60th Annual Drosophila Research Conference – March 27-31, 2019
Full Abstracts

Opening General Session What's love got to do with it? Stimulating reproduction and activating eggs in Drosophila. *M.F. Wolfner* Dept Molec Biol & Gen, Cornell Univ, Ithaca, NY.

What's love got to do with it? Stimulating reproduction and activating eggs in Drosophila

2 Assembly and disassembly of germ plasm localized RNPs. *Elizabeth R. Gavis* Princeton University, Princeton, NJ.

Ribonucleoprotein assemblies (RNPs) organize RNA molecules according to their regulatory requirements. We have investigated how partitioning of mRNAs into different RNPs within the *Drosophila* germ plasm regulates their fate and function. At least two types of RNPs reside within the germ plasm: germ granules and founder granules. Germ granules are segregated to the primordial germ cells as they bud from the posterior of the embryo and contain numerous mRNAs, including several known to be important for germline development. Within germ granules, mRNAs are organized as spatially distinct clusters, called homotypic clusters, which contain multiple copies of an individual mRNA. By combining smFISH, super-resolution imaging, and quantitative image analysis we showed that during oogenesis, nascent granules are first populated by single-transcript RNPs that then act as seeds, recruiting additional like-transcripts to form homotypic clusters. Our data further suggest that RNA self-association drives germ granule mRNA accumulation. We have identified elements within the 3'UTRs of two resident germ granule transcripts, *nanos* and *polar granule component,* that regulate homotypic cluster growth. Our results suggest that regulation of homotypic clustering ensures delivery of requisite quantities of mRNAs to the primordial germ cells.

Founder granules contain *oskar* mRNA and regulate the local production of Oskar protein to initiate germ granule assembly in the oocyte. In contrast to germ granules, founder granules must be excluded from primordial germ cells for their development. We found that unlike germ granule mRNAs, which are protected from degradation, *oskar* mRNA is degraded in advance of germ cell formation. Temporal analysis of *oskar* degradation, the association of degradation machineries with founder granules, and the decrease in founder granule size indicate that degradation of *oskar* leads to founder granule disassembly, thus protecting the germline.

3 The I of the fly. Gerit Linneweber[2], Maheva Andriatsilavo[2], Suchetana Bias Dutta[2], Iryna Mohylyak[1], Mercedes Bengochea[1], *Bassem Hassan*[1,2] 1) ICM, Paris, FR; 2) FU Berlin and Charité, Berlin, DE.

Individual variability in morphology and behavior is the rule, not the exception. The fact that such variability is observed even among genetically identical individuals underscores the existence of epigenetic mechanisms that generate variability during development resulting in innate individual differences within a population. This fundamental feature of biology is as true for brain and behavior as it is for any other aspect of the organism. How epigenetic variability mechanisms operate during brain development and how they generate innate individuality in behavior is unknown. This talk will describe our efforts towards solving this problem. Using the fly visual system as developmental and behavioral model we show that intrinsically stochastic developmental mechanisms generate non-heritable variation in brain wiring, which in turn underlies stable individual differences in innate behavior.

4 The gut microbiome: the driving and driven partners of *Drosophila*. *Angela Douglas* Entomology, Cornell University, Ithaca, NY.

Drosophila is like most other animals, in that various phenotypic traits are influenced by the presence and composition of microorganisms borne within the gut. However, the effects of the gut microbiota can be context-dependent, varying with the genotype, sex and age of the *Drosophila* host, as well as environmental conditions, especially diet. Compounding this complexity, the composition and function of the microbiota can be influenced by host traits, environmental factors and among-microbe interactions. One productive route to understand these interactions is to simplify, i.e. to construct associations with a single or a few microbial taxa in order to investigate microbial drivers of the interaction. Certain microbial taxa have been demonstrated to have substantial effects on *Drosophila* metabolism, especially energy storage, and this can be explained in terms of both microbial-host competition for dietary resources and microbial modulation of the signaling circuits that regulate fly metabolism. The scale of these metabolic effects varies substantially with *Drosophila* genotype, and many of the genes with microbiota-dependent effects on phenotype function in the regulation of metabolism and behavior. An alternative approach to study *Drosophila* interactions with the gut microbiome is to "embrace the complexity" of the un-manipulated microbiota. To date, this approach has been applied predominantly to investigate the drivers of microbiota composition and function. Just as for people, the *Drosophila* microbiota varies widely among individual flies, and much of this variation can be explained by processes that are independent of host genotype and traits. In other words, the *Drosophila* host is not the sole driver of microbiota composition, even though this composition can have substantial effects on *Drosophila* traits and fitness. In summary, there is now overwhelming evidence that the gut microbiota is variable in composition, with substantial effects on the phenotype of *Drosophila* both in the laboratory and under field conditions. Altogether, the gut microbiome is an important consideration for the biology of *Drosophila* - and for *Drosophila* biologists.

5 Interrogating centromere specification mechanisms. Jason Palladino[1], Ankita Chavan[1], Ching-Ho Chang[2], Xiaolu Wei[2], Amanda Larracuente[2], *Barbara Mellone*[1] 1) Molecular and Cell Biology & Institute for Systems Genomics, University of Connecticut, Storrs, CT; 2) Department of Biology, University of Rochester, Rochester, NY.

Centromeres are essential chromosomal regions with a conserved function— to mediate kinetochore assembly and spindle attachments during cell division. Despite their functional conservation, centromeres are surprisingly dynamic and are a driving force of karyotype evolution and speciation. Whether centromere identity is specified by centromeric DNA sequences or by epigenetic mechanisms remains a major outstanding question in the field. Assessing the role of centromere-associated DNA elements in metazoans has focused on known centromeric or pericentromeric satellite repeats, but these represent only a subset of the DNA sequences associated with these regions. To date, the large satellite arrays to which centromeres map have been notoriously refractory to traditional DNA sequencing and assembly, leaving large gaps even for those species with the highest quality genomes, such as *D. melanogaster*. Previous work showed that when chromatin containing the centromere-specific histone mark CENP-A/Cid is formed at non-centromeric sites, it can initiate kinetochore assembly; however, it is unclear whether or not these *de novo* centromeres can sustain centromere function and specification through development. We report the unexpected DNA sequence composition and organization of all *D. melanogaster* centromeres, obtained leveraging long-read sequencing, CENP-A/Cid ChIP-seq, and high-resolution chromatin fiber imaging. We also present our findings on the epigenetic specification and inheritance of *de novo* centromeres through *Drosophila* development, mediated by re-directing the CENP-A/Cid assembly machinery to a variety of targeted genomic locations.

6 Tissue growth and metabolic sensing: from flies to humans. *Aurelio Teleman*[1,2] 1) German Cancer Research Center (DKFZ), Heidelberg, Germany; 2) Heidelberg University, Heidelberg, Germany.

I will present two projects in the lab that started in Drosophila with the aim of understanding how tissue growth is regulated. One focuses on mTORC1 signaling and the other on organismal sensing of a dietary lipid metabolite. These two projects provide examples of how the fly can be used to discover novel, fundamental biology that is relevant to human biology. I will try to present the projects from how they originally started in Drosophila up to the recent completion of a clinical study.

7 Precision and plasticity in animal transcription. *Angela DePace*[1], Jeehae Park[1], Javier Estrada[1,5], Jeremy Gunawardena[1], Kelly Biette[1], Tara Lydiard-Martin[1], Mary Katherine Howard[1], Meghan Bragdon[1,4], Anna Cha[1], Ben Vincent[1,3], Francheska Lopez Rivera[1], Zeba Wunderlich[1,2] 1) Department of Systems Biology, Harvard Med School, Boston, MA; 2) Department of Developmental and Cell Biology, University of California, Irvine, CA; 3) Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA; 4) Department of Biological Engineering and Biological Design Center, Boston University, Boston, MA; 5) Novartis Institutes for Biomedical Research, Cambridge, MA.

The regulatory DNA that controls transcription harbors natural sequence variants at multiple DNA length scales, from single nucleotide polymorphisms to large scale structural variants. Given that changes in gene regulation are critical in development, disease and evolution, a central challenge is to understand how sequence variants at all of these scales impact proximal molecular phenotypes and downstream organismal phenotypes. I will present our work probing the molecular mechanisms that govern transcription in the developing Drosophila embryo, with a view to understanding how regulatory DNA can drive precise gene expression patterns while the underlying sequence is quite plastic. I will discuss examples at different levels of complexity, from single enhancers to entire developmental loci, and emphasize how coupling quantitative measurements to computational models can decipher underlying principles of transcription.

8 Putting a STINGer on *Drosophila*: Evolutionary Conservation of Antimicrobial Defense. M. Martin, A. Hiroyasu, R.M. Guzman, S.A. Roberts, *A.G. Goodman* School of Molecular Biosciences, Washington State University, Pullman, WA.

The innate immune response provides the first line of defence against pathogens by responding to foreign molecules, such as by-products of bacterial and viral infections. The vertebrate protein STING, an intracellular sensor of cyclic dinucleotides, is critical to the innate immune response and the induction of type I interferon during pathogenic infection. In *Drosophila*, circulating hemocytes contain cell surface receptors for the detection of pathogens in the hemolymph. Activation of these receptors stimulates the innate immune response, which is potentiated by the induction of antimicrobial peptides. Here, we show that a STING ortholog (dmSTING) exists in *Drosophila*, which similar to vertebrate STING, associates with cyclic dinucleotides to initiate an innate immune response. Following infection with *Listeria monocytogenes*, a Gram-positive intracellular bacterium that generates cyclic dinucleotides during its life cycle, dmSTING activates an innate immune response potentiated by the NFkB transcription factor Relish, part of the immune deficiency (IMD) pathway. Using flies knocked-down for dmSTING by RNAi or genetic deletion, and flies overexpressing dmSTING, we found that dmSTING-mediated induction of the IMD pathway and antimicrobial peptides reduced the levels of *Listeria*-induced lethality, bacterial load in the host. Of significance, dmSTING triggers an innate immune response in the absence of a known functional cyclic GMP-AMP synthase (cGAS) ortholog in the fly. Together, our results demonstrate that STING is an evolutionarily conserved antimicrobial effector between flies and vertebrates, and it comprises a key component of host defense against pathogenic infection in *Drosophila*. Ongoing studies in the lab are focused on how STING-mediated pathways regulate innate immune responses to other intracellular pathogens, such as *Coxiella burnetii* and West Nile virus.

9 Tradeoffs between immune defense and resistance to environmental stress at a single amino acid polymorphism. *Andrea Darby*, Sarah Mullinax, Robert Unckless Molecular Biosciences, University of Kansas, Lawrence, KS.

Evolutionary trade-offs exist when a trait that enhances one component of fitness decreases another. These tradeoffs can lead to balancing selection, where different alleles are maintained in populations, but rarely can tradeoffs be attributed to a single amino acid change. We identified an amino acid polymorphism in the antimicrobial peptide, Diptericin, segregating in wild populations of *Drosophila*. Flies that have the serine (S) allele survive systemic infection with *Providencia rettgeri* better than those with the arginine (R) allele. Convergent mutations through different codons has led to R alleles segregating in both *D. melanogaster* and *D. simulans*, with the susceptible R alleles at high frequencies in both species. The fitness advantages of the arginine allele are unknown. In nature, *Drosophila* face desiccation and starvation, which are common environmental stressors. We investigated if flies with the arginine allele have better fitness when desiccated or starved. We used CRISPR/Cas9 genome editing to create variant lines of the AMP Diptericin, one line that is homozygous S and the other line is homozygous R. We measured desiccation and starvation resistance in both lines. For the starvation assay, flies were placed in vials of 1% agar at 25^{o}C, and recorded survival every eight hours. For the desiccation assay, flies were placed into vials plugged by a cotton plug, and then filled with two grams of silica gel desiccant 25^{o}C. R flies survive desiccation and starvation significantly longer than S flies (Welch Two Sample t-test $p<0.001$ in both cases). There are at least two possible explanations for this result: a) Diptericin directly impacts metabolites necessary for starvation and desiccation resistance or b) Diptericin indirectly influences resistance to environmental stressors via manipulating the gut microbiota. The gut microbiome is associated with multiple aspects of host fitness like physiology and pathogen resistance, so we looked at the microbiome as one mechanism that causes a difference in survival between R and S flies. We found that the R flies do not contain *Lactobacillus* species when plated on MRS agar, which suggests the phenotypes play a role in microbiome community assembly. Desiccation and starvation assays on axenic flies and gnotobiotic flies will help us understand the trade-offs between the host immune defense and resistance to environmental stressors mediated by the gut microbiota.

10 Role of Circular RNAs in Innate Immunity and Neurodevelopment. X.P. Xiong[1], W. Liu[2], W.H. Liang[2], S. Xu[3], A. Tito[3], J.L. Li[1], J. Situ[1], R. Perera[1,2], S. Zhang[3], *R. Zhou*[1,2] 1) Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA & Orlando, FL ; 2) Department of Oncology, Johns Hopkins University School of Medicine, St Petersburg, FL; 3) The University of Texas Medical School at Houston, Houston, TX.

Circular RNAs (circRNAs) are widely expressed in eukaryotes. However, a critical challenge is a lack of understanding of their physiological functions except for only a handful of circRNAs. We have identified and validated a collection of *Drosophila* circRNAs in response to various pathogen infections. Our analyses revealed that *Edis*, a brain-enriched circRNA, displays a spatiotemporal expression pattern and is upregulated with age. Depletion of *Edis*, but not its linear sibling mRNA, causes hyperactivation of anti-bacterial innate immunity signaling both in cultured cells and *in vivo.* Consequently, *Edis*knockdown flies show enhanced pathogen clearance and resistance to bacterial infection. In addition, restoration of *Edis* expression suppresses the innate immunity phenotype elicited by *Edis* depletion. Furthermore, flies with whole-body or neuron-specific depletion of *Edis* display innate immunity hyperactivation, severely impaired locomotive activity and short lifespan, which correlate with neuronal cell loss and strong defects in brain development. Thus our study establishes the circular RNA *Edis* as an important modulator of both basal and bacteria-stimulated immunity signaling, and suggests that *Edis* may function as a critical integrator of neuronal immune response and the development and survival of neuronal cells.

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11 Diet-induced microbiota adaptation is controlled by NF-kB-dependent regulation of 4EBP in Drosophila. *C. Vandehoef*, J. Karpac Molecular and Cellular Medicine, Texas A&M Health Science Center, College Station, TX.

Diet and nutrition shape all aspects of animal physiology across taxa, including composition, adaptation, and maintenance of intestinal microbiota. These microbiota, in turn, influence host animal metabolic responses. The reciprocal interactions between diet, host signaling networks, and microbiota likely define a rheostat that governs host physiology. Importantly, when these interactions are misregulated, the result is often metabolic dysfunction and disease. Thus, there is a critical need to explore the distinct cellular and molecular host signaling mechanisms that shape diet-microbe interactions and influence host

physiology. This work investigates diet-dependent host signaling mechanisms, driven by the evolutionarily conserved innate immune transcription factor NF-kB, that dictate intestinal microbiota composition and homeostasis. The Drosophila model is exploited to tissue-specifically manipulate host signaling function under various dietary condtions, and microbiota are subsequently surveyed using culture-dependent and independent methods. Here, we provide evidence that NF-kB transcription factor function in the Drosophila intestine can govern microbiota adaptation/composition and metabolic signaling pathway activity in response to specific changes in dietary macronutrients, putatively influencing microbiota-regulated aspects of host health and dietary adaptation. More specifically, in response to high carbohydrate-and-low protein dietary macronutrient ratios, NF-kB activity can modulate transcriptional levels and function of 4EBP a conserved regulator of physiology that couples nutrition and mRNA translation. NF-kB-dependent regulation of 4EBP is required to shift microbiota composition in response to a high carbohydrate-and-low protein diet, subsequently influencing host physiology. This work has uncovered an integrated system involving transcriptional and translational regulation of host signaling, dietary macronutrients, and microbiota composition working together to impact organismal health and physiology. Furthermore, these findings highlight host signaling, shaped by dietary cues, as an active participant in the microbial symbiotic relationship.

12 Modeling Host-Pathogen Interactions with the DNA virus IIV-6. *C. West*[1], Don Gammon[2], Neal Silverman[1] 1) University of Massachusetts Medical School, Worcester, MA; 2) UT Southwestern Medical Center, Dallas, TX.

The fruit fly *Drosophila melanogaster* is a powerful model system for the study of innate immunity in vector insects as well as mammals. For vector insects, it is particularly important to understand all aspects of their antiviral immune defenses, which could eventually be harnessed to control the transmission of human pathogenic viruses. The immune responses controlling RNA viruses in insects have been extensively studied, but the response to DNA virus infections is poorly characterized. Here, we report that infection of *Drosophila* with the DNA virus Invertebrate iridescent Virus 6 (IIV-6) triggers JAK-STAT signaling and the robust expression of the *Turandots*, a gene family encoding small secreted proteins. To drive JAK- STAT signaling, IIV-6 infection more immediately induced expression of the *unpaireds*, a family of IL-6-related cytokine genes, via a pathway that required one of the three Drosophila p38 homologs, p38b. In fact, both Stat92E and p38b were required for the survival of IIV-6 infected flies. In addition, *in vitro* induction of the unpaireds required an NADPH-oxidase, and *in vivo* studies demonstrated *Nox* was required for induction of TotA. These results argue that ROS production, triggered by IIV-6 infection, leads to p38b activation and *unpaired* expression, and subsequent JAK-STAT signaling, which ultimately protects the fly from IIV-6 infection.

Recently, we have also found that IIV-6 infected cells secrete protective factors capable of preventing infection of naive cells challenged with an mCherry-expressing strain of IIV-6.

Additionally, IIV-6 inhibits two major immune responses in *Drosophila*, the Imd and Toll pathways. Stimulation of IIV-6 infected *Drosophila* S2* cells with either Imd or Toll stimulators results in very poor antimicrobial peptide responses. Yet, Imd and Relish are still cleaved upon stimulation in IIV-6 infected cells, indicating that the block is downstream. In support of this finding, IIV-6 infected flies respond very poorly to infection with the enterobacteria *Erwinia carotovora carotovora* compared to mock-injected flies.

13 Two Nimrod receptors, NimC1 and Eater, synergistically contribute to phagocytosis in *Drosophila melanogaster*. *C. Melcarne*[1], E. Ramond[1], A. Bretscher[1], M. Poidevin[2], E. Kurucz[3], I. Andó[3], B. Lemaitre[1] 1) Global Health Institute, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne, EPFL, Lausanne, Switzerland; 2) Centre de Génétique Moléculaire, CNRS/Université Pierre et Marie Curie, Gif-sur-Yvette; 3) Institute of Genetics Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary.

Eater and NimC1 are transmembrane receptors of the *Drosophila* Nimrod family, specifically expressed in hemocytes, the insect blood cells. Previous RNAi studies have pointed to their role in bacterial phagocytosis. Here, we re-evaluated the role of NimC1 alone, or in combination with Eater, in the cellular immune response with the use of a novel null mutant: *NimC1*[1]. We show that NimC1 functions as an adhesion molecule but, in contrast to Eater, is not required for hemocytes sessility. E*x vivo* phagocytosis assays and electron microscopy experiments confirmed that Eater is the main phagocytic receptor for Gram-positive, but not Gram-negative bacteria, and contributes to microbe tethering. *NimC1* deletion did not impair phagocytosis of bacteria, nor their adhesion to the hemocytes. However, phagocytosis of both bacteria type was severely impaired in *NimC1*[1]*;eater*[1] double mutant. In addition, hemocytes lacking both receptors cannot mediate a proper uptake of latex beads and zymosan yeast particles neither. We suggest that both receptors contribute to bacterial phagocytosis, but that Eater can bypass the requirement for NimC1. To our knowledge, *NimC1*[1]*;eater*[1] is the first *Drosophila* viable deletion mutant showing such an impaired ability to phagocytose both immunogenic and non-immunogenic particles, despite showing a mature and differentiated blood cell phenotype.

We demonstrated that analysis of multiple mutants is the best approach to unmask phenotypes corresponding to specific receptors, and therefore to gain deeper knowledge on the contribution of each proteins on biological processes.To conclude, our work provided solid evidences for Eater and NimC1 being two critical receptors for the initial “recognition phase” of *Drosophila* phagocytosis, and that each receptor might play distinct roles in microbial uptake.

14 A resilience function for the Toll pathway in host defense against systemic *Aspergillus fumigatus* infection. Rui Xu[1,2], Chuqin Huang[1], Samuel Liégeois[1,2,3], Zi Li[1], *Dominique Ferrandon*[1,2,3] 1) Sino-French Hoffmann Institute, Guangzhou Medical University, Guangzhou, China; 2) Université de Strasbourg, Strasbourg, France; 3) IBMC, CNRS UPR 9022, Strasbourg, France.

Host defense encompasses two complementary dimensions, resistance, the immune response that results in the neutralization and killing of invading pathogens, and resilience, the homeostatic reactions that participate in enduring and repairing damages inflicted either by pathogen's virulence factors or the host's own immune response.

Aspergillus fumigatus is a major human opportunistic pathogen that causes high morbidity and mortality in immunodeficient patients. We are currently performing a large-scale screen to identify *Drosophila* host defense genes. A first step was to better characterize the infection model, which we have optimized for our purpose. As reported previously, *A. fumigatus* does kill *MyD88*- Toll pathway immunodeficient- flies but not wild-type flies. However, we observed that the fungal burden hardly increases in the mutant flies, even upon death, in contrast to other fungal infections. Some 250 injected conidia suffice to kill *MyD88* flies in the absence of invasion of most fly tissues. We have therefore tested whether some of the many toxins known to be secreted by *A. fumigatus* might be involved in the pathogenesis. We shall report that some but not all such toxins differentially kill *MyD88* and not wild-type flies. Therefore, the Toll pathway is not only a major pathway in the resistance against fungal infections but also appears to be required for resilience against such infections by protecting the host from the action of pathogen-secreted toxins.

