trait variation.