15 Integrating cis and trans changes to analyze the evolution of Bcd dependent patterning network. *P. Onal*, J. Ling, Y. Umezawa, S. Small Biology, New York University, New York, NY.

Major changes in body plans evolve by numerous mechanisms including the appearance of novel genes and protein neo-functionalization in addition to cis-regulatory changes. The transcription factor Bicoid (Bcd) is an evolutionary novelty in the anterior patterning network in flies. It emerged after a gene duplication event more than 150 MYA, which also gave rise to Bcd's sister protein Zen. It then rapidly diverged in amino acid sequence to become an instructive morphogen that patterns anterior structures of *Drosophila* embryo by regulating more than fifty mostly conserved target genes. To identify the historical steps in the evolution of Bcd as an anterior patterning factor we combined ancestral gene reconstruction algorithms with biochemical and gene

replacement assays. Our experiments define a precise number of amino acid substitutions that were critical for the evolution of the Bcd's transcriptional activities. We suggest that these substitutions gradually led Bcd to bind to and activate more target genes, which we are testing using chromatin immunoprecipitation experiments. Importantly, one of Bcd's most conserved targets, *hunchback (hb)*, shows a gradual increase in expression that accompanies the gradual improvement in the ability of the ancestral protein to rescue the *bcd* mutant phenotype, suggesting a critical role for Hb in mediating Bcd's functions. Using reporter genes, we have shown that increasing the affinity of Bcd sites in a *hb* enhancer can enhance the binding of partially active substituted ancestral proteins, leading to an increase in expression. We are now mimicking these experiments using CRISPR-mediated gene editing of the *hb* enhancer *in vivo*. Our ultimate goal is to investigate how much of the bcd mutant phenotype can be rescued by cis-regulatory mutations that increase the response of the *hb* regulatory sequences to Bcd-mediated activation. These studies will critically test the idea that coordinated changes in the Bcd protein and in the cis-regulatory elements of its target genes were critical for the evolution of the anterior patterning network in *Drosophila*.

16 Genetics and genomics of gene expression variation in the *D. melanogaster* early embryo. *Nicolas Svetec*[1,2], Li Zhao[1,2], Hayley Sheehy[2], David Begun[2] 1) Laboratory of Evolutionary Genetics and Genomics, The Rockefeller University, New York, NY; 2) Department of Evolution and Ecology, University of California, Davis, CA.

A key question in biology is to understand how genetic and phenotypic diversity arise and are maintained in natural populations. Spatially varying selection is one of the forces shaping such diversity. Latitudinal clines like the ones observed in *D. melanogaster* represent an ideal model system for investigating the genetic basis for local adaptation. Our previous work showed that a particular aspect of the biology of *D. melanogaster* oocyte (UV tolerance) differs drastically between temperate and equatorial fly populations. Using genomic tools and phenotypic experiments, we demonstrated that UV tolerance is shaped by spatially varying selection acting on the DNA damage repair pathway and that both structural and expression changes contribute to the observed patterns. Here, we analyze allele specific expression from mature oocytes in a set of DGRP lines to characterize the genetics of expression variation. We integrate these data with data from geographic differentiation of early embryo transcriptomes and existing eQTL data to present a portrait of the influence of spatially varying selection on the early embryo transcriptome. Finally, we studied a number of eQTL SNPs variants putatively causal to the observed expression variation between populations. Our results connect spatially varying selection to gene expression regulatory circuit, and provide novel insights on the extent to which local adaptation can shape and maintain variation for the maternal provisioning of RNA into the oocyte.

17 Convergent evolution of sex-limited pigmentation alleles in *Drosophila*. *E.K. Delaney*[1], Masayoshi Watada[2], Artyom Kopp[1] 1) Evolution and Ecology, University of California-Davis, Davis, CA; 2) Ehime University, Matsuyama, Ehime, Japan.

Convergent evolution of sexually dimorphic phenotypes provides natural replicates that can be harnessed to study how alleles evolve to be sex-specific. To understand how alleles gain sex-specific activity, we identified a set of sexually monomorphic and sexually dimorphic alleles involved in a convergent, female-limited color polymorphism in the *Drosophila montium* subgroup. In this clade, females of some species are polymorphic for abdominal pigmentation (i.e. they are light or dark) while the males are monomorphic regardless of genotype (i.e. they are only light or only dark) regardless of genotype. We performed a genome-wide association analysis in three distantly-related species with polymorphic female pigmentation and either light males (*D. serrata* and *D. kikkawai*) or dark males (*D. rufa*). We found that color alleles repeatedly mapped to introns of the same autosomal gene—*POU domain motif 3* (*pdm3*)—a transcription factor that represses dark pigment. In each species, light and dark alleles occur at small (< 2 kb) structural variants (SV) where the dark allele is longer and divergent in sequence. The SV in *D. serrata* and *D. rufa* occurs at the same position in the first intron of *pdm3*, potentially indicative of a trans-species polymorphism, but the SV in *D. kikkawai* occurs in the second intron of *pdm3*. Our identification of SV alleles in different introns of the same gene suggests that (1) sexually dimorphic alleles may have evolved via repeated propagation of an ancestral polymorphism and selection on *de novo* mutations, and (2) the alleles likely affect distinct abdominal enhancers of *pdm3*. We use population genomic data and reference genomes from 26 *montium* species to assess the evolutionary histories of light and dark alleles across the *montium* clade, and expression analysis and CRISPR experiments to test the phenotypic effects of these SV alleles on female and male pigmentation. By integrating genomic and functional analyses of alleles underlying a convergently-evolved female-limited color polymorphism, we determine the steps and DNA sequences that lead to sexual dimorphism.

18 Recurrent losses and rapid evolution of the condensin II complex in insects. *Thomas King*[1], Chris Leonard[1], Jacob Cooper[1], Son Nguyen[2], Eric Joyce[2], Nitin Phadnis[1] 1) School of Biological Sciences, University of Utah, Salt Lake City, UT ; 2) Department of Genetics, University of Pennsylvania, Philadelphia, PA.

Condensins play a crucial role in the organization of genetic material by compacting and disentangling chromosomes. The condensin I and condensin II complexes are widely considered to have distinct functions based on studies in a few model organisms, although the specific functions of each complex are yet to be fully understood. The condensin II complex is critical for genome organization in Drosophila, and is a key anti-pairing factor that separates homologous chromosomes in somatic cells. Intriguingly, the Cap-G2 subunit of condensin II is absent in *Drosophila melanogaster*, and this loss may be related to the high levels of homologous chromosome pairing in somatic cells seen in flies. Here, we find that this Cap-G2 loss predates the origin of Dipterans, and other Cap-G2 losses have occurred independently in multiple insect lineages. Furthermore, the Cap-H2 and Cap-D3 subunits have also been repeatedly and independently lost in several insect orders, and some taxa lack condensin II-specific subunits entirely. We used Oligopaint DNA-FISH to quantify pairing levels in ten species across seven orders, representing several different configurations of the condensin II complex. We find that all non-Dipteran insects display near-uniform low pairing levels, suggesting that some key aspects of genome organization are robust to condensin II subunit losses. Finally, we observe consistent signatures of positive selection in condensin II subunits across flies and mammals. These findings suggest that these ancient complexes are far more evolutionarily labile than previously suspected, and are at the crossroads of several forms of genomic conflicts. Our results raise fundamental questions about the specific functions of the two condensin complexes and the interplay between them in taxa that have experienced subunit losses, and open the door to further investigations to elucidate the diversity of molecular mechanisms that underlie genome organization across various life forms.

19 Patterns of genetic and transcriptional selection response under stress. *S Forsberg*, L Pallares, J Ayroles Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ.

For nearly a century, experimental evolution has been a favorite tool of biologists seeking to test various evolutionary models. Experimental evolution is no less relevant today, having been reinvigorated by advances in DNA and RNA sequencing that allow the evolutionary dynamics of genotypes and phenotypes to be monitored in real time. In an ongoing evolutionary experiment, we have exposed multiple outbred fly populations to a diet with high sugar content, while control populations are kept on a normal diet. Flies exposed to the high sugar diet initially display various detrimental symptoms, such as hyperglycemia, abnormal fat accumulation, and delayed developmental time. After more than 100 generations of adaption to this stressful environment, we are now in a position to ask questions about the general principles and modalities of selection response.

Through DNA and RNA sequencing of flies at the onset of this selection regime, we have a picture of the transcriptional response to this dietary stress. We also find multiple cases of genotype-by-environment interactions, where the stress response is genotype specific. Through pooled DNA sequencing of flies at different time points during selection, we have also quantified the changes in allele frequency over time. We find many strong signatures of selection, some implicating well studied genes involved in response to insulin stimulus, metabolic processes, and feeding behavior to name a few. Other selection signatures

implicate genes with as yet unknown functions. Multiple parallel populations were exposed to the high sugar and control diets, allowing us distinguish true selection signatures from genetic drift with very high statistical power. In addition, this design allows us to ask how genetic interactions shape evolutionary trajectories. We find examples of highly correlated changes in allele frequencies at physically unlinked loci, indicating that certain allele combinations convey a synergistic selective advantage. This also implies that the trajectories can not always be accurately predicted based on the standing genetic variation in the base population.

We are currently sequencing more time points in order to paint a clearer picture of how genetic interactions can shape evolution, as well as to increase our mapping resolution. Through RNA sequencing at later time points, we also hope to answer whether the architecture of the transcriptional network is maintained by selection, or if the transcriptional modules are reorganized with a changing selective environment.

20 New gene formation in hybrid *Drosophila*. *R.L. Rogers*[1], N. B. Stewart[2], C.M. Moore[1] 1) Bioinformatics and Genomics, UNC Charlotte, Charlotte, NC; 2) Biological Sciences, Ft Hays State University , Hays, KS.

Gene expression changes in hybrids can be a source of phenotypic variation. Transgressive gene expression occurs when hybrid offspring have expression changes beyond variation within parent species. Qualitative changes in expression have been well studied in a number of hybrid systems. However, the ability of hybrids to create new genes or activate existing genes has been largely overlooked. We have assayed expression changes in *D. santomea* x *D. yakuba* hybrids. We observe as many as 56 cases of new gene formation in a single cross. These new genes appear when trans acting factors from one species bind to a cis regulatory module that was present in the other species. We find that the number of new transcripts created depends on the direction of the cross. In *D. yakuba* ♀*x D. santomea* ♂ crosses, a greater number of transcripts are formed in female offspring (21 vs 9). In *D. santomea* ♀*x D. yakuba* ♂ crosses, a greater number of transcripts are formed in male offspring (56 vs 5) (χ^2=34.671, P=3.905 x 10^{-9}). These new transcripts formed in hybrids might potentially spread through populations via introgression. This source of genetic novelty may offer a source of new variation that could influence natural populations.

21 Adaptive evolution at a meiosis gene mediates species differences in the rate and patterning of recombination. *Cara Brand*[1,2], Daven Presgraves[2] 1) Biology Department, University of Pennsylvania, Philadelphia, PA; 2) Biology Department, University of Rochester, Rochester, NY.

Crossing over between homologous chromosomes during meiosis repairs programmed DNA double-strand breaks, ensures proper segregation, enhances the efficacy of natural selection among genetically linked sites, and determines the genomic distribution of nucleotide variability in populations. Little however is known about the molecular genetic changes or population genetic forces involved in the evolution of recombination rates between species. We show that a dicistronic meiosis gene, *mei-217/mei-218*, with a history of rapid evolution acts as a global, trans-acting modifier of the rate and chromosomal distribution of crossing over between two closely related *Drosophila* species. Using transgenic flies, we find that species differences in crossing over are attributable to changes in the strengths of crossover assurance, crossover interference, and centromeric suppression of crossing over. We speculate that rates of crossing over evolved in part to mitigate fluctuating, species-specific risks of ectopic recombination between non-homologous transposon insertions. Regardless of its causes, the evolution of *mei-217/mei-218*-mediated changes in recombination landscapes may contribute to downstream species differences such as the chromosomal distribution of nucleotide variability and rates of nondisjunction. Finally, we investigate the deeper phylogenetic history, causes, and consequences of evolution of our meiosis gene and its interactors over the *Drosophila* phylogeny.

22 Flies in the Diagnosis of Rare Disease: The Model Organisms Screening Center for the Undiagnosed Diseases Network. *M.F. Wangler*[1,2,3,4], Jonathan Andrews[1], Scott Barish[1], Hsiao-Tuan Chao[1,2,3], Hyunglok Chung[1], Samantha Deal[1,4], Jake Harland[1], Sharayu Jangam[1], Oguz Kanca[1], Yoon Wan-Hee[1], Xi Luo[1], Ning Liu[1], Dongxue Mao[1], Paul C. Marcogliese[1], Matthew Moulton[1], Thomas A Ravenscroft[1], Mumine Senturk[1], Julia Wang[1], Shinya Yamamoto[1,3,4,5], Hugo J. Bellen[1,3,4,5,6], Members of the Undiagnosed Diseases Network 1) Molecular and Human Genetics, Baylor College of Medicine (BCM), Houston TX, 77030; 2) Department of Pediatrics, BCM, Houston TX, 77030; 3) Jan and Dan Duncan Neurological Research Institute, Houston, TX 77030; 4) Program in Developmental Biology, BCM, Houston TX, 77030; 5) Department of Neuroscience, BCM, Houston TX, 77030; 6) Howard Hughes Medical Institute, BCM, Houston, TX, 77030, USA.

Patients with rare undiagnosed diseases often undergo genomic sequencing to identify potential causative variants and genes involved in their medical condition. Sequencing interpretation is complex and requires knowledge of gene function. Predicting how the specific genetic variation from a patient's genome might impact the encoded protein and the clinical phenotype can be difficult. Many of these diagnostic challenges posed by undiagnosed diseases have solutions in model organism research as animal studies provide the medical research community with a wealth of detailed biological information. The Undiagnosed Diseases Network (UDN) is a multi-center effort of the top clinical centers in the United States aimed at solving the most challenging medical mysteries with advanced medical technology. As part of this effort, our team was tasked with establishing a Model Organisms Screening center (MOSC) that uses *Drosophila* and Zebrafish for functional studies of the genes and variants identified in UDN patients by whole-exome sequencing (WES) or whole-genome sequencing (WGS). In Phase I (2015-2018) of the UDN over 2000 patients applied and 907 were accepted leading to sequencing in 868 cases. We established a multidisciplinary team of clinicians, human geneticists and Drosophila researchers at Baylor College of Medicine to establish the MOSC Drosophila Core a center for studies in flies to aid in the diagnosis of UDN cases. We created a process for variant submission that went from patient evaluation by the clinician to model organism studies. Our team developed the MARRVEL informatics online tool and worked to match patients with similar phenotypes and mutations in the same gene across human genomic studies. From the study our team analyzed 236 variants in 179 genes for 118 UDN cases for consideration of fly studies. From these, we prioritized 72 genes for functional studies in model organisms of which 58 were studied in *Drosophila*. Our studies in flies employed a number of technologies including use of T2A-GAL4 elements to test human rescue and to study the impact of specific point mutations from UDN cases. We also engineered genomic transgenes with patient specific amino acid changes. Our work led to a number of novel disease gene discoveries including *EBF3*, *ATP5F1D, TBX2* and *IRF2BPL*, while we characterized new human disease phenotypes for *CACNA1A, ACOX1* and *NR5A1* amongst others. These discoveries required in depth studies in flies as well as identification of additional cases in human databases and contributed to over 11% of the successful diagnoses in the UDN, a significant fraction considering flies contributed to diagnosis for patients that had eluded a specific diagnosis despite extensive previous evaluations. *Drosophila* studies of human genomic variation are therefore an effective tool in undiagnosed disease research.

23 Zika virus protein NS4A inhibits Ankle2, a primary microcephaly locus that regulates asymmetric division. *N. Link*[1], P. Shah[2], N. Krogan[3], H.J. Bellen[1] 1) Howard Hughes Medical Institute, Department of Molecular and Human Genetics, Neurological Research Institute, Texas Children's Hospital, Program in Developmental Biology, Baylor College of Medicine, Houston, TX; 2) Department of Chemical Engineering, Department of Microbiology and Molecular Genetics, University of California, Davis; 3) Department of Cellular and Molecular Pharmacology, California Institute for Quantitative Biosciences, University of California, San Francisco.

Primary microcephaly, or reduced head size, is often the result of a neurodevelopmental disease with associated cognitive and neurological defects. We discovered that human *ANKLE2* variants are associated with primary microcephaly, and that mutations in *Drosophila Ankle2* cause cell loss in the central nervous system, mimicking microcephaly phenotypes found in humans. In addition, *Ankle2* mutants contain fewer neuronal stem cells that divide less

frequently. We showed that *Ankle2* is required for progenitor cell maintenance, division, and survival in the nervous system. We find that *Ankle2* is required for proper asymmetric localization of polarity components during neuroblast division. Ankle2 negatively regulates the kinase Ballchen (Ball), the fly orthologue of VRK1, and removing one copy of *Ball/VRK1* in *Ankle2* mutant animals rescues microcephaly and lethality. Both Ankle2 and Ball/VRK1 interact with L(2)gl, a negative regulator of aPKC activity, and removing one copy of *l(2)gl* in *Ankle2* mutants rescues microcephaly. Furthermore, we also show that Zika virus protein NS4A interacts with and inhibits the Ankle2 pathway, leading to a small brain phenotype. Moreover, expression of human ANKLE2 or modulation of the *Ankle2* pathway rescues NS4A induced microcephaly. Hence, NS4A inhibits Ankle2, which regulates polarity components and asymmetric division. Our data indicate a mechanism for Zika induced microcephaly in humans. Hence, by discovering and studying a rare human genetic disease in flies, we can now propose a mechanisms for a much more common diseases induced by ZIka virus infections.

24 Micropipette harpooning reveals a loss of physical coupling between the nucleus and cytoplasm in Drosophila models of muscular dystrophy. G.H. Fedorchak[1,2], N.M. Shaw[4], G. Coombs[3], J. Lammerding[1,2], *L.L. Wallrath*[4] 1) Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY; 2) Nancy E. and Peter C. Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY; 3) Biology Department, Waldorf University, Forrest City, IA; 4) Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA.

Mutations in the human genes *LMNA* and *SYNE1* cause rare types of muscular dystrophy by mechanisms that are not well understood. Both genes encode structural proteins of the nuclear envelope and are important for nuclear-cytoplasmic interactions. *LMNA* encodes lamins, intermediate filaments that line the inner membrane of the nuclear envelope. *SYNE1* encodes nesprins, KASH-domain proteins that represent one component of the LINC (Linker of Nucleoskeleton and Cytoskeleton) complex. Nesprins reside in the outer nuclear envelope where they connect to SUN domain proteins across the perinuclear space and to actin and microtubules in the cytoplasm, thereby facilitating physical coupling between the nucleus and the cytoplasm. To model muscular dystrophy, human disease-causing variants in Drosophila *Lamin C* and a transgene encoding an RNAi against *Msp300* (an orthologue of *SYNE1*) were expressed in Drosophila larval body wall muscles, which are strikingly similar to human skeletal muscles. Expression of mutant Lamin C and depletion of Msp300 caused reduced larval motility and premature death at the pupal stage. To understand the molecular basis of the muscle pathology, we applied micropipette harpooning to muscle filets from mutant and control larvae. This novel assay involves live imaging during precise force application to the perinuclear cytoskeleton by a fine microneedle to measure the physical coupling between the nucleus and the cytoplasm and the deformability of the nucleus. These results showed that specific mutant lamins and reduced levels of Msp300 caused a loss of coupling between the nucleus and cytoplasm and changes in nuclear envelope deformation. Immunohistochemistry of affected muscles revealed a loss of astral microtubules that normally cage the nucleus for protection from the forces of muscle contraction. Furthermore, muscle-specific expression of a fluorescently labeled cGAS (a cytosolic DNA-sensing protein cyclic-GMP-AMP synthase) that binds to cytoplasmic DNA, showed evidence of nuclear envelope rupture and DNA leakage from the nucleus, indicative of a weakened nuclear envelope. Taken together, these findings provide insights on disease mechanisms and suggest that approaches to facilitate and re-force connections between microtubules and the nucleus may protect against nuclear envelope rupture and improve muscle health.

25 The cathepsin Cystein protease-1/Cathepsin V regulates α-synuclein mediated accumulation and neurotoxicity in a synucleinopathy model. *Tom Lee*[1,2], Gabriel Velez-Vazquez[2,3], Aleksander Bajic[2,3], Katherine Allison[1,2], Ismael Al-Ramahi[2,3], Juan Botas[2,3], Huda Zoghbi[2,3], Joshua Shulman[1,2,3] 1) Department of Neurology, Baylor College of Medicine, Houston, TX; 2) Duncan Neurologic Research Insititute, Texas Children's Hospital, Houston, TX; 3) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Synucleinopathies are one of the most common forms of neurodegenerative disease and characterized by the abnormal accumulation of α-synuclein in the adult brain. Based on human genetic studies, we identified *Cathepsin V* (*CtsV*) as a candidate regulator of α-synuclein pathology. Utilizing the fruit fly for functional validation, we show that *Cysteine protease-1* (*Cp1*), the fly ortholog of *CtsV,* is expressed thoughout the adult fly brain including dopaminergic neurons. In the adult fly, *Cp1* inhibits α-synuclein induced neurotoxicity and behavioral defects in an age dependent manner. In addition, *Cp1* inhibits α-synuclein induced dopaminergic neuron loss in aged adult brains. We show that *Cp1* and *CtsV* regulate α-synuclein protein turnover in flies and human differentiated neurons, respectively. Moreover, altering levels of *Cathepsin B1* (*CtsB1*) or the pan-cathepsin inhibitor, *crammer* (*cer*) indicates that *Cp1* may function redundantly with other cathepsins to mediate α-synuclein proteostasis. Taken together, our data indicates that *Cp1/CtsV* functions as a modifier of α-synuclein induced neurotoxicity and implicates an important role of the lysosome in regulating aberrant α-synuclein accumulation and disease pathology.

26 Common and differential pathogenic mechanisms caused by mutant Huntington expression in glia and neurons. *Tarik Onur*[1,2], Andrew Laitman[1,2], Hyemin Kim[1,2], Jennifer Wang[1,2], Ying-Wooi Wan[1,2], Ismael Al-Ramahi[1,2], Zhandong Liu[1,2], Juan Botas[1,2] 1) Baylor College of Medicine, Houston, Texas; 2) Jan and Dan Duncan Neurological Research Institute, Houston, Texas.

Huntington's disease (HD) is a rare neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in the gene *Huntingtin*. Much of the molecular pathogenesis that occurs during HD has been studied in the context of neurons, whereas relatively little is known about the effect of mutant Huntingtin (mHTT) protein in glia. To better understand the biological processes disrupted in glia during HD, we mined transcriptomic data to compare the effect on gene expression upon the introduction of mHTT glia to what occurs in neurons. We made a detailed comparison of the transcriptome of fruit flies, mice, and patients affected by the mHTT protein defining concordantly altered, conserved alterations to gene expression. The addition of the *Drosophila* transcriptome in this cross-species analysis allowed us to characterize and compare the evolutionarily conserved alterations to gene expression in the central nervous system that results from expressing mHTT in glia versus neurons. Network analysis on these conserved differentially expressed genes (DEGs) defined biological processes that could be underlying the pathogenesis of HD. Next, we wanted to experimentally determine which of these DEGs associated with HD are involved in the disease pathogenesis. We defined compensatory DEGs as those changes which ameliorate glial/neuronal dysfunction phenotypes in *Drosophila*when gene expression is further altered in the same direction. Likewise, pathogenic alterations are those changes to gene expression that aggravate a disease-related phenotype when that gene is further altered in the same direction. We screened DEGs from our network using an automated, high-throughput behavioral assay system. We have defined alterations to gene expression that represent important components of the molecular progression of HD in glia, such as genes involved in synapse assembly, potassium regulation, and endocytosis. Moreover, there are pathogenic and compensatory changes in glia shared with neurons such as *LMO7, ADCY5, and LRP2*. These modifiers represent particularly high-potential therapeutic targets that consistently improve toxicity caused by mHTT. We will perform homogenous time-resolved fluorescence (HTRF) to measure which of these modifiers could be reducing mHTT levels. Finally, some common DEGs that result from neuronal or glial mHTT expression had differing effects when manipulated in neurons and glia such as *TGF-beta*. These differential effects in represent important physiological differences that are being simultaneously disrupted during HD or cross-talk between neurons and glia. Overall, we have defined alterations to gene expression changes observed during HD pathogenesis that affect glial function, and have compared these results to what occurs in neurons.

27 Hap40 is a conserved binding partner of HTT in *Drosophila*. *S. Zhang*[1,3,4,5], S. Xu[1], G Li[1], B Hu[1], E Stimming [2] 1) The Brown Foundation Institute of Molecular Medicine ; 2) Department of Neurology, HDSA Center of Excellence; 3) Department of Neurobiology and Anatomy ; 4) McGovern Medical School at the University of Texas Health Science Center at Houston (UTHealth), Houston, Texas, 77030; 5) Programs in Human and Molecular Genetics and Neuroscience, The University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences (MD Anderson UTHealth GSBS), Houston, Texas,

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Huntington's disease (HD) is caused by an abnormal expansion of the glutamine tract (polyQ) in Huntingtin (HTT) protein. As a large scaffold protein with numerous reported binding partners (HAPs), HTT is implicated in a growing list of cellular processes from vesicular transport, transcription to autophagy. However, little is known how these diverse functions are integrated through HTT and how HTT itself is regulated.

Converging evidence support that HAP40 is a *central*regulator of HTT. HAP40 was originally isolated from rat and mouse brains as the "*most significantly correlated*" partner of endogenous HTT protein, and binds HTT in a stochastic 1:1 molar ratio. Recently, HAP40 was found to stabilize the structure of HTT, converting it from a conformational heterogeneity status to a well-defined globular structure. Importantly, in both primary fibroblasts and striatal tissues, a ~10-fold increase of the levels of endogenous HAP40 were observed in samples from HD patients as compared to healthy controls, implicating a pathogenic role in HD. Despite these strong biochemical and structural evidence, there is no reported functional evaluation of HAP40 in any physiological setting, its role on HTT functions, mutant HTT toxicity and HD pathogenesis remains unclear.

In a proteomic study for the HTT homolog (**dHtt**) in *Drosophila*, we isolated a novel ~40Kda protein as the strongest binding partner for endogenous dHtt. This 40Kda protein has significant sequence homology with mammalian HAP40 and is renamed as **dHap40 (***Drosophila*Hap40**)**. The co-evolution of Hap40 and HTT in evolutionarily distant species from flies to humans not only supports the functional importance of HAP40 in HTT regulation, but also establishes *Drosophila*as a relevant genetic model to evaluate the physiological and pathological roles of HAP40. To this end, we have created several *dhap40*knockout mutants and also transgenic flies for dHap40 overexpression, and currently are characterizing the resulting phenotypes. Given the complex pathogenic mechanisms underlying HD, a clear understanding of central HTT regulators such as HAP40 is essential for "HTT-lowering" and other novel therapeutic strategies against HD.

28 Phagocytic glia mediate prion-like spreading of mutant huntingtin aggregates in Drosophila brains. K.M. Donnelly[1], W. Thu[1], G.E. Pisano[1], L. Luo[2,3], R.R. Kopito[2], *M.M.P. Pearce*[1] 1) Department of Biological Sciences, University of the Sciences, Philadelphia, PA; 2) Department of Biology, Stanford University, Stanford, CA; 3) Howard Hughes Medical Institute, Stanford University, Stanford, CA, USA.

A key pathological feature of neurodegenerative disease is the accumulation of misfolded proteins into insoluble aggregates, which appear as proteinaceous deposits in or near dying neurons. Accumulating evidence supports the hypothesis that pathogenic protein aggregates associated with many neurodegenerative diseases behave similarly to infectious prions--they spread between cells and nucleate the aggregation of natively-folded versions of the same protein. We have recently developed a *Drosophila* model of Huntington's disease that demonstrates "prion-like" spreading of mutant huntingtin (Htt) aggregates in an intact central nervous system (CNS). Mutant Htt aggregates generated in olfactory receptor neurons (ORNs) nucleate the aggregation of cytoplasmic wild-type Htt expressed in post-synaptic partner projection neurons (PNs) or in nearby glial cells. Remarkably, ORN-to-PN and ORN-to-glia transfer of mutant Htt aggregates requires the glial scavenger receptor Draper and is blocked by expressing anti-apoptotic proteins in ORNs. These findings suggest that Draper-dependent phagocytic glia recognize apoptotic "eat me" signals on aggregate-containing ORN axons, leading to their engulfment. However, a portion of the phagocytosed mutant Htt aggregates escape from the phagolysosomal system to seed aggregation of wild-type Htt in the glial cytoplasm. In addition, trans-synaptic (ORN-to-PN) transfer of mutant Htt aggregates is enhanced by silencing ORNs through inhibition of shibire/dynamin or SNARE-mediated synaptic vesicle fusion. Together, our findings suggest a central role for Draper-dependent phagocytosis and impaired exo/endocytic processes in the prion-like spreading of mutant Htt aggregates between synaptically-connected neurons and phagocytic glia in the fly CNS.

29 A Course-based Undergraduate Research Experience to investigate the neuronal subtype specificity of iPLA$_2$-beta function. R. Delventhal[1], M. Avracen[2], A. Burg[2], B. Chernigoff[2], E. Fogel[2], J. Friedman[2], M. Haller[2], I. Leventer[2], E. Levy[2], Y. Malkiel[2], D. Mamet[2], Z. Narrowe[2], E. Shamsian[2], B. Shulman[2], J. Stiefel[2], L. Wiener[2], *J. Steinhauer*[2] 1) Department of Genetics & Development, Columbia University Irving Medical Center, New York, NY, USA; 2) Department of Biology, Yeshiva College, New York, NY, USA.

Phospholipid homeostasis is critical for proper nervous system development and maintenance, and human mutations in the phospholipid acyl chain remodeling enzyme iPLA$_2$-beta (Group 6 PLA$_2$) are associated with several neurodegenerative disorders. Null *Drosophila* mutants for *iPLA$_2$-beta* (*CG6718*) have reduced lifespan and develop severe locomotor defects with age, which can be phenocopied with pan-neuronal RNAi knockdown using *elav-GAL4*. In order to investigate whether specific subsets of neurons underlie iPLA$_2$-associated neurodegeneration and associated phenotypes, we performed RNAi knockdown of *iPLA$_2$-beta* using the following neurotransmitter specific *GAL4* lines: *ple-GAL4* (dopaminergic), *VGlut[OK371]-GAL4* (glutaminergic), *ChAT[7.4]-GAL4* (cholinergic), and *tdc2-GAL4* (octopaminergic and tyraminergic), as well as *Dilp2-GAL4* to target insulin producing median neurosecretory cells. The experiment was performed as a Course-based Undergraduate Research Experience (CURE) in the Yeshiva University undergraduate Genetics course laboratory. After learning basic *Drosophila* husbandry, each pair of students established crosses between a single *GAL4* line and the *UAS*-RNAi flies, collected and aged F1 knockdown progeny, and evaluated F1 locomotor activity using climbing assays. At the end of the term, students presented their results both orally and in written lab reports. Pre- and post-CURE surveys, adapted from those published by D. Lopatto, were used to assess student attitudes toward scientific research and experimentation. This CURE, the first at Yeshiva University, has the potential to engage a large number of students in novel research, is aligned with current efforts to remodel undergraduate STEM education around active learning strategies, and exposes students to the use of *Drosophila* as a model for human neurodegenerative disease.

30 Using Theatre to Teach and Learn Biology: an Interdisciplinary Experiment in Science Communication. *Z. Payne*, A. Sodeinde, A. Arsham, The Performance-Enhanced Biology Collective Biology Baccalaureate Partnership, Bemidji State University, Brooklyn Park, MN.

Communicating with non-specialist audiences is an important aspect of both science and theater. Although superficially different, these two disciplines share core intellectual and expressive tools: curiosity, collaboration and teamwork, communication, specificity, creativity, and risk. Performance-Enhanced Biology is an interdisciplinary collaboration that creates connections between first-year community college students and graduating university biology majors, develops the scientific acumen of the former, and broadens the expressive palette of the latter. Over the course of 8 weeks, collaborating faculty Kathy Hendrickson (Theatre, North Hennepin Community College) and Andy Arsham (Biology, Bemidji State University) merged their intro-level Acting 1 and senior capstone Gene Expression courses once a week for 80 minutes. Students in Gene Expression are seniors who have completed multiple upper-division genetics electives and participate in a semester-long Drosophila CURE. The merged group of approximately 40 students were coached in ensemble theater and storytelling techniques. Students collaborated in mixed groups to clearly and effectively translate scientific theories into performance using the tools of the performing artist: rhythm, gesture, voice, poetic movement, and the creation of images. By workshopping and rehearsing in a classroom, a black box theatre, a traditional proscenium theatre, and a *Drosophila* lab, students explored the idea that environment changes expression, in theatre as in genetics. After seven weeks, final performances were shared on the theater stage with the campus community. Biology students demonstrated improvement in communicating complex biological concepts to the general public by integrating multiple performing arts techniques, and theatre students expressed

changed attitudes towards science and scientists. Student feedback indicated that each group began the collaboration with fear and apprehension of the techniques and physical environments of the other and that co-creating the work led to a mutual demystification, accessibility, and respect.

31 iCURE: Interdisciplinary Course-based Undergraduate Research Experiences for all. *J. Hackney*[1], P. Marshall[1], J. Broatch[1], J. Foltz-Sweat[1], K. Sweat[1], A. Falsetti[2] 1) School of Mathematical and Natural Sciences, Arizona State University, Glendale, AZ; 2) College of Science, George Mason University, Fairfax, VA.

Science writ large is about doing (asking questions, carrying out experiments, analyzing data, solving problems, and designing solutions) not memorizing a series of isolated facts. STEM (science, technology, engineering, and mathematics) by its very nature is interdisciplinary with scientists from many fields coming together to solve problems large and small. Contrary to many other disciplines, however, students majoring in science disciplines often do not participate in the central activities of the careers of the disciplines. Most students do not engage in authentic experiences in which "they think like a scientist", such as asking questions, designing experiments, testing hypotheses, carrying out literature searches to develop testable hypotheses, generating and analyzing data, repeating experiments, making observations, and presenting their work. We have developed high impact interdisciplinary CUREs (iCUREs) that enroll students from not just our biology majors, but from math, computing, and statistics majors as well. Furthermore, we have created a framework of a common course outline, with concomitant curriculum, activities, and assessments that can be used by any instructor who wishes to develop and teach a CURE. I will describe our team and process, share the topics of our iCUREs including a module about *Drosophila* development, delineate some of the materials we have designed, and describe preliminary assessment of these iCUREs

32 The Genomics Education Partnership: A community of practice that enhances research opportunities for students and faculty at diverse institutions. *M. Manier*[1], A. Arsham[2], M. Burg[3], C. Jones[4], J. DiAngelo[5], J. Jemc[6], L. Kadlec[7], J. Kennell[8], J. Leatherman[9], H. Mistry[10], A. Nagengast[10], C. Reinke[11], C. Small[12], J. Stamm[13], N. Valesquez Ulloa[14], S. Elgin[15], L. Reed[16], Genomics Education Partnership 1) Dept. of Biological Sciences, George Washington University, Washington, DC; 2) Dept. of Biology, Bemidji State University, Bemidji, MN; 3) Depts. of Biomedical Sciences and Cell & Molecular Biology, Grand Valley State University, Allendale, MI; 4) Dept. of Biological Sciences, Moravian College, Bethlehem, PA; 5) Division of Science, Penn State Berks, Reading, PA; 6) Dept. of Biology, Loyola University, Chicago, IL; 7) Dept. of Biology, Wilkes University, Wilkes-Barre, PA ; 8) Dept. of Biology, Vassar College, Poughkeepsie, NY ; 9) School of Biological Sciences, University of Northern Colorado, Greely, CO ; 10) Dept. of Biology, Widener University, Chester, PA ; 11) Dept. of Biology, Linfield College, McMinnville, OR ; 12) Medgar Evers College,The City University of New York, NY; 13) Dept. of Biology, University of Evansville, IN ; 14) Biology Department, Lewis & Clark College, Portland, OR; 15) Dept. of Biology, Washington University in St Louis, MO; 16) Dept. of Biology, University of Alabama, Tuscaloosa, AL.

Since 2006, the Genomics Education Partnership (GEP) has incorporated authentic genomics research experiences into the undergraduate curriculum, introducing thousands of students to eukaryotic gene structure, comparative genomics, and the evolution of the Drosophila Muller F element. Our 100+ participating institutions include community colleges, primarily undergraduate institutions, minority-serving institutions, historically black colleges and universities, and research-intensive PhD-granting institutions. For many faculty and their students, the accessible and immersive curriculum and custom bioinformatics tools provided by the GEP represent a unique chance to participate in research. GEP faculty benefit from membership in a national network of like-minded colleagues and professional development opportunities that include training, research, and publication in peer-reviewed journals. Students who resolve sequencing/assembly problems and generate high-quality gene models for GEP analyses are eligible to be co-authors on the resulting scientific publications based on their contributions. GEP has partnered with Galaxy to develop G-OnRamp, an open-source platform for constructing UCSC Assembly Hubs and JBrowse/Apollo genome browsers for eukaryotic genomes, thereby enabling crowd-sourced gene annotation using the GEP curriculum. G-OnRamp allows for more varied research projects to be incorporated into the GEP science portfolio, including a current investigation of venom evolution in parasitoid wasps. There is also a new research project investigating the evolution of insulin pathway genes across 27 Drosophila genomes. Our ongoing work in science education finds that a bioinformatics CURE fosters experiences of "formative frustration" in which students can safely fail in their original analysis, adjust, recover, and succeed. This iterative process allows students to gain deeper insight into annotation and can occur fairly quickly within an inexpensive, online framework. The GEP is actively recruiting additional faculty members to use the GEP curriculum in their classrooms, science partners who can collaborate with GEP members to fund and develop additional projects, and science education partners to assist with curriculum development and assessments. Supported by NSF IUSE-1431407 to SCRE.

33 Mechanics of Asymmetric Cell Division. *Tri Pham*[1], Arnaud Monnard[1,2], Jonne Helenius[3], Nicole Lee[1], Erik Lund[1], Daniel Mueller[3], Clemens Cabernard[1] 1) Biology, University of Washington, Seattle, WA; 2) Biozentrum, University of Basel, SWITZERLAND; 3) D-BSSE, ETH Zürich, SWITZERLAND.

Asymmetric cell division (ACD) generates cellular diversity and is an important process during development. Stem cells in particular utilize ACD in order to self-renew the stem cell yet generate differentiating siblings. Some stem cells undergo both physical and molecular ACD and it is unknown how biophysical parameters, such as cortical tension, stiffness or osmotic pressure affect the formation of sibling cell size asymmetry. We use *Drosophila* neural stem cells (neuroblasts) to study the contribution of biophysical parameters on ACD. We are combining fluorescence microscopy with atomic force microscopy, particle image velocimetry (PIV) and genetically encoded tension sensors to elucidate the biophysical forces involved in the establishment of physical asymmetry. We show that the force driving initial apical cortical expansion is provided by hydrostatic pressure, peaking shortly after anaphase onset, and enabled by a relieve of actomyosin contraction on the apical cell cortex. The subsequent increase in contractile tension at the cleavage furrow, combined with the relocalization of basally located Myosin results in basal membrane extension and sustained apical expansion. We propose that spatiotemporally controlled actomyosin contractile tension and hydrostatic pressure enable biased cortical expansion to generate sibling cell size asymmetry.

34 FIP is a novel Chromosomal Passenger Protein that Regulates Fascetto (PRC1) to Ensure Proper Cytokinesis and Ploidy. *R. Ng*[1], Z. Swider[2], R. Varadarajan[1], C. Fagerstrom[1], N. Rusan[1] 1) National Heart, Lung, and Blood Institute, NIH, Bethesda, MD; 2) University of Wisconsin, Madison, WI.

Mitosis is a fundamental process required for cell proliferation and development. The final stages of mitosis include the segregation of the duplicated genome in anaphase and the physical separation of daughter cells by cytokinesis. Defects in either of these late mitotic stages have profound implications for human health such as tumorigenesis and birth defects associated with aneuploidy. Both chromosome segregation and cytokinesis rely, in part, on intricate microtubule (MT)-based structures, which are organized by MT-associated proteins (MAPs), molecular motors, and other regulatory elements. One critical MAP, Fascetto (Feo), crosslinks MTs in anaphase to form the central spindle and localizes key protein complexes such as the Chromosomal Passenger Complex and Centralspindlin for anaphase progression. Feo localization in telophase is also required for midbody formation and proper cell abscission. We identified Septin-Interacting Protein-2 (SIP-2) as a novel Feo-Interacting Protein, and therefore renamed SIP-2 to FIP. We used yeast two-hybrid analysis and co-immunoprecipitation to show that the FIP-Feo interaction is direct and exclusively occurs in mitosis. Furthermore, high resolution live cell imaging revealed that FIP and Feo colocalize dynamically from the central spindle in anaphase to the midbody in telophase and that FIP functions upstream of Feo. Loss of function analysis of *fip-* animals revealed chromosome segregation errors, disorganized midbodies, unstable microtubules, and cytokinesis failures. These defects resulted in highly polyploid neuroblasts in the *Drosophila* larval brain. Codepletion of Feo in the *fip-* mutants increased the number and severity of polyploid neuroblasts, ultimately leading to tumor-like masses. Conversely, overexpression of Feo in the *fip-* mutants rescued the polyploid neuroblast phenotype, further indicating a genetic interaction between the two proteins. Thus, we have identified a novel mechanism that relies on the FIP-Feo interaction for regulating late-stage cell division and ploidy.

35 Neuronal ribosomal protein function regulates *Drosophila* growth and development. *L.Patricia. Deliu*[1,2,3,4], Abhishek Ghosh[1,2,3,4], Savraj Grewal[1,2,3,4] 1) Clark H Smith Brain Tumour Centre, Alberta, CA; 2) Arnie Charbonneau Cancer Institute, Calgary, Alberta, CA; 3) Alberta Children's Hospital Research Institute, Calgary, Alberta, CA; 4) Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta, CA.

Stimulation of ribosome biogenesis is a conserved mechanism of growth control. Studies, mostly in yeast and cell culture, have shown how ribosome synthesis controls cell growth. However, less is know about how ribosome synthesis promotes body growth and development. We have been investigating this issue by studying the *Minutes*. These are a class of dominant ribosomal protein (*rp/+)* mutants that exhibit a characteristic delay in larval development. This phenotype is classically thought to be due to an overall reduction in ribosome numbers and protein synthesis. However, when we examined three *Minutes* (*rpS13/+, rpS26/+* and *rpL38/+*) we saw little or no change in either global ribosome numbers or in protein synthesis rates, when compared to wild-type controls. Instead, as discussed below, we found evidence of a cell type-specific function for one RP (S13) in the control of development.
Termination of the larval period is controlled by a neuroendocrine circuit that leads to a pulse of secretion of the steroid hormone ecdysone from the prothoracic gland (PG) in response to signals from specific CNS neurons. We found that *rpS13/+* animals had a delayed ecdysone pulse as seen by delayed expression of the 'Halloween' genes, *spooky* and *phantom*, which are required for ecdysone synthesis in the PG. We postulated that these effects might reflect a specific role for S13 in the CNS-PG neuroendocrine circuit. To test this we used the GAL4/UAS system to see if tissue selective expression of S13 could rescue the delayed development seen in *rps13/+* animals. Expression of S13 in the PG had no effect. However we found that expression of S13 in neurons (using either elav-Gal4, or nSyb-Gal4) could rescue the delay in development in *rpS13/+* animals by ~40%. Furthermore, we discovered that expression of S13 in serotonergic neurons alone (TRH-Gal4) lead to the same rescue in developmental timing as pan-neuronal S13 expression. Three pairs of 5-HT innervate the PG to control ecdysone release, and S13 expression in these neurons (using the R29H01-GAL4) driver also partially rescued the delay in development in *rpS13/+* animals. These findings suggest that the overall developmental delay seen in one *Minute* reflects a selective neuronal ribosome function in a neuroendocrine circuit that controls systemic steroid hormone signaling.

36 Headcase regulates tissue growth and cell cycle progression in response to nutrient restriction. Naren Li, Qinfang Liu, Yulan Xiong, *Jianzhong Yu* Department of Anatomy & Physiology, Kansas State University College of Veterinary Medicine, Manhattan, KS.

Nutrient restriction (NR) decreases the incidence and growth of many types of tumors. To date, the anti-tumorigenic effects of NR have been well established and its potential implications in both cancer prevention and treatment have been suggested. Despite these advances, our understanding of the molecular mechanisms underlying the anti-tumorigenic effects of NR remain fragmented. It has been shown that cells with PI3K activation are resistant to NR in both mammalian and *Drosophila* tumor models. And TORC1 activation has been shown to play a key role in NR resistance of PTEN or TSC-null tissues in *Drosophila*. Unexpectedly, recent studies revealed that the *in vivo* TORC1 activity, detected by S6 phosphorylation, is selectively increased in S-phase cells and is necessary for proper cell cycle progression in *Drosophila*. The functional relevance of this spatially regulated TORC1 activation on insulin/TORC1 mediated NR resistance is unclear.
Here we identified Headcase (Hdc) as a tumor suppressor that regulates tissue growth in response to NR. We found that *hdc* mutant cells do not show apparent growth advantage under normal nutrient conditions but proliferate much faster than wildtype cells under NR. Our results suggested that Hdc regulates tissue growth through its regulation on cell cycle progression. We further found that Hdc binds to Unkempt (Unk) and forms a protein complex with TORC1 component Raptor. Unk was first identified as a TORC1 binding protein in a systematic protein interaction study in *Drosophila* Kc167 cells. Both Hdc and Unk were later reported to regulate neuronal cell differentiation downstream of mTOR pathway, but surprisingly, not tissue growth nor any known TORC1 downstream targets. Here we demonstrated that both Hdc and Unk spatially regulate TORC1 target S6 phosphorylation *in vivo*. Taken together, our study suggests a functional link between Hdc/Unk and insulin/TORC1 signaling on cell cycle progression and NR resistance.

37 Single cell RNA-sequencing reveals a metabolic aspect of apoptosis in *Rbf* mutant. Majd Ariss[1], Abul Islam[2], Meg Critcher[1], Maria Paula Zappia[1], *Maxim Frolov*[1] 1) Biochem Molec Gen, Univ Illinois Chicago, Chicago, IL; 2) Department of Genetic Engineering and Biotechnology, University of Dhaka, Dhaka 1000, Bangladesh.

The function of Retinoblastoma tumor suppressor (pRB) is greatly influenced is by the cellular context and therefore the consequences of pRB inactivation are cell-type-specific. However, conventional methods for profiling of the mutant tissue often characterize it at the population level. Single-cell RNA-seq platforms have been recently developed to overcome this hurdle. In our laboratory, we adopted Drop-seq to analyze transcriptional profiles of thousands of individual cells simultaneously. Here, we employed Drop-seq to profile the impact of a mutation in the *Retinoblastoma* gene (*Rbf*) during *Drosophila* eye development. First, we built a cell atlas of wild type larval eye disc with 1x cellular coverage that identifies major cell types and reveals a novel transcriptional switch during photoreceptor differentiation at the time of axonogenesis. Next, we utilized this resource to examine the *Rbf* mutant phenotype and identified a small, mutant-specific cell population that shows intracellular acidification due to increase in glycolytic activity. Genetic experiments demonstrate that such metabolic changes, restricted to this unique *Rbf* mutant population, sensitize cells to apoptosis and define the pattern of cell death in *Rbf* mutant eye disc. Thus, these results illustrate how scRNA-seq can be applied to dissect mutant phenotypes.

38 Investigation of intratumor heterogeneity in a Drosophila tumor model through single-cell transcriptomic analysis. Tiantian Ji[1,2], Lina Zhang[1,2], Shengshuo Huang[1,3], Mingxi Deng[1,2], Ying Wang[1,2], Jiguang Wang[1,2,3], *Yan Yan*[1,2] 1) Division of Life Science, HKUST, Kowloon, Hong Kong, China; 2) Center of Systems Biology and Human Health, HKUST, Kowloon, Hong Kong, China; 3) Department of Chemical and Biological Engineering, HKUST, Kowloon, Hong Kong, China.

Loss of cell polarity has been linked with disruption of proliferation control. The members of the Scribble (Scrib) cell polarity complex were originally discovered in Drosophila as "neoplastic tumor suppressor genes" (nTSGs). Drosophila larvae homozygous mutant for any of the nTSGs grow into giant larvae with tumorous imaginal discs and optic lobes. These mutant tumors fail to differentiate and grow into masses that survive serial transplantations and kill the hosts. Interestingly, while the *scrib* mutant tumors have successfully modeled many aspects of human epithelial cancers over the past decades, it was noted that for the *scrib* mutant tumors that progress rapidly over days, a single gene mutation is sufficient to cause tumorigenesis, contrary to human tumors which display genetic and epigenetic intratumor heterogeneity that fuels evolution. Whether the rapid-developing fly *scrib* tumors also exhibit a certain degree of intratumor heterogeneity and evolution capacity has remained unclear.
We have found that the *scrib* mutant tumors display changes in growth rates, cell cycle profiles and multiple cell signaling signatures, indicative of an arrest-to-proliferation transition over time. Through longitudinal single-cell transcriptomic analysis we have also found that the *scrib* mutant tumors harbor heterogeneous cell populations that potentially provide a ground for selection. The genetically-accessible fly tumor experimental model allows us to systematically dissect the sources of intra-tumor heterogeneity, to establish markers and track the proliferation of heterogeneous cell populations and to explore how cells of distinct proliferative states can be defined by a combinatory code of multiple growth-regulatory signaling activities.

39 Cell-type specific patterned stimulus-independent neuronal activity in the *Drosophila* visual system during synapse formation. *O. Akin*[3], B.T. Bajar[1], M.F. Keles[2], M.A. Frye[2], S.L. Zipursky[1] 1) Department of Biological Chemistry, Howard Hughes Medical Institute, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA ; 2) Department of Integrative Biology and Physiology, University of California, Los Angeles, Los Angeles,

CA ; 3) Department of Neurobiology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA.

Stereotyped synaptic connections define the neural circuits of the brain. In vertebrates, stimulus-independent activity contributes to neural circuit formation. It is unknown whether this type of activity is a general feature of nervous system development. Here, we report patterned, stimulus-independent neural activity in the *Drosophila* visual system during synaptogenesis. Using *in vivo* calcium, voltage, and glutamate imaging, we found that all neurons participate in this spontaneous activity, which is characterized by brain-wide periodic active and silent phases. Glia are active in a complementary pattern. Each of the 15 examined of the over 100 specific neuron types in the fly visual system exhibited a unique activity signature. The activity of neurons that are synaptic partners in the adult was highly correlated during development. We propose that this cell type-specific activity coordinates the development of the functional circuitry of the adult brain.

40 Postsynaptic differentiation controlled by a specific Pix isoform mediates scaling growth of the neuromuscular junction. *Cheuk Hei Ho*, Jessica Treisman Skirball Institute of Biomolecular Medicine , NYU School of Medicine, New York, NY.

The peripheral nervous system undergoes scaling growth, a phenomenon in which dendritic receptive fields and axonal arborizations grow in proportion to the size of their target tissues. We have used the *Drosophila* larval neuromuscular junction (NMJ) to study how the target tissue communicates its size to the innervating neuron. When we autonomously changed the growth of larval muscles by modifying the level of Insulin Receptor (InR) signaling, we observed a proportional adjustment of NMJ size. This regulation of NMJ size by muscle growth is a local phenomenon, since changing the growth of a single muscle only affected motor neuron branches that directly formed synapses on that muscle. Scaling growth of the NMJ represents a novel form of synaptic plasticity independent from the known synaptic activity pathway, because scaling growth is still observed in a *mothers against dpp* (*mad)*mutant in which structural change of the NMJ cannot be induced by changes in synaptic activity.

Postsynaptic differentiation plays a critical role in the scaling growth of the NMJ. We found that postsynaptic differentiation was proportional to InR signaling levels in muscle, and removing the postsynaptic scaffolding protein Discs-large (Dlg) abolished scaling growth. We identified Pix (RtGEF), a Rho-type guanine nucleotide exchange factor, as a protein that is necessary both to recruit Dlg and other postsynaptic components, and to enable scaling growth of the NMJ. *Pix* overexpression is also sufficient to induce ectopic postsynaptic differentiation that is not associated with synaptic boutons. The function of Pix in NMJ scaling growth is independent of Git, which acts in a complex with Pix in many other contexts. Of the six different protein-coding isoforms encoded by the *Pix* locus, we found that only isoforms H or F could rescue postsynaptic differentiation, and isoform H was sufficient to induce ectopic postsynaptic differentiation and synaptic growth. Moreover, other isoforms appear to antagonize the function of isoform H in postsynaptic differentiation. These data suggest that the expression or localization of different Pix isoforms plays a critical role in adjusting the growth of the NMJ to match the size of its target muscle.

41 Post-transcriptional regulation by Syncrip/hnRNP Q modulates activity-dependent synaptic plasticity at the larval NMJ. Josh Titlow[1], Francesca Robertson[1], Carlas Smith[2], *David Ish-Horowicz*[3], Ilan Davis[1] 1) Dept. of Biochemistry, University of Oxford, UK; 2) Centre for Neural Circuits and Behaviour, University of Oxford, UK; 3) MRC Lab for Molecular Cell Biology, University College London, UK.

During memory and learning, neuronal activity remodels synaptic connections by elevating the levels of many synaptic proteins. How gene expression is regulated locally during synaptic plasticity remains poorly understood. Here, we show that the conserved RNA binding protein (RBP) Syncrip/hnRNP Q regulates activity-dependent translation and synaptic plasticity at the larval neuromuscular junction (NMJ). A key target of Syncrip is *msp300/nesprin1*, an Actin-binding protein that accumulates around new synaptic boutons and is essential for their formation. We found that *msp300* is regulated post-transcriptionally by Syncrip. Steady-state and activity-induced production of Msp300 protein is significantly decreased in the absence of Syncrip, however *msp300* transcription and cytosolic mRNA levels are unchanged, suggesting that Syncrip regulates *msp300* translation. To determine how Syncrip regulates *msp300* translation we generated a Syncrip-GFP fusion protein and assessed its interaction with *msp300* mRNA using smFISH and super resolution microscopy (Airyscan), and in live NMJ synapses using cross correlation raster imaging correlation spectroscopy. We found that Syncrip protein levels around the synapse increase in response to elevated activity. We also observed that Syncrip forms small, ribosome-containing granules that physically interact with *msp300* mRNA *in vivo.* The mobility of Syncrip granules decreases in response to elevated activity. Together, these findings provide a direct link between an RBP and activity-dependent regulation of a key scaffolding gene during synaptic plasticity, filling an important gap in our understanding of local gene regulation at the synapse.

42 Effects of altered gravity on the central nervous system of *Drosophila melanogaster*. *Siddhita Mhatre*[3,5], Janani Iyer[4], Amber Paul[1,2], Jhony Zavaleta[6,7], Ravikumar Hosamani [3,5], Karen Ocorr[8], Sharmila Bhattacharya[1] 1) Space Biosciences, NASA Ames Research Center, Mountain View, CA; 2) NASA Postdoctoral Program, USRA, Moffett Field, CA; 3) University of New Mexico, FILMSS, Moffett Field, CA; 4) NAMS, USRA, Moffett Field, CA; 5) FILMSS/Wyle, Moffett Field, CA; 6) Blue Marble Space Institute of Science, Moffett Field, CA; 7) San Francisco State University, San Francisco, CA; 8) Sanford Burnham Prebys Medical Discover Institute, La Jolla, CA.

A comprehensive understanding of the effects of spaceflight and altered gravity on human physiology is necessary for continued human space exploration and long-term space habitation. Spaceflight includes multiple factors such as microgravity, hypergravity, ionizing radiation, physiological stress, and disrupted circadian rhythms and these have been shown to contribute to pathophysiological responses that target immunity, bone and muscle integrity, cardiovascular and nervous systems. In terrestrial conditions, some of these factors can lead to cancer and neuroimmunological disorders. In this study, we used a well-established spaceflight model organism, *Drosophila melanogaster,* to assess spaceflight-associated changes in the nervous system. We hypothesize that exposure to altered gravity triggers the oxidative stress response, leading to impairments in the nervous system. To test this hypothesis, we used two experimental paradigms: 1) hypergravity, using the ground-based chronic acceleration model, and 2) spaceflight conditions, which includes exposure to microgravity and in-flight space 1g controls. In our ground studies, acute hypergravity resulted in an induction of oxidative stress-related genes with an increase in reactive oxygen species (ROS) in fly brains. Additionally, we observed a depressed locomotor phenotype in these flies ($p<0.05$). These flies also show a decreased dopaminergic neuron counts in the fly brain upon exposure to acute hypergravity ($p<0.05$). Thus, the data suggest that altered gravity has a profound effect on the fly nervous system. Similarly, we observe behavioral impairments ($p<0.001$) and synaptic deficits, including decreased synaptic connections ($p<0.05$), in 3rd instar larvae which were developed in space. Furthermore, space-grown adults show a decrease in neuronal ($p<0.05$) and dendritic field ($p<0.01$) in adult brains coupled with an increased number of apoptotic cells ($p<0.001$), suggesting increased neuronal loss under spaceflight conditions. In summary, we observe that altered gravity leads to gross neurological deficits. To better understand the long-term effects of spaceflight on the nervous system, longitudinal and multigenerational changes were also identified. This study will help identify targets for countermeasures to prevent nervous system dysfunction in astronauts during spaceflight, while also contributing to a better understanding of the pathways that are related to some CNS disorders on Earth.

43 Hereditary Spastic Paraplegia proteins model a continuous dynamic network of ER tubules in Drosophila motor neurons. Lu Zhao[1], Belgin Yalç?n[1], Anood Sohail[1], Ayesha Riaz[1], Juanjo Perez Moreno[1], Megan Oliva[1], Zeynep Öztürk[1], Valentina Baena[2], Mark Terasaki[2], *Cahir J O'Kane*[1] 1) Department of Genetics, University of Cambridge, Cambridge, GB; 2) UConn Health Center, Farmington, CT.

In neurons, ER continuity throughout axons, dendrites and cell bodies potentially allows it to be a channel for long-distance communication, independent of

action potentials or microtubule transport, comparable to a "neuron within a neuron". We want to understand the mechanisms that set up and maintain the unbroken architecture of ER over distances that are massive on a subcellular scale, as well as the functional roles of ER over such distances.

Axonal ER comprises a network of mainly smooth and tubular ER. Several proteins with intramembrane hairpin domains that model ER membranes, of the spastin, atlastin, REEP and reticulon families, are candidates to establish and maintain this network, since mutations affecting them cause an axon degenerative disease, hereditary spastic paraplegia (HSP). To test the roles of these proteins, we identified new markers for axonal ER, and then showed that loss of *Drosophila*REEP or reticulon proteins can cause partial loss of ER from distal axons, and that loss of members of both families leads to occasional discontinuities in axonal ER. Live imaging and FRAP also show a continuous network of axonal ER that is interrupted in reticulon and REEP multiply mutant larvae, and in addition reveal dynamic features of the network. Serial reconstruction of EM sections revealed larger and fewer tubules, and an ER network with sporadic gaps in continuity, in larvae that lack reticulon and REEP proteins, consistent with loss of membrane curvature.

We hypothesise that (1) the occurrence of occasional gaps in HSP mutants provides a potential explanation for susceptibility of longer axons for degeneration; (2) additional HSP proteins may contribute to forming axonal ER tubules; (3) homeostatic mechanisms both maintain sufficient dynamic ER tubules in axons to avoid gaps in the network, and prevent accumulation of excess local ER.

We are now testing the roles of additional HSP proteins in organising the axonal ER network, and the effects of altered ER architecture on axonal and presynaptic cell physiology.

44 Hox miRNAs: tuning behavior to gene regulation. *Daniel L. Garaulet*[1], Binglong Zhang[1], Elena Li[1], Roumen Voutev[2], Richard S. Mann[2], Eric C. Lai[1] 1) Memorial Sloan-Kettering Cancer Center, New York City, NY; 2) Columbia University, New York City, NY.

Whether to sleep when tired, to drink when thirsty, or to eat when hungry, reproductive and survival success depend on the ability to adjust behavior to internal state. The nervous system is crucial to this process, integrating external and internal inputs to orchestrate appropriate behaviors. However, much remains to be learned about the genetic control of these adaptive processes.

Using a combination of genetics, genomic engineering and transcriptome profiling, we have approached this question in fly virgins. Unlike mated females, the genetic factors that determine virgin behavior are unknown, commonly understood as the default state. Surprisingly, we found that deletion of the Hox miRNA locus, *mir-iab-4/mir-iab-8*, results in selective loss of virgin behaviors and a gain of mated conducts. This inversion in behavior, from virgin to a post-mated like state, occurs in the absence of copulation and delivery of seminal proteins and indicates that *mir-iab-4/8* are crucial to tune behavior to internal state in virgin flies. Furthermore, we can restore virgin behavior in mutant females by increasing activity in specific clusters of abdominal neurons, suggesting a role for miRNA regulation in the homeostasis of the post-mating circuit.

Genetic and immunohistochemical evidence indicate that miR-iab-4/8 repress multiple homeotic TFs in abdominal neurons: *Ultrabithorax*, *abdominal-A* and distinct isoforms of *homothorax* (*hth*). We used *CRISPR/Cas9* to surgically eliminate *mir-iab-4/8* regulation from this entire network, by mutating >50 conserved binding sites of the 3'UTRs of these Hox factors. This allows us to explore the effects of loss of miRNA regulation from single or combinations of targets, and compare them to the loss of the miRNA per se. Interestingy, we find that deletion of binding sites in the *hth-HD* 3'UTR isoform is sufficient to recapitulate the defects observed in virgin fly behavior, indicating that an individual target can account for substantial aspects of miRNA function in vivo. Lastly, we are in the process of profiling the transcriptome of *mir-iab-4/8* neurons in wild type and mutant females, and also in the *hth-HD 3'UTR* binding sites KO's. With these data, we will compare the global consequences of miRNA regulation in neurons with phenocritical events downstream of a single miRNA target like *hth*.

Altogether, our work provides an integrated picture of the role of post-transcriptional gene regulation in the context of animal behavior: from expression of a miRNA, its physical interaction with 3'UTRs in an entire gene network, its impact in the transcriptional landscape of neurons, and the ultimate consequences of this in neuronal physiology and fly behavior.

45 Continued activity of the pioneer factor Zelda is required to drive zygotic genome activation. *T. Gibson*, S. McDaniel, K. Schulz, M. Nevil, S. Jain, P. Lewis, M. Harrison Department of Biomolecular Chemistry, University of Wisconsin School of Medicine and Public Health, Madison WI.

During the initial stages of metazoan development, the sperm and the egg must transition from the differentiated germ-cell state to the totipotent state of the early embryo. In the early embryo, there is no transcription from the zygotic genome, and development is controlled by maternal mRNAs and proteins deposited into the egg prior to fertilization. Zygotic genome activation (ZGA) is driven by transcription factors that must gain access to the genome and remodel the zygotic transcriptome. ZGA is a gradual process with an initial minor wave of transcription followed by a dramatic increase in transcriptional activity later (major wave). In Drosophila, the transcription factor Zelda is required for ZGA. Prior to the major wave of ZGA, Zelda is already bound to the regulatory regions of thousands of genes and marks these genes for subsequent activation. Nonetheless, it remained unclear whether Zelda activity is required throughout ZGA or whether it is only required early to establish *cis*-regulatory regions. To address this question, we introduced the optogenetic CRY2 tag on the endogenously encoded Zelda protein and demonstrated that this allowed us to rapidly inactivate Zelda upon exposure to blue light. Blue-light mediated inactivation of Zelda coupled with single-embryo RNA-sequencing revealed that continuous Zelda activity is required for transcriptional activation of zygotic genes. Furthermore, Zelda activity during only the major wave of ZGA was sufficient to drive transcription of hundreds of genes. Together this demonstrated that continued Zelda activity was required throughout both the minor and major waves of ZGA. Because early development is characterized by a series of rapid mitotic divisions, this indicated that Zelda must quickly regain access to its target sites after mitosis. We demonstrated that Zelda bound to nucleosomal targets *in vitro*, suggesting a potential mechanism allowing Zelda to rapidly reoccupy the genome after it is evicted during mitosis. We propose that pioneering reprogramming factors, like Zelda, require nucleosome binding to enable them to continuously access the genome even during rapid division cycles.

46 Promoter-specific histone methylation and post-transcriptional regulation of the *foraging* gene modulate food-associated behavior in *Drosophila*. *Ina Anreiter*[1,2], Zixuan Xiao[1], Jamie Kramer[3,4], Marla Sokolowski[1,2] 1) Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario, Canada; 2) Child and Brain Development Program, Canadian Institute for Advanced Research, Toronto, Ontario, Canada; 3) Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada; 4) Department of Biology, Faculty of Science, Western University, London, Ontario, Canada.

Individuals of the same species often display remarkable variation in behavior even in identical contexts, but the molecular mechanisms that underlie this variation are still poorly understood. We show that the interaction of two genes, *foraging* (a protein kinase) and *G9a* (a histone methyl transferase) mediates strain differences in feeding behavior and metabolism in *Drosophila*. The *for* gene encodes a cGMP-dependent protein kinase that is involved in regulating

many independent phenotypes, likely through the spatially and temporally restricted expression pattern of it's 4 promoters and 21 different transcripts. We found that adult feeding behavior differences in the rover and sitter variants of the *for* gene are mediated by allele-specific histone methylation of *for's* promoter 4 (pr4) by *G9a*. Our results show that rovers have higher levels of pr4 H3K9me, and lower levels of pr4 expression than sitters. This expression pattern underlies rover-sitter differences in adult foraging behavior, as rover-sitter differences can be transgenically abolished by reducing pr4 expression in sitters. The regulation of feeding behavior by G9a-mediated methylation of *for's* pr4 is tissue specific, and other *for*-related phenotypes are not regulated by this interaction. We also found a novel role for the RNA-binding protein Pumilio in regulating *for* and it's associated larval phenotypes, independently of G9a. Our findings provide insight into the molecular mechanisms underlying the pleiotropic functions of the *for* gene, and the regulation of behavioral variability.

47 Investigating cis-regulatory evolution in *Drosophila*: Learning the rules of regulatory logic. *A. D Buffry*[1], J Ling[2], S Small [2], T Gregor[3], A. P McGregor[1] 1) Department of Health and Life Sciences, Oxford Brookes University, Oxford, GB; 2) Department of Biology, New York University, NY; 3) Department of Physics, Lewis-Sigler Institute for Integrative Genomics, Princeton University, NJ.

Enhancers are cis-regulatory elements of DNA that contain binding sites for specific transcription factors. They play a crucial role during development by facilitating precise spatial and temporal patterns of gene expression. It is generally accepted that mutations in cis-regulatory DNA is one of the principal mechanisms underlying phenotypic evolution. However, enhancer sequences can also turnover while maintaining the same output, which can lead to developmental systems drift. The aim of this project is to better understand how enhancer sequences evolve. To do this we are studying the consequences of sequence turnover in the Bcd-dependent P2 enhancer of *hunchback* within and between *Drosophila* species. This enhancer is known to have evolved in sequence but still maintains its function in anterior patterning in the embryo. We surveyed natural variation in number, spacing and orientation of known binding sites in the P2 enhancer. We then tested the functional equivalence of enhancers from different species as well as synthetic enhancers in *Drosophila melanogaster,* through direct replacement of the endogenous P2. We have found that the P2 enhancer from *D. pseudoobscura*and *D. yakuba* is functionally equivalent to that from *D. melanogaster* consistent with previous results with *D. virilis*. We are currently using the MS2-MCP system to measure the spatial, temporal and quantitative expression driven by enhancer variants in live embryos. The data collected from these experiments will improve the design of synthetic enhancers and mathematical modelling of enhancer function and evolution. Overall our study will greatly improve our understanding of the functionality and evolution of cis-regulatory sequences and their impact on gene regulation during development.

48 A novel tudor-domain protein promotes germline differentiation through post-transcriptional gene regulation in cytoplasmic RNA granules. *C. Pozmanter*[1], S. Primus[1], H. Curnutte[1,2], M Van Doren [1] 1) Department of Biology, Johns Hopkins University, Baltimore, MD; 2) Department of Biology, McDaniel College, Westminster, MD.

Tudor-domain containing proteins are conserved across the animal kingdom for their necessary functions in germline development including post-transcriptional gene regulation. Recent work in our lab identified the previously uncharacterized tudor protein, Tudor5-prime (Tdrd5p), which promotes male germline identity in germline stem cells (GSCs) in the testis, but is repressed by the RNA binding protein Sex lethal (SXL) in female GSCs. Interesting, Tdrd5p is also expressed in the differentiating germline in both sexes, indicating that it may also act to control germline differentiation in both sexes.
Previously we reported that Tdrd5p localizes to the periphery of a previously uncharacterized germline RNA granule in both sexes. In the males, numerous smaller Decapping protein 1 (Dcp1) granules co-localize with the periphery of the Tdrd5p granule. This suggests that Tdrd5p granules are docking with processing bodies, and could play a role in post-transcriptional gene regulation. Additionally a subset of Tdrd5p granules of smaller size co-localize with Survival Motor Neuron (SMN), which is a hallmark of the U-body. This data suggests that in addition to a role in post-transcriptional gene regulation, Tdrd5p might function in RNA processing. To understand what RNA regulatory pathway Tdrd5p functions in we conducted RNAi against the deadenylase *twin* in mutant gonads, which revealed a genetic interaction between *tdrd5p* and the CCR4-NOT deadenylation complex. In both males and females, knockdown of *twin* in *tdrd5p* mutants results in sterility. Additionally, we found similar genetic interactions between *tdrd5p* and both *dcp1* and *gawky(gw)* further suggesting a role for *tdrd5p* in post-transcriptional gene regulation. Recent investigation into the role Tdrd5p plays in the female germline suggests that Tdrd5p could function in maternal RNA deposition. Interestingly, Tdrd5p localizes to a single large granule at the anterior of the oocyte directly adjacent to the ring canals. To further investigate this possibility we are testing for the mis-regulation of maternally deposited RNAs such as *gurken, bicoid, and nanos.* Taken together, our data suggests TDRD5P functions to ensure the proper development of the germline, however the mechanism by which it regulates differentiation is different between males and females.

49 *The contributions of optimal and suboptimal Bcd and Otd DNA binding sites to enhancer activity in the Drosophila embryo*. *R.R. Datta*[1], R Levina[2], S Small[2] 1) Department of Biology, Hamilton College, Clinton, NY; 2) Department of Biology, New York University, New York, NY.

Anterior-posterior patterning of the *Drosophila* embryo by Bicoid (Bcd) and its transcriptional targets has long been a paradigm in developmental biology. More recently, this system has been studied to gain a deeper understanding of gene regulation and enhancer architecture. Bcd is distributed as a long-range maternal gradient and activates transcription of a large number of target genes, including *orthodenticle* (*otd*). Otd is critical for anterior patterning and brain and eye development in most metazoans. In *Drosophila*, Bcd has evolved to replace Otd's ancestral function in embryo patterning. Bcd and Otd are K50 homeodomain transcription factors bind similar DNA sequences *in vitro*, but how their transcriptional activities are integrated to pattern anterior regions of the embryo is unknown.

We used ChIP-sequencing, *in vitro* binding arrays, gene replacement, and transgenic reporters to define classes of enhancers that are differentially sensitive to binding and transcriptional activation by Bcd and Otd. We show that critical developmental enhancers are initially activated by Bcd, and activation is transferred to Otd via a feed-forward relay that involves sequential binding of the two proteins to the same DNA motif. The specific activities of these enhancers are mediated by DNA motif variants preferentially bound by Bcd or Otd, and the presence or absence of sites for cofactors that interact with these proteins. Our results define specific patterning contributions of optimal and suboptimal binding sites for Bcd and Otd, as well as contributions from the early maternal pioneer factor Zelda (Zld), that coordinate the precise timing of gene expression patterns during embryonic development.

50 Activating and repressing stochastic gene expression between chromosomes. *Chaim Chernoff*, Elizabeth Urban, Kayla Viets, Robert Johnston, Jeong Han, Adrienne Chen Biology, Johns Hopkins University, Baltimore, MD.

Cell fate specification during development is often thought of as a highly reproducible process, in which tight regulation of gene expression determines precise cell fates. Cellular diversity can also arise from stochastic gene expression, as in the specification of bacterial competence states, visual and olfactory receptors, motor neuron subtypes, immune cells, and stem cells. In flies, a stochastic fate choice in the R7 photoreceptors of the retina is controlled by the transcription factor Spineless (Ss). Stochastic expression of *ss* is controlled by a complex *cis*-regulatory logic, including a promoter, two activating enhancers, and two repressive silencers, resulting in expression of Ss in 65% of R7 cells. Homologous chromosome pairing enables transvection between copies of *ss*, a phenomenon in which *cis*-regulatory elements such as enhancers on one chromosome regulate copies of the gene on the other homologous chromosome. However, the mechanisms that regulate transvection are poorly understood. Here, we show that transvection between naturally occurring alleles

of *ss* activates and represses Ss expression frequency to yield intermediate ratios of Ss^{ON} to Ss^{OFF} cells, providing a biological role for transvection. Activation of *ss* between chromosomes requires an intact enhancer and promoter on the same chromosome, suggesting that enhancer-promoter looping in *cis* promotes gene activation in *trans*. Repression of *ss* requires Polycomb Response Elements (PREs) in the *ss* silencer. Unlike activation between chromosomes, repression between chromosomes does not require an intact enhancer and promoter on the same chromosome, showing that repression and activation between chromosomes are separable mechanisms. Previous work has shown that large chromosomal aberrations often ablate homologous chromosome pairing and transvection. However, using FISH we confirmed that *ss* PREs still colocalize with the endogenous locus in the presence of a chromosomal inversion that causes them to be 12Mb away from the locus, this shows that homologous pairing persists despite these chromosomal aberrations. In addition, *ss* PREs repress endogenous *ss* expression even when translocated to a different chromosome, indicating that transvection and *ss* pairing still occur in the presence of chromosomal rearrangements. Together, our findings provide mechanistic insight into how DNA elements work between chromosomes to activate and repress stochastic gene expression.

51 Visual detection of parasitoid wasps is mediated through the lobula columnar 11 neurons. *S. Davis*, T. Schlenke Department of Entomology, University of Arizona, Tucson, AZ.

The survival of an organism depends on the correct recognition of environmental threats. A major threat to natural *Drosophila* populations are the parasitoid wasps that lay their eggs inside fly larvae. The wasp eggs hatch and the wasp larvae grow inside the fly larvae, eventually consuming the flies from the inside out. Adult flies are not themselves attacked, but adult females recognize the presence of wasps and alter their oviposition rates, which is thought to reduce the potential for offspring infection. Blind adult flies do not respond to the presence of wasps, suggesting that vision is a critical sensory modality for detecting wasps, but the underlying neurocircuitry remains unknown. We are investigating the function of the lobula columnar 11 (LC11) neurons in mediating the adult behavioral response to wasps. These neurons are activated when flies are presented with a small moving dark object, which might be similar to how flies see wasps. Silencing LC11 neurons in females group-housed with wasps resulted in no oviposition change, mimicking the phenotype of blind flies. Conversely, activating LC11 neurons in the absence of wasps phenocopied fly oviposition behavior in the presence of wasps. Our results suggest that the LC11 neurons are necessary and sufficient for the fly to detect the threat of parasitoid wasps, and that they serve as a bridge between the visual sensory neurons and the brain to alter female oviposition behavior.

52 Regulation of modulatory cell activity across olfactory neuropil in *Drosophila melanogaster*. Xiaonan Zhang[1], Kaylynn Coates[2], Andrew Dacks[2], Cengiz Gunay[3], Scott Lauritzen[4], Feng Li[4], Steven Calle-Schuler[4], Davi Bock[4], *Quentin Gaudry*[1] 1) Department of Biology, University of Maryland, College Park, MD 20742, USA; 2) Department of Biology, West Virginia University, Morgantown, WV 26506, USA; 3) School of Science and Technology, Georgia Gwinnett College, Lawrenceville, GA 30043, USA; 4) Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA 20147, USA.

Virtually all neural circuits are innervated by centrifugal modulatory neurons that project broadly across brain regions. As a result, most cognitive tasks, including the formation of sensory precepts, involve neuromodulation at each subsequent stage of processing. A central question in the field of neuromodulation is how the activity of such neurons correlates across brain regions involved in sensory processing. In most model organisms such modulatory systems comprise large populations of heterologous neurons, thus complicating their analysis. Here we use *Drosophila*to examine how the activity of a modulatory neuron spanning multiple olfactory neuropils is regulated. In flies, only one serotonergic neuron, the contralaterally projecting, serotonin-immunoreactive, deuterocerebral neuron (CSDn) innervates the antennal lobe (AL) and lateral horn (LH); first and second order olfactory processing regions, respectively. Remarkably, we find that CSDn processes have opposite polarity olfactory responses in the AL and LH. Using a combination of 2-photon microscopy, GRASP, computational modeling, and EM microscopy, we reveal the network connectivity that results in the strong inhibition of CSDn neurites in the AL and the excitation of CSDn processes in the third order LH. The AL inhibition scales with odor strength and the spatial pattern of inhibition is odor independent. The spatial pattern of CSDn neurite activation in the LH is odor specific and mediated in part by direct feed-forward input from PNs terminals in the LH. Next, we use a passive compartmental model and laser axotomy to demonstrate that the inhibition of the CSDn's processes in the AL propagates to its dendrites in the LH. As the inhibition of CSDn dendrites in the AL increase with odor strength, the peak responses of CSDn neurites in the LH are shunted. We propose that this circuit architecture allows the CSDn to modulate olfactory responses in the LH in an odor-dependent manner across a wide range of intensity of olfactory stimulation.

53 Starvation differentially modulates GABA signaling in olfactory receptor neurons. *E. Slankster*[1], D. Baria[1], R. Jain[1], S. Odell[1,2], D. Mathew[1,2] 1) Department of Biology, University of Nevada, Reno, Reno, NV; 2) Integrated Neuroscience Graduate Program, University of Nevada, Reno.

Starvation increases olfactory sensitivity that encourage animals to find food. The molecular mechanisms that underlie starvation dependent modulation of olfactory neuron sensitivity are unclear. Breakdown in these mechanisms lead to abnormal feeding habits, which, in turn, lead to disease states such as obesity and diabetes. Insulin signaling via insulin receptors (InR) was previously shown to regulate metabolic glucose levels as well as impact neuronal function in the brain. Differential expression of $GABA_B$ receptors ($GABA_BR$) in adult *Drosophila* olfactory receptor neurons (ORNs) was shown to differentially impact ORN function. A study of healthy human patients showed that dietary GABA supplementation increased insulin levels during fed and fasted states suggesting a possible interaction between the two signaling pathways. Components of insulin and GABA signaling pathways are conserved throughout the animal kingdom. We used two different assays to analyze the chemotaxis responses of starved and fed *Drosophila* larvae to a panel of odorants. We found that the larva's starved state altered behavioral sensitivity to some but not all odorants. Using immunocytochemistry techniques we determined that $GABA_BR$ localized to terminals of larval ORNs. Genetic manipulation of $GABA_BR$ and InR levels specifically in ORNs, and GABA levels in surrounding local neurons (LNs) impacted the starvation dependent changes observed during larval chemotaxis. To systematically characterize the molecular players involved, we quantified gene expression in the ORNs of starved and fed larvae. We observed that lower $GABA_BR$ levels in specific neurons were crucial to starvation dependent increase in odor response. Our results also suggested that a potential target of these signaling events is differential modulation of Orco gene expression levels in specific ORNs. Based on our results, so far, we propose that interactions between the GABA and insulin signaling pathways are critical to starved-state dependent modulation of ORN sensitivity. Our results offer a mechanistic understanding of how environmental signals are translated into different behavioral outputs based on the animal's physiological state.

54 Feeding control via multimodal taste integration in pharyngeal taste neurons in adult *Drosophila*. *Yu-Chieh David Chen*[1], Ryan Joseph[2], Anupama Dahanukar[1,2] 1) Interdepartmental Neuroscience Program, University of California, Riverside, CA 92521; 2) Department of Molecular, Cell and Systems Biology, University of California, Riverside, CA 92521.

Gustatory inputs play instrumental roles in feeding behavior, including food choice and intake. In adult *Drosophila*, tastants are detected via taste sensilla in multiple organs throughout the body, present externally in the labellum, legs, wings and internally in the pharynx. Each taste sensillum houses several gustatory receptor neurons (GRNs) that express different chemoreceptors for sensing different tastants. Taste inputs originating from multiple classes of GRNs in different taste organs is thought to be processed and integrated in higher-order brain circuits for mediating behavioral output, yet how distinct GRN classes in different organs contribute to feeding behavior remains unclear. We employed a *Pox-neuron* (*Poxn*) mutant as a minimal taste system model in which all the external taste sensilla are transformed into mechanosensory sensilla, while all pharyngeal GRNs remain intact. In *Poxn*mutants, we genetically silenced all

pharyngeal GRNs with *Ir25a>Kir2.1* and found that feeding attraction to appetitive tastants (sugar, amino acids) and avoidance of aversive tastants (bitter, acid, high salt) were both abolished in binary choice assays. These results indicate a key role for pharyngeal GRNs in food selection and confirm that taste input is essential for food choice in short-term feeding assays. Taking advantage of our recent chemoreceptor reporter map of all pharyngeal GRNs, we genetically protected single classes of pharyngeal GRNs via selective expression of the GAL80 suppressor in a molecularly defined class of pharyngeal GRNs in *Poxn, Ir25a>Kir2.1* taste-blind flies, allowing a unique opportunity to test principles of taste coding and behavior in animals that possess only one type of taste neuron. Using single-fly quantitative FLIC assay to measure micro-feeding parameters such as the number and duration of interactions with tastants, we found that flies with distinct single classes of pharyngeal GRNs exhibited distinct, and in some cases opposing, micro-feeding behaviors in response to the same tastants. Further, Ca^{2+}imaging of selected pharyngeal GRNs revealed overlap in tastant sensitivity, as well as multimodal tastant sensing properties in some GRNs. Together, these results suggest that distinct populations of pharyngeal GRNs can control micro-feeding parameters in synergistic or antagonistic ways. To understand where pharyngeal taste input is conveyed in higher-order brain circuits, we used the circuit tracing technique, trans-Tango, to map second-order pharyngeal neurons. We found that pharyngeal second-order neurons projected to two main brain regions - pars intercerebralis and lateral protocerebrum - that also receive input from external taste neurons. Now, we are examing how tastants are represented in pharyngeal second-order neurons and whether pharyngeal input is transmitted to neuroendocrine cells in the brain.

55 What makes a meal? Defining meals from bouts and identifying regulators of meal size. *S. J. Park*[1,2,3], K. Murphy[2,3,4], M. Ehrlich[2,3], W. Ja[2,3] 1) Skaggs Graduate School, The Scripps Research Institute, Jupiter, FL; 2) Department of Neuroscience, The Scripps Research Institute, Jupiter, FL; 3) Center on Aging, The Scripps Research Institute, Jupiter, FL; 4) Program in Integrative Biology and Neuroscience, Florida Atlantic University, Jupiter, FL.

Animals feed in bouts, observed as pecks, licks, bites, and visits to the food source. Series of feeding bouts make up meals—larger structures that reflect satiation. Although previous works have used arbitrary criteria to cluster feeding bouts into meals, there has yet to be an unbiased, consistent way of defining meals. We reasoned that ethologically relevant meals should reflect the concept of satiety: the larger the previous meal, the longer it would take for the animal to reinitiate feeding. Using a recently published assay, the Activity Recording CAFE (ARC), we collected a large number of high-resolution feeding bouts from freely-moving individual flies. From this dataset, we calculated an inter-bout interval cutoff for meal classification that maximizes the relationship between meal size and time to the next meal, an approach that could also be applied to feeding patterns in other organisms. This definition reveals that the size of a meal is tightly regulated by numerous factors, including circadian rhythm, hunger state, and diet composition, but also that their contribution to long-term food intake is limited. In addition, we identified a population of neurons that regulate meal termination. These neurons, labeled by CCKLR-17D3 enhancer fragments, are found in the fan-shaped body and the sub-esophageal zone in the adult fly. Constitutive and temporally-restricted silencing of the cells resulted in an increase in meal size, whereas activation decreased meal size and inhibited proboscis extension response, all without affecting 24-hr total food consumption. Optogenetic stimulation of the CCKLR-17D3 neurons acutely during feeding events, using a modified, closed-loop version of the ARC, resulted in premature meal cessation without changing inter-meal intervals. Numerous agents—such as taste, diet composition, and volume ingested—are known to influence meal size. The specific signals to which the CCKLR-positive neurons respond are under further investigation.

56 The *nervy* gene modulates aggression levels through its function in the octopaminergic neurons. Kenichi Ishii, Matteo Cortese, *Kenta Asahina* Molecular Neurobiology Laboratory, Salk Institute for Biological Studies, La Jolla, CA.

Proper adjustment of the intensity of aggressive behavior is important for maximizing animal fitness. Aggression against inappropriate targets, such as females or animals of different species, can cause waste of resources (energy, time, etc.), while inappropriate escalation of aggression can cause injury or death, which decreases fitness. Thus, a mechanism that keeps the levels of aggression at check must be as important as a mechanism that promotes aggression. However, a “brake” mechanism of aggression has received relatively little attention.

Through an RNAi-based behavioral screen, we discovered that the neuronal knockdown of a gene called *nervy* caused unusually high levels of aggression. We generated a CRISPR/Cas9-mediated mutant allele of *nervy*, and confirmed that the homozygous mutants of this allele showed increased aggression. These data suggest that *nervy* is required to suppress excessive aggression. In fact, the *nervy* mutant males are so aggressive that they even attack a female target fly, which wild type male flies almost never do.

The hyper-aggressive phenotype of the *nervy* mutation can be reversed by expressing a wild type *nervy* gene pan-neuronally, suggesting that *nervy* is required in neurons to control the levels of aggression. The “knock-in” allele of *nervy* that express a bacterial transcription factor LexA instead of *nervy* itself revealed a broad expression of *nervy* gene throughout the nervous system. To identify a specific subpopulation of neurons in which *nervy* functions to regulate the levels of aggression, we screened GAL4 lines in combination with the *nervy* RNAi transgene. We found that *nervy* knockdown under the control of Tdc2-GAL4, which labels octopaminergic and tyraminergic neurons, significantly increased aggression, indicating that *nervy* in Tdc2-expressing neurons is necessary for a proper control of aggression.

nervy is an evolutionarily conserved gene, and 4 domains of the NERVY protein show particularly high homology across animal species (called *nervy* homology regions, or NHRs). We created transgenes that expresses mutant proteins in which one of the 4 NHRs is missing. The loss of NHR2 abrogated its capacity to reverse the hyper aggressive phenotype of the *nervy* mutants. This is consistent with previous observations in mammals that NHR2 is particularly important for NERVY protein’s cellular function. Lastly, we expressed human *nervy* homologues, MTG8, MTG16, and MTGR1, in the neurons of the nervy mutant flies, and found that MTG8 and MTG16 reversed the high level of aggression. This suggests that *nervy* protein’s cellular function is conserved between human and flies.

57 FOXO is a hypoxia-inducible transcription factor necessary for *Drosophila* tolerance to low oxygen. *E. Barretto*[1], B. Lee[1], A. Beevor-Potts[1,2], S. Grewal[1] 1) Clark H. Smith Brain Tumour Centre, Arnie Charbonneau Cancer Institute, Alberta Children's Hospital Research Institute, Department of Biochemistry & Molecular Biology, and Cumming School of Medicine, University of Calgary, Calgary, Alberta, CA; 2) Bachelor of Health Sciences Program, University of Calgary, Calgary, Alberta, CA.

Animals often live in conditions where environmental oxygen levels fluctuate. When oxygen is abundant, growth is promoted, but when oxygen is scarce, metabolic processes are altered to limit growth and promote survival. One important mechanism controlling cellular responses in hypoxia is the regulation of metabolic gene expression. In this context, the conserved hypoxia-inducible factor (HIF) has been established as a transcriptional regulator of genes important for hypoxia-responses in different model organisms. However, less is known about other transcription factors important in hypoxia adaptation. We have explored this question in *Drosophila*.

Drosophila larvae and adults can tolerate low oxygen conditions. At 5% oxygen, larvae slow their growth and development, but show little effect on overall viability. At 1% oxygen, both larvae and adult animals exhibit a state of ‘suspended animation’, but can survive for up to 12 (larvae) or 30 (adults) hours. We found that upon switching larvae from normoxia to hypoxia, the transcription factor FOXO was rapidly relocalized from the cytoplasm to the nucleus in larval tissues such as the fat body and intestine, even though animals maintain normal feeding. Moreover, we saw that many known FOXO target genes were induced in hypoxic animals. FOXO is important for regulating starvation and stress responses, and can regulate aging in *Drosophila* and *Caenorhabditis*

elegans. We found that both *foxo* null mutant larvae and adults show reduced survival in hypoxia. We also identified two downstream FOXO target genes involved in hypoxia tolerance. The first is the translational repressor 4E-BP. Upon exposure to hypoxia, 4E-BP expression is increased in a FOXO-dependent manner, and overall protein synthesis is reduced. We found that while *4e-bp* mutants only showed a modest decrease in hypoxia survival, overexpression of 4E-BP was sufficient to increase larval survival under low (1%) oxygen. The second factor is the NF-KappaB transcription factor Relish, which has been best studied as a mediator of innate immune responses. We found that *relish* expression was increased in a FOXO-dependent manner in hypoxia, and that FOXO could also induce expression of anti-microbial peptides in low oxygen. Finally, we observed that *relish* mutant animals showed reduced survival in hypoxia. Together, these data indicate that FOXO is a hypoxia inducible factor that mediates tolerance to low oxygen by controlling protein synthesis and immune signaling.

58 *Drosophila* HNF4 directs a switch in fatty acid metabolism that supports the transition to adulthood. *G. Storelli*[1], H-J. Nam[1], J. Simcox[2], C.J. Villuaneva[2], C.S. Thummel[1] 1) Department of Human Genetics, University of Utah School School of Medicine, Salt Lake City, UT; 2) Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, UT 84112, USA.

Metabolic adjustments must accompany developmental transitions to meet the changing needs of each stage in the life cycle. We identified a role for the *Drosophila Hepatocyte Nuclear Factor 4* nuclear receptor (*dHNF4*) in directing the transition into the adult metabolic state. *dHNF4* mutants develop normally through larval stages but die shortly after adult emergence. Newly-eclosed *dHNF4* mutant adults become rapidly hyperglycemic and display a steady increase in triglyceride levels, while controls mobilize these stores. We investigated the impact of these metabolic defects on the fitness of *dHNF4*mutant adults. Despite their inability to use lipids, young *dHNF4* mutants are not starvation-sensitive compared to controls, and both genotypes survive for weeks under humid conditions in the absence of food. Rather than contributing to energy metabolism, our data demonstrate that lipids serve a more essential function at this stage in development. They are processed by oenocytes in a *dHNF4*-dependent manner to produce Very Long Chain Fatty Acids (VLCFAs) and VLCFA-derived hydrocarbons, which waterproof the animal and reduce water loss by transpiration. *dHNF4* coordinates the transcriptional induction of genes involved in VLCFA/hydrocarbon biosynthesis with the onset of adulthood, supporting rapid hydrocarbon production in newly-emerged adults. Consistent with their lack of hydrocarbons, *dHNF4* mutants show increased rates of water loss and die within a day in the absence of water, while oenocyte-specific *dHNF4* genetic rescue suppresses the sensitivity of the mutant to dry conditions and hyperlipidemia. More surprisingly, *dHNF4* is required in oenocytes to suppress diabetic phenotypes. Oenocyte-specific expression of wild-type *dHNF4* transgene rescues hyperglycemia and dietary sugar toxicity in *dHNF4* mutants, suggesting that these diabetic phenotypes arise from an imbalance in body fluid homeostasis. Consistent with this, rearing *dHNF4* mutants in increased humidity restores euglycemia and resistance to dietary sugar. Our work thus provides unexpected links between oenocyte lipid metabolism, fluid homeostasis, and glucose homeostasis at the onset of *Drosophila* adulthood. This work was funded by the NIH (R01DK108941).

59 Analysis of the Diurnal Transcriptomes of Young and Old Drosophila Heads Reveals Metabolic Shifts During Aging. B. Sebastian[1], R. Fey[2], E. Chow[3], D. Long[3], P. Reardon[4], J. Giebultowicz[3], *D. Hendrix*[2,5] 1) Department of Mathematics, Oregon State University, Corvallis, OR; 2) Biochemistry and Biophysics, Oregon State University, Corvallis, OR; 3) Department of Integrative Biology, Oregon State University, Corvallis, OR; 4) Nuclear Magnetic Resonance Facility, College of Science, Oregon State University, Corvallis, OR; 5) School of Electrical Engineering and Computer Science, Oregon State University, Corvallis, OR.

Aging is associated with significant changes in the fly transcriptome. Early studies of aging measured gene expression in samples collected at a random time of day; however, expression of hundreds of genes fluctuate in a daily pattern due to circadian regulation in both mammals and *Drosophila*. To understand age-related changes in rhythmically expressed genes, we performed around-the-clock RNA-seq and small RNA-seq, and NMR metabolomics in the heads of 5-day-old (young) and 55-day-old (old) flies. Here, we present an analysis of the obtained data. Our methods build upon Fourier-based detection of changes in rhythmic expression, and can also detect rhythmic bursts of expression that are not detected with other Fourier-based approaches. Although the net level of rhythmicity is similar in young and old flies, we observe a "rewiring" of the circadian system. First, we observe changes in the core clock genes, such as the expression of *period*, which exhibits an increase in the amplitude of mRNA expression and a reduction in protein expression. We observe several stress-response genes, late life cyclers (LLCs), that gain rhythmicity during aging. We observe that LLCs are also rhythmically activated in young flies by oxidative stress, a known cause of neurodegeneration, resulting in expression patterns that recapitulate expression in old. Consistent with LLC expression changes, NMR metabolomic data shows changes in metabolites such as lactate that are consistent with age-altered gene expression such as lactate dehydrogenase, which implicate some of these genes in neurodegeneration—with both potentially harmful and neuroprotective roles. In contrast, we also observe other genes we call early life cyclers (ELCs), which lose rhythmicity with age. The ELC genes are enriched for pathway annotations that include translation and mRNA processing. We also detected hundreds of robust life cyclers (RLC) with sustained rhythmic patterns of expression throughout lifespan and include vital pathways such as the regulation of mitochondrial translation. Finally, we observe a statistically significant genome-wide reduction in microRNA expression with age, and also observe age-onset rhythmic piRNA expression. Taken together, these data shed new light on the widespread shifts in gene regulation and metabolic pathways to cope with the changing cellular environment during aging.

60 Genome-wide analyses of lifespan and healthspan reveal a role for *decima* as a novel regulator of neuronal insulin-like peptide production. *K.A. Wilson*[1,2], J.N. Beck[1], C.S. Nelson[1], R.B. Brem[1,2], P. Kapahi[1,2] 1) Buck Institute for Research on Aging, Novato, CA; 2) Leonard Davis School of Gerontology, University of Southern California, Los Angeles, CA.

Dietary restriction (DR) robustly extends lifespan and delays age-related diseases across species. An underlying assumption in aging research has been that DR mimetics extend both lifespan and healthspan jointly, though this has not been rigorously tested in different genetic backgrounds. Furthermore, nutrient response genes important for lifespan or healthspan extension remain underexplored, especially in natural populations. To address these gaps, we utilized over 150 strains from the *Drosophila* Genetic Reference Panel to measure nutrient-dependent changes in lifespan and age-related climbing ability to measure healthspan. DR extended lifespan and delayed decline in climbing ability on average, but there was no evidence of correlation between these traits across individual strains. Through GWAS, we then identified and validated *CG34351* (which we rename *decima*) and *Ferredoxin* as determinants of diet-dependent lifespan, and *CG33690* (which we rename *Daedalus*) for diet-dependent physical activity. Modulating these genes produced independent effects on lifespan and climbing ability, further suggesting that these age-related traits are likely to be regulated through distinct genetic mechanisms. Our research shows that neuronal RNAi of *decima*, the human homolog of *RGS7BP* and a downstream component of GPCR signaling, is capable of extending life only in *ad libitum* (AL) dietary conditions. We further show that expression of *decima* specifically in the GABA receptor neurons is responsible for regulating insulin-like peptides production and longevity under AL conditions.

61 Neural mechanisms underlying energy homeostasis: hormonal regulation of synaptic plasticity in fat-sensing neurons. *A.E. Brent*, Z. Goldberg, A. Rajan Basic Sciences, FHCRC, Seattle, WA.

Energy homeostasis is the ability of an organism to sense nutrient flux and alter its internal physiology such that physiologic parameters, such as blood glucose and fat stores, are maintained within a permissible range. Our lab is interested in understanding how the two centers regulating physiology--the fat body (FB), which stores energy in the form of triacylglycerol (TAG), and the brain, which orchestrates a systemic insulin-dependent response—communicate

with each other.

The FB sends out fat store information to the brain via release of the adipokine Upd2. The Upd2 signal is received by a group of STAT-expressing GABAergic neurons located near the brain's insulin producing cells (IPCs). These GABAergic STAT neurons provide tonic inhibitory tone to the insulin-producing cells (IPCs), ensuring that levels of insulin release reflect the status of energy stores. Upd2 promotes fat storage by reducing the extent of this tonic GABAergic inhibition. Understanding how Upd2-dependent regulation of inhibitory tone occurs is central to our picture of FB-brain communication.

We performed an RNAi-based screen to identify potential STAT target genes that might be responsible for regulating the extent of IPC inhibition by GABA neurons. From our screen we identified candidate genes previously shown to be required for the dynamic cytoskeletal organization that underlies neuronal plasticity. These observations suggest that in Drosophila, adipokines control the level of GABA neuron activity on the IPCs via modulation of cytoskeleton-dependent synaptic organization and activity. In this way, the energy stores themselves determine the extent of inhibitory tone on the IPCs, ensuring that a basal level of insulin is released to maintain nutrient storage and promote energy expenditure.

62 Peroxisome-mediated inter-tissue communication during *Drosophila* aging. *K. Huang*, Q. Han, H. Bai Department of Genetics, Development, and Cell Biology, Iowa State University, Ames, IA.

Cardiovascular diseases (CVDs) are one of the major causes of death in the western world. Age represents the largest risk factor for CVDs, as the prevalence of these pathologies increase significantly with age. In addition, there is strong association between liver abnormality and cardiac dysfunction in clinical settings, suggesting there is communication between two organs. Yet the understanding for cardiovascular aging and its relationship with liver aging is limited. In the present study, we discover that liver-heart communication and aging-induced cardiac dysfunction are mediated by an inflammatory cytokine unpaired 3 (*Upd3*), the homolog of mammalian interleukin-6. We find that during aging and oxidative stress, *Upd3* expression is highly up-regulated in liver-like tissue oenocytes. Over-expressing *Upd3* specifically in oenocytes can induce premature cardiac aging in young flies. Remarkably, knockdown of *Upd3* in oenocytes, decreases JAK-STAT signaling in the heart, and alleviates cardiac arrhythmia induced by oxidative stress and aging. Intriguingly, *Upd3* in oenocytes can be transcriptionally up-regulated by either catalase (*Cat)* knockdown or disruption of peroxisome function. Recently, it has been shown that peroxisome function declines with age. We find that oenocyte-specific knockdown of peroxin 5 (*Pex5*), the key peroxisome importing factor, induces cardiac arrhythmia via increased *Upd3* in young flies. Taken together, our findings suggest that age-dependent induction of *Upd3* in oenocytes, due to a dampened peroxisome activity, is responsible for mediating cardiac dysfunction in old fly hearts.

63 The *Drosophila* pioneer factor Zelda modulates the nuclear microenvironment of a Dorsal target enhancer to potentiate transcriptional output. *P.H. Whitney*[1], S. Yamada[1], S. Huang[1], E.C. Eck[2], H.G. Garcia[2,3,4,5], C.A. Rushlow[1] 1) Biology, New York University, New York, NY; 2) Biophysics Graduate Group, University of California at Berkeley, Berkeley, CA; 3) Department of Molecular and Cellular Biology, University of California at Berkeley, Berkeley, CA; 4) Department of Physics, University of California at Berkeley, Berkeley, CA; 5) Quantitative Biosciences-QB3, University of California at Berkeley, Berkeley, CA.

Connecting the developmental patterning of tissues to the mechanistic control of RNA polymerase II remains a long term goal of developmental biology. Many key elements have been identified in the establishment of spatial−temporal control of transcription in the *Drosophila* early embryo, a model system for transcriptional regulation. The dorsal/ventral axis of the *Drosophila* embryo is determined by the graded distribution of Dorsal (DL), a homologue of the NF−κB family of transcriptional activators found in humans. A second maternally deposited factor, Zelda (ZLD), is uniformly distributed in the embryo and is thought to act as a pioneer factor, increasing enhancer accessibility for transcription factors such as DL. Here we utilized the MS2 live imaging system to evaluate the expression of the DL target gene short gastrulation (*sog*) to better understand how a pioneer factor affects the kinetic parameters of transcription. Our experiments indicate that ZLD modifies probability of activation, the timing of this activation, and the rate at which transcription occurs. Our results further show that this effective rate increase is due to an increased accumulation of DL at the site of transcription, suggesting that transcription factor 'hubs' induced by ZLD functionally regulate transcription.

64 Regulatory crosstalk between ecdysone-induced transcription factors confers temporal specificity to chromatin-state & gene expression during metamorphosis. *S.L. Nystrom*[1,2,3,4], D.J. McKay[2,3,4] 1) Genetics & Molecular Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC; 2) Department of Biology; 3) Department of Genetics; 4) Integrative Program for Biological and Genome Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Organismal development requires proper temporal and spatial coordination of gene expression by transcription factors (TFs). Binding of TFs *in vivo* is strongly influenced by packaging of DNA into chromatin; accessible binding sites are permissive for transcription factor binding, whereas inaccessible sites are a barrier to TF binding. Despite the central role of chromatin acessibility in regulating dynamic gene expression programs during development, the underlying mechanisms remain unclear.

Our work has identified the ecdysone pathway as playing a central role in regulation of chromatin accessibility over time. Pulses of ecdysone initiate transitions between developmental stages by inducing expression of a suite of temporal-specific TFs (tTFs) which activate & repress target genes. Prior work from our lab revealed thousands of changes in chromatin accessibility in the developing wing during the larval to pupal transition. However, most of these changes fail to occur in mutants of the ecdysone-induced tTF E93. Interestingly, E93 appears to perform two opposing functions. In the absence of E93, late-acting target enhancers fail to open & activate and early-acting target enhancers fail to close. How E93 performs these two opposing actions is unclear. We identified DNA binding motifs for the early-acting tTF, broad (br), at sites which are opened by E93 later in development, suggesting the potential for regulatory crosstalk between early and late-acting tTFs.

To test whether br plays a role in repressing regulatory activity of these late-acting sites, we assayed activity of late-acting enhancer reporter constructs in brRNAi wings. We observe that loss of br results in precocious activity of late-acting enhancers, suggesting br acts during larval stages to repress activity of late-pupal enhancers. In support of these findings, RNA-seq in brRNAi wings identifies hundreds of late-acting genes which are precociously expressed during larval stages.

To identify whether br, like E93, is required for regulating accessibility at dynamic chromatin sites, we performed FAIRE-seq in brRNAi wings during late larval and early pupal stages. We identify hundreds of late-opening sites which precociously open in brRNAi wings many of which are bound and opened by E93 during pupal stages. Therefore, we hypothesize that br actively prevents TF access to chromatin at late-acting enhancers to provide temporal specificity to target gene expression.

Finally, we find that E93 is both necessary & sufficient to repress *br* during wing development, supporting a model where E93 acts to both repress *br* expression and antagonize br repressive activity at target enhancers to promote a forward developmental transtion.

65 *Trans* regulatory changes produce differences in maternal transcript deposition between closely related species of Drosophila. *E. Cartwright*, S. Lott University of California, Davis.

During the early development of all Metazoa, maternal mRNAs and proteins are deposited into the early embryo, which are necessary to carry out developmental processes prior to activation of the zygotic genome. Maternal products are degraded as the zygotic genome is activated and takes over control of the rest of development. The handoff of developmental control from the maternal to the zygotic genome, a process known as the maternal to zygotic transition (MZT), is conserved, highly regulated, and necessary for development and organism survival. While the MZT is conserved across all animals and is critical for development, there are differences in the maternally deposited transcripts and zygotically transcribed genes between species. The MZT has been studied in a number of model systems, but these organisms are too evolutionarily diverged to make direct comparisons in how gene regulation during this critical time in development can evolve. In this analysis, we used three closely related species of *Drosophila, D. simulans*, *D. sechellia*, and *D. mauritiana*, to study how gene regulation evolves across species during this critical early stage of development. To identify changes in *cis* and *trans* regulatory elements that lead to differences in maternal deposition of mRNAs, degradation of these maternal mRNAs, and transcription of early zygotic genes between these species, we utilized genetic crosses and conducted allele-specific RNA-sequencing of single embryos, both before and after zygotic genome activation. Using Poisson-Gamma models to identify differentially expressed genes, we find a surprisingly high proportion of *trans* regulatory changes amongst genes that are differentially maternally deposited between these species and have identified candidate transcription factors that may be responsible for these differences. In contrast, evolved changes in zygotic gene expression are due to a mix of regulatory changes in *cis*, *trans*, and the combination of both. These patterns of gene regulatory changes indicate that gene expression evolves via different mechanisms at different developmental timepoints, and that maternal gene expression may have unique features that make it more likely to evolve through regulatory changes in *trans*.

66 Dynamic identification of the dosage-compensated *Drosophila* male X-chromosome during early embryogenesis. *L.E. Rieder*[1,2], W.T. Jordan, III[1], E.N. Larschan[1] 1) Molecular Biology, Cellular Biology and Biochemistry Department, Brown University, Providence, RI; 2) Biology Department, Emory University, Atlanta, GA.

All heterogametic species face a chromosome imbalance. For example, in species with the XY system of sex determination, including humans and *Drosophila*, males have half the X-linked gene dosage compared to females. Mechanisms of dosage compensation therefore evolved to restore balanced expression of X-linked genes between the sexes. In *Drosophila,* the zinc-finger transcription factor Chromatin-Linked Adaptor for MSL Proteins (CLAMP) localizes to X-linked High Affinity Sites (HAS), which contain GA-rich *cis*-elements. CLAMP facilitates Male Specific Lethal (MSL) complex recruitment, which increases transcription of active genes by depositing the H4K16ac active chromatin mark. Investigation into these interactions has largely been confined to steady-state systems such as cultured cells or larval polytene chromosomes, yet the mechanisms of dosage compensation are established dynamically during early embryogenesis.

To capture the dynamics of male X-chromosome identification in the early embryo in real-time, we used a meiotic drive system to generate pools of male embryos, precisely staged them by nuclear cycle, and performed small scale ChIP-seq for CLAMP, MSL complex, and the H4K16ac chromatin mark. Our results reveal that CLAMP localizes to HAS on the male X-chromosome long before MSL complex is recruited. We observe MSL complex localization at the time of widespread zygotic genome activation (late nuclear cycle 14), but not before. Although previous hypotheses suggest that MSL complex first arrives at HAS and then spreads to nearby active genes in two- or three-dimensions, our results indicate that there is no observable time point when MSL is confined to HAS. Instead, MSL complex coats X-linked active gene bodies, although it is enriched at HAS.

Moreover, we demonstrated that CLAMP is an early transcription factor that can open chromatin in a large domain surrounding its GA-rich binding sites, thereby establishing a more open environment on the male X-chromosome compared to autosomes. Our results suggest that MSL does not directly recognize CLAMP or the chromatin at HAS, but rather the permissive chromatin environment of the male X-chromosome that is generated by CLAMP. We propose a new model in which CLAMP localizes to HAS very early during embryogenesis and establishes a chromatin environment that is permissive for MSL complex recruitment which occurs during zygotic genome activation.

67 Unidirectional fork movement coupled with strand-specific histone incorporation ensures asymmetric histone inheritance. *M.I. Wooten*[1], Jonathan Snedeker[1], Zehra Nizami[2], Xinxing Yang[3], Jie Xiao[3], Joe Gall[2], Xin Chen[1] 1) Biology, Johns Hopkins University, Baltimore, MD; 2) Carnegie Institution for Science, Baltimore, MD; 3) Biophysics and Biophysical Chemistry, Johns Hopkins University, Baltimore, MD.

Epigenetic mechanisms play a crucial role in specifying and maintaining proper cell identity throughout organismal development. Stem cells are unique in

their ability to both self-renew and differentiate into a variety of specialized cell types. However, little is known regarding whether stem cells selectively retain their epigenetic information during cell divisions, and, if so, whether the loss of stem cell epigenetic memory could contribute to the genesis and progression of disease.

The *Drosophila* germline provides an excellent system to study asymmetric cell division and stem cell function *in vivo*. Male germline stem cells (GSCs) divide asymmetrically to produce one daughter cell that self-renews, and another daughter cell that differentiates. We have demonstrated that histone proteins, which represent crucial carriers of epigenetic information, are asymmetrically inherited during GSC division such that old histones are segregated towards the future stem cell, whereas new histones are segregated towards the differentiating daughter. Proper histone inheritance is essential for germline maintenance, as disruption of asymmetric histone inheritance can lead to cell death and tumorigenesis.

During DNA replication, old histones are recycled and new histones are deposited onto nascent DNA. In order to better understand how histones are recycled and segregated during DNA replication, our lab developed a chromatin fiber technique which allows us to directly visualize histone segregation patterns on newly synthesized sister chromatids. Using the chromatin fiber technique, we have been able to observe that old and new histones segregate asymmetrically such that a majority of old histones are segregated towards the leading strand, while a majority of newly synthesized histones are segregated towards the lagging strand.

We have further utilized chromatin fibers to track replication fork movement in the *Drosophila* germline. Using the thymidine analogues EdU and BrdU, we can sequentially label replicating regions of chromatin fibers. We have observed that fibers derived from germline tissue show a significantly higher incidence of unidirectional fork progression than did fibers derived from somatic tissue. We hypothesize that in the *Drosophila* germline, unidirectional fork movement coupled with strand preferences in histone incorporation act together to establish asymmetric histone inheritance in preparation for asymmetric cell division.

68 The global, multilayer structure of homolog pairing reflects a level of functional organization in the *Drosophila* genome. *Jumana AlHaj Abed*[1], Jelena Erceg[1], Anton Goloborodko[2], Bryan R. Lajoie[3,4], Geoffrey Fudenberg[2,5], Nezar Abdennur[2], Maxim Imakaev[2], Ruth B. McCole[1], Son C. Nguyen[1,6], Wren Saylor[1], Eric F. Joyce[1,6], T. Niroshini Senaratne[1,7], Mohammed A. Hannan[1], Guy Nir[1], Job Dekker[3], Leonid A. Mirny[2,8], Chao-ting Wu[1,9] 1) Department of Genetics, Harvard Medical School, Boston, MA, USA; 2) Department of Physics, Massachusetts Institute of Technology (MIT), Cambridge, MA, USA; 3) Howard Hughes Medical Institute and Program in Systems Biology, Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA, USA; 4) Illumina, San Diego, CA, USA; 5) Gladstone Institutes of Data Science and Biotechnology, San Francisco, CA, USA; 6) Department of Genetics, Penn Epigenetics Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; 7) Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles, CA 90095, USA; 8) Institute for Medical Engineering and Science, Massachusetts Institute of Technology (MIT), Cambridge, MA, USA; 9) Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA, USA.

Chromatin organization encompasses chromosome territories, compartments of active (A-type) and inactive (B-type) chromatin as well as topologically associating domains as delineated by conformation capture technology or Hi-C. These layers of organization include *cis* and *trans* interactions that determine the 3D organization and function of the genome. *Trans* homologous interactions are particularly special in *Drosophila*, where homologous chromosomes are paired in somatic cells from embryogenesis through adulthood, and pairing-dependent gene regulation, or transvection, is well-documented. Although the relationship between homolog pairing and gene function has been studied extensively in a locus specific manner, never before has the structure of homolog pairing, nor its functional consequence been elucidated globally. In this study, we utilize *Drosophila melanogaster*hybrid embryos and a hybrid cell line to survey *trans* homolog interactions genome-wide in unprecedented detail using a robust haplotype-resolved Hi-C approach, discovering that homologs pair extensively and relatively precisely genome-wide. Our results also reveal a multi-layer, highly structured architecture of homolog pairing that includes *trans*-homolog domains for the first time, loops, and A- and B- type compartments that are concordant with *cis* structures. We also document significant variation in pairing across the genome and present different forms of pairing: tight pairing, consisting of small domains, and loose pairing, consisting of single larger domains. Additionally, we find that global homolog pairing correlates significantly with gene expression, A compartments, and active epigenetic marks. In embryos, homolog pairing seems to be related to early chromatin accessibility and pioneer factor Zelda. In summary, this study provides a robust tool for investigating global *trans*-homolog interactions in haplotype-resolved genomes, delineates them from other *trans* and *cis* interactions and uncovers a new level of structural and functional organization in the *Drosophila* genome.

69 A personalized approach to treat a KRAS mutant colorectal cancer patient using Drosophila. *E. Bangi*[1,2], P. Smibert[2], A. Teague[2], A. Uzilov[2], Y. Antipin[2], M. Donovan[2], C. Ang[2], K. Misiukiewicz[2], E. Schadt[2], M. Posner[2], R. Cagan[2] 1) Florida State University, Tallahassee, FL; 2) Icahn School of Medicine at Mount Sinai, New York, NY.

Personalized cancer genomics is providing unprecedented access into the genetic complexity and diversity of human tumors. Current efforts at 'personalized medicine' primarily focus on using genomic analysis to select the best therapeutic target. This has shown some success but overall, focusing on individual cancer driver genes is not always sufficient to identify effective therapies. In recent years, we have been using Drosophila to explore the importance of increased complexity in our models. To this end we established a platform designed to generate and drug-screen large numbers of genetically complex, personalized fly models to identify treatments for individual patients in a clinical study. Here we report our efforts to develop a personalized therapy for a patient with treatment resistant metastatic KRAS-mutant colorectal cancer. Briefly, we first carried out an extensive genomic analysis of the patient's tumor. We then generated a 9-hit Drosophila model by genetically manipulating the Drosophila orthologs of genes altered in the patient's tumor. A robotics-based screen of an FDA approved drug library identified trametinib plus zoledronate as a candidate treatment combination. Treating the patient with this drug combination led to a significant response where lesions displayed a strong partial response initially and remained stable for 11 months. This approach leverages sophisticated genetic tools and high throughput drug screening methods in Drosophila to address a disease's genomic complexity and may provide personalized treatment options for recalcitrant diseases such as KRAS-mutant colorectal cancer.

70 Diet-enhanced *Drosophila* Tumors Induce Muscle Wasting as a Nutrient-Scavenging Metabolic Program. *H.L. Newton*, L. Camplese, S. Hirabayashi Metabolism and Cell Growth Group, MRC London Institute of Medical Sciences, London, UK.

Cancer cells demand excessive nutrients to support their proliferation, but how cancer cells sense and exploit extracellular nutrients remain incompletely understood. Feeding *Drosophila* high dietary sugar was previously demonstrated to not only direct obesity and organismal insulin resistance, but also transform Ras/Src-activated cells into aggressive tumors[1].

Here, we demonstrate that diet-enhanced Ras/Src-tumors induce progressive skeletal muscle wasting reminiscent of cancer-associated cachexia. We identify branchless, a *Drosophila* Fibroblast Growth Factor as a tumor- and fat body-derived mediator of muscle wasting. Muscle-specific activation of a *Drosophila* Fibroblast Growth Factor Receptor breathless promoted muscle wasting through increased Ras-MAPK signaling. The result was muscle protein degradation and release of free circulating amino acids. Increased availability of amino acids in turn promoted Ras/Src-tumorigenesis, which was functionally dependent on elevated tumor-expression of SLC-family amino acid transporters.

This tumor metabolic response reveals two layers of coordination: (i) at the whole organism level – by promoting cachexia-like muscle wasting and systemic amino acid availability, and (ii) at the tumor-autonomous level - by promoting amino acid transporter expression. This coordinated tumor-host interplay acts to fuel tumors with nutrients, thereby promoting tumor growth. Altogether our findings illustrate a systemic amino acid-utilizing circuit whereby tumors induce cachexia-like muscle wasting as a nutrient-scavenging metabolic program to drive tumorigenesis.

1. Hirabayashi S, Baranski TJ & Cagan RL. (2013). Transformed *Drosophila* Cells Evade Diet-Mediated Insulin Resistance through Wingless Signaling. *Cell*, 154, 664-675.

71 A tumor-microbe self-enforcing loop promotes intestinal tumorigenesis. *J. Zhou*, F. Port, M. Boutros Signaling and Functional Genomics, German Cancer Research Center, Heidelberg, Baden Württemberg, DE.

The intestinal barrier epithelium regulates nutrients absorption, gut-microbiota symbiosis and prevents infections, whereas barrier dysfunction is linked to commensal dysbiosis. The imbalance of the gut flora has been correlated with tumorigenesis, but how tumors alter the microbiota and how a dysbiotic microbiota contributes to tumor growth remains largely unknown. In this study, we create an inducible intestinal tumor model by tissue-specific CRISPR/Cas9 mutagenesis on BMP signaling to investigate host-microbe interactions. We show that intestinal tumorigenesis induces JNK activation that causes intestinal barrier dysfunction followed by commensal imbalance. A dysbiotic microbiome then triggers a regenerative response, which further stimulating tumor growth. We find that reducing JNK activity is sufficient to restore the function of the intestinal epithelium and to re-establish host-microbe homeostasis and to inhibit tumor growth. Taken together, these experiments identify a self-enforcing feedback mechanism, rather simply a correlative relationship between intestinal tumors and the gut microbiome in *Drosophila*. Our findings also highlight the importance of controlling JNK signaling for the maintenance of epithelial barrier function and host-microbe homeostasis as an essential factor for overall organismal health and longevity.

72 Transgenerational inheritance model of high fat diet-induced lipotoxic cardiomyopathy. *M. Clara Guida*, Ryan Birse, Alessandra Dall'Agnese, Nathan Troup, Paula Coutinho, Lorenzo Puri, Rolf Bodmer Development, Aging and Regeneration, Sanford Burnham Prebys Medical Discovery Institute, San Diego, CA.

Obesity is strongly correlated with lipotoxic cardiomyopathy, heart failure and thus mortality. The incidence of obesity has reached alarming proportions worldwide, and increasing evidence suggests that the parents' nutritional status may predispose their offspring to lipotoxic cardiomyopathy. To date, mechanisms underlying intergenerational heart disease risks have yet to be elucidated. Using the *Drosophila* model, we found strong evidence that cardiac dysfunction induced by high-fat diet (HFD) persists for two generations of progeny and is associated with reduced expression of two key metabolic regulators, the main adipose triglyceride lipase (ATGL) encoded by *brummer* (*bmm*) in flies, and the transcriptional co-factor PGC1α, encoded by the fly gene *spargel* (*srl*). Reducing *bmm* or *srl* gene dosage mimics the effects of HFD in causing cardiac lipotoxicity. Moreover, lipotoxic heart dysfunction observed in the *srl* heterozygous mutant flies persists also in those progenies that are genetically *wildtype*. Conversely, cardiac, or adipose expression of *bmm* in the offspring of HFD-fed parents protects them – and the subsequent generation – from HFD-induced heart dysfunction. Interestingly, through an *in vivo* screen of heart function in heterozygous mutants for epigenetic regulators, we identified the involvement of the Polycomb Repressive Complex 2 (PRC2) in the intergenerational inheritance of lipotoxic cardiomyopathy. Indeed, high-fat diet induced lipotoxic cardiomyopathy correlates with elevated systemic histone3-lysine27 trimethylation (H3K27me3). Lowering systemic H3K27me3 genetically by overexpressing the H3K27me3 demethylase, UTX, or pharmacologically by inhibiting the enzymatic sub-unit EzH_2 of the PRC2 in the offspring of HFD-fed parents prevents cardiac pathology, suggesting that metabolic homeostasis is epigenetically regulated across generations. Thus, cardio-protection can be achieved by targeted metabolic or epigenetic manipulations that lead to re- and even pre-programming of the metabolic state of a developing or adult individual.

73 A conserved role for the N-glycosylation pathway in sleep and seizures. *B. Leger*[1,2], S. Gill[2], C. Aonbangkhen[3], C. Woo[3], M. Muona[4], A-E. Lehesjoki[4], B. Baykan[5], S. Schreiber[2], R. Saxena[1,2,6], J. Walker[1,2,7] 1) Center for Genomic Medicine, Massachusetts General Hospital, Boston, MA; 2) Broad Institute, Cambridge, MA; 3) Department Of Chemistry And Chemical Biology, Harvard University, Cambridge, MA; 4) Folkhälsan Institute of Genetics, University of Helsinki, Helsinki, Finland; 5) Department of Neurology, Istanbul Faculty of Medicine, Istanbul University, Capa/Istanbul, Turkey; 6) Department of Anesthesia, Harvard Medical School, Boston, MA; 7) MGH Department of Neurology, Harvard Medical School, Boston, MA.

Protein N-glycosylation is a protein post-translational modification carried out in the endoplasmic reticulum (ER) that is important for the folding, stability and secretion of proteins. The assembly of the precursor glycan is carried out by a set of ALG enzymes. Mutations that disrupt the function these ALG enzymes cause a family of rare metabolic disorders called congenital disorders of glycosylation (CDGs). No CDG has yet been reported for mutations in either *ALG10* or *ALG10B* (alpha-1,2-glucosyltransferases), two paralogous ALG enzyme coding genes that were created by an intrachromosomal duplication in Hominoidea 25 million years ago. Using UK Biobank data, GWAS of human sleep disturbance revealed variants near both *ALG10* and *ALG10B*, that affect multiple sleep and chronotype traits, consistent with the paralogy. Further, a patient who has a quadruple mutant genotype for *ALG10*and *ALG10B* exhibits epilepsy, among other symptoms.

We are taking advantage of *Drosophila* as a model system to study the role of Alg10 and the N-glycosylation pathway in seizure and sleep. *Drosophila* has a single *Alg10* gene with no previously reported phenotypes. Neuron-specific *Alg10* knockdown using RNAi leads to sleep defects and mechanically-induced seizures. Both imprecise excision of a P-element insertion upstream of *Alg10* and CRISPR/Cas9 gene editing have been used to generate independent genetic evidence supporting a role for Alg10 in seizures and sleep. Additionally, transgenic flies expressing human ALG10 and ALG10B (both wild type and with the human patient's missense and truncating mutations) have been tested for rescue of the RNAi phenotypes, thus addressing their pathogenicity. Mechanistic studies using chemical biological tools are being used to identify the target protein(s) whose glycoregulation is disrupted in *Alg10* mutant flies leading to neurological deficits. Further supporting our hypothesis, we found that other enzymes in the N-glycosylation pathway are also important for neurological function, as RNAi knock down of these genes also show sleep and seizure phenotypes. In summary, by making use of the unique lack of genetic redundancy in *Drosophila*, we have not only identified a gene (*ALG10*) and a pathway (N-glycosylation) that play a role in human and *Drosophila* sleep and seizures, but also have the ability to understand an ultra-rare human neurological disorder. Future mechanistic work is expected to provide a path to targeted therapeutics.

74 The intellectual disability-associated SWI/SNF chromatin remodeling complex regulates structural plasticity of the *Drosophila* mushroom body during critical developmental transitions. Kevin Nixon[1], Melissa Chubak[1,2], Max Stone[1,3], *Jamie Kramer*[1,2,3] 1) Deptartment of Physiology and Pharmacology, Western University, London, Ontario, Canada; 2) Department of Biology, Western University, London, Ontario, Canada; 3) Division of Genetics and Development, Children's Health Research Institute, London, Ontario, Canada.

Advances in DNA sequencing technology have led to the rapid identification of nearly 1000 genes involved in intellectual disability (ID), but our functional understanding of the causative genes is lagging. We and others have shown that ID genes are functionally connected, suggesting that a limited number of cellular processes are disrupted in ID. Understanding these processes and how their disruption leads to reduced cognitive functioning is a critical hurdle towards the development of therapies. Using gene ontology enrichment analysis for known ID genes, we identified the SWI/SNF chromatin remodeling complex as the most overrepresented cell component in ID. The SWI/SNF complex was originally identified in yeast and contains 10-15 protein subunits that

utilize energy from ATP to alter nucleosome position. This impacts gene expression by changing the accessibility of DNA regulatory elements that interact with transcription factors. Our goal was to advance our understanding the SWI/SNF complex in the regulation of neuronal processes that might be relevant to ID. The SWI/SNF complex is essential for cell-type specification during development, however, individuals with SWI/SNF related ID do not show widespread defects in cell-type specification. Therefore, cognitive dysfunction in SWI/SNF related ID disorders may be due to defects in post-mitotic neurons. To learn more about the post-mitotic neuronal function of the SWI/SNF complex we systematically investigated all SWI/SNF subunits in post-mitotic memory forming neurons of the *Drosophila* mushroom body (MB). Flies with MB-specific knockdown were screened for defects in morphology, short-term memory (STM), and long-term memory (LTM). Using this approach, we identified novel differential roles for components of the two main SWI/SNF subcomplexes known as BAP and PBAP. PBAP is required post-mitotically for neuron remodeling and is essential for both STM and LTM. In contrast, the BAP conformation of the SWI/SNF complex has a highly specific role in the formation of long-term stable memories. Cell type specific RNA-seq data suggests that SWI/SNF is essential for MB gene regulation at critical developmental transitions when MB neurons undergo structural plasticity in response to environmental or developmental signals. These structural changes are critical for different aspects of normal memory formation in mature adult flies. Our data provides potential insight into processes that might be disrupted in individuals with SWI/SNF related intellectual disability.

75 A whole-animal platform to advance a clinical kinase inhibitor into new disease space. *M. Sonoshita*[1,2], R. Cagan[2], A. Dar[3] 1) Institute for Genetic Medicine, Hokkaido University, Sapporo, JP; 2) Department of Cell, Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, NY; 3) Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY.

Synthetic tailoring of approved drugs for new indications is often difficult, as the most appropriate targets may not be readily apparent, and therefore few roadmaps exist to guide chemistry. Here, we present a multidisciplinary approach for accessing novel target and chemical space starting from an FDA-approved kinase inhibitor. By combining chemical and genetic modi- fier screening with computational modeling, we identify distinct kinases that strongly enhance ('pro-targets') or limit ('anti- targets') whole-animal activity of the clinical kinase inhibitor sorafenib in a Drosophila medullary thyroid carcinoma (MTC) model. We demonstrate that RAF—the original intended sorafenib target—and MKNK kinases function as pharmacological liabilities because of inhibitor-induced transactivation and negative feedback, respectively. Through progressive synthetic refinement, we report a new class of 'tumor calibrated inhibitors' with unique polypharmacology and strongly improved thera- peutic index in fly and human MTC xenograft models. This platform provides a rational approach to creating new high-efficacy and low-toxicity drugs.

76 Phosphatidic acid as a limiting host metabolite for the proliferation of the microsporidium *Tubulinosema ratisbonensis* in *Drosophila* flies. Adrien Franchet[1,2], Sebastian Niehus[1,2], Gaëtan Caravello[1,2], *Dominique Ferrandon*[1,2] 1) Université de Strasbourg, Strasbourg, France; 2) IBMC, CNRS UPR 9022, Strasbourg, France.

Microsporidia are located at the base of the fungal evolutionary tree. They are obligate intracellular parasites and harness host metabolism to fuel their growth and proliferation. However, how the infestation of cells impacts the whole organism and how the organism contributes to parasite proliferation remain poorly understood. We have developed a *Tubulinosema ratisbonensis* systemic infection model in the genetically-amenable *Drosophila melanogaster*host in which parasite spores obtained in a mammalian cell culture infection system are injected into adult flies. The parasites proliferate within flies and ultimately kill their hosts. As commonly observed for microsporidia infecting insects, *T. ratisbonensis* preferentially grows in the fat body and ultimately depletes the host metabolic stores. We find that supplementing the fly diet with yeast does not benefit the host but the parasite that increases its proliferation. Unexpectedly, fatty acids and not carbohydrates nor amino-acids are the critical components responsible for this phenomenon. Our genetic dissection of host lipid metabolism identifies a crucial compound hijacked by *T. ratisbonensis:* phosphatidic acid. We propose that phosphatidic acid is a limiting precursor for the synthesis of the parasite membranes and hence of its proliferation.

77 *Drosophila rab27* mediates longevity in mushroom body by downregulating TOR signaling. *Yi-Jhan Li*[1], Wen-Yu Lien[1], Chia-Lin Wu[2], Shu-Yi Huang[3], Chih-Chiang Chan[1] 1) Graduate Institute of Physiology, National Taiwan Univ., Taipei, Taiwan; 2) Graduate Institute of Biomedical Sciences, Chang Gung Univ., Taoyuan, Taiwan; 3) Dept. Medical Research, National Taiwan University Hospital, Taipei, Taiwan.

The mushroom body (MB) is an integration center in *Drosophila* brain, regulating homeostasis by controlling learning, memory, sleep, etc. Whether MB regulates lifespan extension is not clear. Here, we show that loss of Rab27 extend lifespan. *rab27* expresses in adult brain neurons including MB. Knockdown of *rab27* specifically in the α/β posterior (αβp) subset neurons of MB extended lifespan. Rab27 is an evolutionarily conserved Rab GTPase implicated in the regulation of vesicle exocytic machinery in neuroendocrine cells. However, we show that the lifespan extension regulated by Rab27 is independent of neuronal exocytosis. Loss of *rab27* deactivates TOR signaling, as indicated in the decreased level of phosphorylated S6 (p-S6). We show that, within the αßp neurons, Rab27 stabilizes the Tor effector S6K by anchoring S6K in the postsynaptic region. Therefore, loss of *rab27* may lead to the mislocalization of S6K, hence attenuating protein synthesis in the αßp neurons. Rab27 represents a novel node of lifespan regulation in a specific neural circuitry

78 With no lysine (WNK) Kinase: A Potassium Sensor. *J. Pleinis*[1], J. Sosa-Pagan[1], J. Humphreys[2], R. Akella[2], H. He[2], E. Goldsmith[2], A. Rodan[1] 1) Internal Medicine, University of Utah, Salt Lake City, UT; 2) Biophysics, University of Texas Southwestern Medical Center, Dallas, TX.

Plasma or hemolymph electrolyte concentrations are maintained within a narrow range in flies and humans through the regulation of ion transport in renal epithelia, implying the ability to sense deviations from normal. WNK mutations in mice and humans result in abnormal potassium concentrations, and WNKs have been proposed to indirectly sense plasma potassium via effects on intracellular chloride. Here, we investigate whether WNKs can directly sense potassium. WNKs phosphorylate and activate two downstream kinases, SPAK and OSR1, which phosphorylate and regulate renal ion transporters. Our lab has previously shown that this cascade is conserved in *Drosophila* Malpighian tubules. Here we show that potassium directly binds to the kinase domain of DmWNK (*Drosophila* WNK) and HsWNK3 (human WNK3) *in vitro* by differential scanning fluorimetry. Potassium also inhibits autophosphorylation, required for kinase activation, of DmWNK and HsWNK3 kinase domains *in vitro*, as analyzed by mass spectrometry and ProQ Diamond phospho-protein stain. Next, we examined the activity of DmWNK or HsWNK3 in Malpighian tubules, using phosphorylation of transgenically expressed kinase-dead rat SPAK as a readout. There was no change in kinase activity in low potassium bath, compared to a normal potassium bath, while kinase activity was inhibited in a high potassium bath ($p<0.0001$). Inductively coupled plasma mass spectrometry (ICP-MS) showed no change in intracellular potassium in low potassium bath compared to normal potassium bath, but showed significantly higher intracellular potassium in the high potassium bath ($p<0.001$). Chloride is also a known regulator of WNKs. The L295F mutation in the chloride-binding pocket of HsWNK3, which decreases chloride sensitivity, did not impair the inhibitory effect of potassium on HsWNK3 activity, suggesting a different binding location ($p<0.001$). In conclusion, our data suggest WNKs directly sense potassium to maintain ion homeostasis.

79 An intestinal zinc sensor couples micronutrient availability with developmental growth through Tor signalling. *Siamak Redhai*[1], Claire Pilgrim[1], Olena Riabinina[1], Tatiana Lopes[1], Farah Dahalan[2], Bhavna Chanana[1], Michaela Yuan[3], Michaela Wilsch-Brauninger[3], Elisabeth Knust[3], Li Wei-Hsiang [4], Richard Baines[4], Nikolai Windbichler[2], Irene Miguel-Aliaga[1] 1) MRC London Institute of Medical Sciences, Hammersmith Hospital Campus, Du Cane Road, London, W12 0NN, United Kingdom; 2) Sir Alexander Fleming Building, South Kensington Campus, Imperial College London, SW7 2AZ, United Kingdom; 3) Max Planck

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Nutrient sensors play key roles in maintaining homeostasis and driving nutritional adaptations. Nutrient-sensing mechanisms may lead to increase food intake when energy stores are low, but may also initiate satiety signals that lead to cessation of feeding when such stores have been replenished. In the digestive system, enteroendocrine cells have been previously shown to act as nutrient sensors, but it is unclear whether their siblings - the digestive/absorptive enterocytes – can also sense nutrients. We have performed a candidate RNAi screen to investigate possible nutrient-sensing mechanisms in enterocytes. This screen has uncovered a gene (which we have named *hodor*) required for normal larval growth and adult survival, particularly in nutrient-scarce conditions. Predicted structural analysis suggests that Hodor is a ligand-gated ion channel. To deorphanize the channel, we performed electrophysiology experiments in *Xenopus* oocytes, which revealed that Hodor is a pH-sensitive zinc-gated chloride channel. Immunohistochemical analyses using newly generated tools indicate that Hodor expression is confined to a very restricted subset of midgut enterocytes of the copper and iron regions, and to the proximal portion of the Malpighian tubules in *Drosophila* larvae. Cell-type specific knockdowns indicate that Hodor controls systemic growth from the interstitial cells – specialised enterocytes in the copper cell region. In interstitial cells, Hodor is required to maintain osmolarity and luminal acidity, but the systemic growth phenotypes can be uncoupled from these functions and result from a striking reduction in food intake and consequent depletion of energy stores. The reduced food intake and slow growth rates of *hodor* mutants can be partially rescued by activating Tor specifically in interstitial cells, or by dietary supplementation with zinc chloride. Together, these findings reveal a key and previously unrecognised role for this subset of enterocytes in regulating food intake by coupling micronutrient availability with Tor signalling and systemic growth.

80 *Drosophila melanogaster* sex peptide is a key regulator of female midgut morphology and physiology. *M. White*[1], A. Bonfini[2], M.F. Wolfner[1], N. Buchon[2] 1) Molecular Biology and Genetics, Cornell University, Ithaca, NY; 2) Cornell Institute of Host-Microbe Interactions, Cornell University, Ithaca, NY.

In *Drosophila melanogaster* females, the completion of mating and subsequent increase in egg production represents a large shift in energy homeostasis. To cope with the large nutrient demands spurred by reproduction, mated females undergo extensive physiological and behavior changes, including increased food intake and altered digestive processes. The mechanisms by which the female gut senses the shift from a virgin to a mated state remain poorly understood. We demonstrate that the male seminal fluid protein sex peptide (SP) is a key modulator of post-mating gut size and gene expression. SP triggers long-term post-mating responses via its binding to and gradual release from sperm and short-term post-mating responses through unbound SP. We find that SP is both necessary and sufficient to induce female gut growth. We also show that post-mating gut enlargement is a long-term post-mating response, requiring SP to be bound to and released from sperm. Additionally, receipt of SP is required for the transcriptional changes that occur in the gut upon mating, such as the up-regulation of protein and lipid metabolic genes. These transcriptional shifts likely reflect gut metabolic changes which help supply the female with the nutrients needed for egg production. Thus, we describe a novel role for SP in regulating post-mating female gut morphology and physiology

81 Activity of the nuclear receptor Seven up in different tissues controls distinct processes of oogenesis. *L. Weaver*, D. Drummond-Barbosa Department of Biochemistry and Molecular Biology, Johns Hopkins University, Baltimore, MD.

Reproduction is essential for species survival and is intimately linked to the physiology of an organism. Nuclear receptors (NRs) are widely expressed transcription factors that mediate the effects of many circulating factors to modulate physiology and reproduction. While many studies have focused on the roles of NRs directly in the ovary, it remains largely unknown how the actions of NRs in peripheral tissues influence oogenesis. To identify NRs with roles in somatic cells of the ovary and/or other organs to control processes during *Drosophila* oogenesis, including germline stem cell (GSC) maintenance and proliferation, early germline cyst survival, follicle growth, vitellogenesis, and ovulation, we performed an RNAi-based screen. We ubiquitously knocked down all *Drosophila* NRs individually in somatic cells of adult *Drosophila* females and analyzed each step of oogenesis. Our screen identified several NRs with potentially novel roles in oogenesis, including the NR encoded by *seven up* (*svp*). Svp and its mammalian homologs (COUP-TFs) have known roles in eye and nervous system development, tumorigenesis, and angiogenesis. We found that global somatic reduction of *svp* leads to reduced rates of egg laying. Furthermore, *svp* knockdown increases the rate of GSC loss, and the death of early germline cysts and of vitellogenic follicles. Interestingly, tissue-specific knockdown experiments revealed that *svp* remotely controls different processes during oogenesis through separate mechanisms involving distinct tissues. Specifically, we found that adipocyte-specific *svp* knockdown impairs GSC maintenance and germline cyst survival, whereas oenocyte-specific *svp* knockdown increases the death of vitellogenic follicles without any effects on GSC number. These results suggest that NRs can have multiple parallel roles in controlling reproduction, for instance by acting on separate tissues to regulate distinct aspects of oogenesis. Future studies will identify critical *svp* transcriptional targets (i.e. secreted molecules and/or proteins that regulate downstream systemic factors) in adipocytes and oenocytes that influence specific processes during oogenesis. Understanding the full set of mechanisms through which NR signaling regulates oogenesis will provide new insight into how whole-body physiology influences reproduction.

82 Male-female differences in Dilp2 secretion contribute to sexual size dimorphism in *Drosophila*. *Elizabeth Rideout*, Jason Millington, George Brownrigg, Paige Basner, Kitty Sun Dept. Cellular and Physiological Sciences, University of British Columbia, Vancouver, British Columbia, CA.

Female flies are visibly larger than male flies due to an increased rate of larval growth. Several studies have implicated the insulin/insulin-like growth factor signaling pathway (IIS) as a key determinant of this sex difference in the rate of larval growth and body size. Yet our knowledge of the sex-specific regulation and function of IIS during larval development remains incomplete. Our analysis of larval growth revealed a key role for *Drosophila insulin-like peptide 2* (*dilp2*) in creating the sex difference in larval growth. Our previous data showed that Dilp2 secretion is normally higher in female larvae than in males. We now show that this sex-biased Dilp2 secretion affects sexual size dimorphism, as females, but not males, lacking *dilp2* are smaller than control animals. Since Dilp2 is secreted in response to dietary protein, we investigated whether females were more sensitive to changes in dietary protein than males. Indeed, a 50% reduction in dietary protein, but not sugar, reproduced the effects of *dilp2* loss on female body size, but had no effect on male growth. Since a simultaneous reduction in dietary protein and loss of *dilp2* had non-additive effects on body size, this finding suggests a model in which a protein-rich diet enhances Dilp2 secretion to promote female growth and body size.

83 Stromalin constrains memory acquisition by developmentally limiting synaptic vesicle pool size. *A. Phan*[1], C.I. Thomas[2], M. Chakraborty[1], J.A. Berry[1], N. Kamasawa[2], R.L. Davis[1] 1) Neuroscience, The Scripps Research Institute, Jupiter, FL; 2) Electron Microscopy Core Facility, Max Planck Florida Institute for Neuroscience, Jupiter, FL.

Cohesin complex protein Stromalin was recently identified as a novel memory suppressor gene from a large learning and memory RNAi screen. However, its mechanism of action was unknown. Surprisingly, we found that Stromalin functions to constrain synaptic vesicle pool size in Drosophila neurons. RNAi mediated Stromalin knockdown in dopamine neurons during a critical developmental period enhanced memory acquisition and increased synaptic vesicle pool size without altering the number of dopamine neurons. However, Stromalin knockdown did not affect dopamine axons, presynaptic numbers, or presynaptic volume. This developmental effect of Stromalin persisted into adult flies, leading to strengthened synaptic connections between dopamine and Kenyon cells, enhancing olfactory memory acquisition in adult flies. Impairing the anterograde synaptic vesicle motor protein Unc104/KIF1A corrected the synaptic vesicle content in dopamine neuron axon terminals and rescued the enhanced learning phenotype in Stromalin knockdown flies. Our results reveal a

novel mechanism for memory suppression and also provide evidence that the size of the synaptic vesicle pool is controlled genetically and independently from other aspects of neuron structure and function through Stromalin.

84 Timing temporal transitions during brain development. *A.M. Rossi*, C Desplan Biology, New York University, New York, NY.

The brain is comprised of diverse neuron types that when wired together result in our rich repertoire of behaviors. The proper construction of neural networks is dependent on neuron types being produced at the correct time (temporal patterning) and place (spatial patterning). In some *Drosophila* lineages, which are cells that are produced from a single stem cell, the temporal patterning mechanism, or clock, that controls when a neuron type is produced is the result of the sequential expression of transcription factors, which feedforward and feedback on each other to make the clock tick. However, this mechanism has not been shown to operate in all lineages, particularly long-lived lineages like those in the developing *Drosophila* central brain. In those lineages, opposing gradients of two RNA binding proteins, Imp and Syp, are expressed by stem cells and inherited by their undifferentiated daughter neurons to produce different neuron types in a sequential manner. To interrogate how this clock ticks and how the switch from high-Imp/low-Syp to low-Imp/high-Syp is achieved, we use the mushroom body lineage since it is comprised of only three, sequentially born neurons (γ, then α'β' and finally αβ) and since we know a great deal about how they are specified during development. Using Mosaic Analysis with a Repressible Cell Marker (MARCM) we demonstrate that when reception of extrinsic cues is blocked, particularly TGFβ (Activin) and ecdysone signaling, the lineage produced from a mutant mushroom body stem cell is less diverse than surrounding wild type stem cells in that it no longer contains α'β' neurons. We show by immunofluorescence that the loss of α'β' neurons is the result of an altered Imp/Syp ratio in mutant stem cells during late-L3, the larval stage when this neuron type is produced. We propose that timely extrinsic cues act on the Imp/Syp gradients to define a temporal window in which Imp and Syp can coexist, allowing α'β' neurons to be specified. In the absence of these cues, and since Imp and Syp inhibit each other, these RNA binding proteins are free to function as a bistable switch, effectively skipping the second temporal window but still achieving the low-Imp/high-Syp state during which αβ neurons are born. In vertebrates, it is well established that extrinsic cues play an important role in specifying when specific neurons are produced. The results we present illustrate that extrinsic cues also play an important role in *Drosophila* brain development.

85 The beta-alanine transporter *BalaT* localizes to visual lamina and sustains vision in extended light conditions. *A. Moehlman*[1], D. Stenesen[2], H. Kramer[1,3] 1) Department of Neuroscience, UT Southwestern Medical Center, Dallas, TX; 2) Department of Biology, University of Dallas, Dallas, TX; 3) Department of Cell Biology, UT Southwestern Medical Center, Dallas, TX.

The initial synaptic event in the Drosophila compound eye requires release of the neurotransmitter histamine from photoreceptor axons to trigger histamine-gated Cl^-channels in lamina neuron dendrites. Although histamine can be synthesized via the enzyme Hdc, this *de novo* synthesis is not sufficient to maintain proper visual transmission. Thus, the visual system utilizes a multi-step recycling pathway to clear excess histamine from the synaptic cleft and return it to photoreceptors neurons. This recycling requires the addition of beta-alanine to histamine to yield the transport metabolite carcinine, a reaction performed by the enzyme Ebony in lamina epithelial glia. Carcinine is taken up by photoreceptors through the photoreceptor-specific organic cation transporter CarT, presumably after its proposed glial counterpart mediates the release from glia. A previous study demonstrated that BalaT (CG3790), a close homolog of CarT, transports beta-alanine (1). Our data indicate that BalaT is expressed in lamina epithelial glia. This conclusion is based on CRISPR/Cas9-mediated tagging of the endogenous BalaT N-terminus with tdTomato or its C-terminus with a Ty1 epitope. Furthermore, a *BalaT-T2A-Gal4* line drives expression specifically in lamina glia. *BalaT*-null mutants exhibit synaptic transmission defects by ERG (1). In addition, we identified a role for BalaT in maintaining photoreceptor depolarization following constant light rearing. Consistent with a defect in the histamine recycling pathway, we observe an accumulation of carcinine in lamina epithelial glia in *BalaT*-null flies. Thus, our data suggest a requirement for BalaT in the Drosophila visual lamina that supports the histamine-carcinine recycling pathway.

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86 Sleep need is driven by a neural circuit involving stress-sensing peripheral neurons and the central brain. Lawrence Satterfield, Glen Seidner, James Robinson, Meilin Wu, Erika Barrall, Vanessa Lambatan, Tianhao Qiu, *William Joiner* University of California San Diego, La Jolla, CA.

The sleep/wake cycle is thought to be controlled by two main processes: a circadian clock that primarily regulates timing of arousal and a homeostatic mechanism that regulates duration of arousal according to sleep need. Although the mechanisms underlying circadian clock function have been studied in detail, the homeostatic process by which sleep need is sensed and discharged remains unknown. Several labs, including ours, have reported that only select populations of wake-promoting neurons drive sleep need after extended arousal. However, there is no consensus as to the identity of these neurons or the neural circuits that they comprise. Here we use a combination of thermogenetics and Gal4/Gal80-based mapping to demonstrate that environmental stress-sensing peripheral ppk neurons are an essential feature of many of these circuits. Furthermore, using trans-Tango and a circuit epistasis approach, we identify putative effectors of sustained ppk neuronal activity in the central brain. Thermogenetic activation of these effector neurons leads to a profound increase in sleep, whereas thermogenetic inhibition of them drives waking, thus demonstrating that these neurons are sufficient and necessary for sleep. Collectively our results suggest a simple model in which central sleep-promoting neurons are acutely inhibited by arousal-promoting signals but are homeostatically upregulated following prolonged suppression by ppk neurons to drive sleep need.

87 The non-nuclear splice isoform of NFκB gene Dif modulates sensitivity to ethanol sedation in Drosophila melanogaster. *T.P. Wijesekera*, L.K. Lew, N.P. Stephens, N.S. Atkinson University of Texas at Austin, Austin, TX.

NFκBs are canonically described as transcription factors and are the outputs of the Toll and IMD (immune deficiency) signaling pathways that modulate innate immunity in Drosophila. Toll signaling pathway functions in the fat body in immunity, through the NFκB gene Dif (Dorsal-like-immunity factor). Interplay between the immune system's NFκB genes, and alcohol consumption is extensively demonstrated in rodent models and humans. Ethanol activates some signaling pathways involved in infection and in mammals, the activation of these pathways was shown to contribute to addictive drinking and ethanol-induced neurodegeneration. The primary NFκB of the Toll pathway in adult flies is Dif, and was previously shown to contribute to normal ethanol sensitivity. Dif was hypothesized to be expressed in two splice isoforms, a nuclear Dif A and a non-nuclear Dif B. Using mutations that eliminate one splice form at a time, this study shows that only the putative non-nuclear B isoform functions in providing resistance to ethanol-induced sedation in flies. The resistance phenotype is recessive and shows no sexual dimorphism. The gene is not required to acquire functional tolerance to ethanol. Using immunohistochemistry, we demonstrate the neuronal and non-nuclear distribution of Dif B in the adult mushroom bodies and antennal lobes, which are structures significant in ethanol response, and its absence in the fat body. Furthermore, an increase in the expression of Dif B is observed upon exposure to ethanol. Dif A, while being absent from the brain, was distributed in the cytoplasm and nucleus of the fat body, indicating that this isoform of Dif mediates Toll signaling in this tissue upon immune challenge. This describes the interesting finding of a mutually exclusive cellular and tissue distribution, and functioning of two splice isoforms of the same NFκB gene. We successfully tagged the isoforms with fluorescent reporter tags and epitope tags in separate fly lines using the CRISPR/Cas9 technology. The tagged proteins were observed to have similar expression patterns to the native proteins. These tagged proteins could provide information on whole body

distribution and gene expression under different treatments and conditions, as well as downstream signaling pathways through protein-protein interactions and co-localization, leading to new discoveries on the functioning of these important transcription factors in ethanol response and beyond.

88 Serotonergic modulation of goal-directed habituation during exploration in *Drosophila*. *M. de la Flor*[1], S. Zhang[1], L. Jen[1], G. Gunaratne[1], B. Dauwalder[1], S. Garcia[1], M. Wang[1], R. Roman[2] 1) Department of Biology and Biochemistry, University of Houston, Houston, TX; 2) Department of Biology, The University of Mississippi, Oxford, MS.

Exploration is a complex behavior through which animals learn about features in their environment that may impact survival and reproduction. However, the neuromodulatory mechanisms of exploration are unclear. Novelty and a lack of information motivate *specific* exploration allowing animals to gather vital information about their surroundings. When introduced to a novel open-field arena *Drosophila melanogaster* display robust locomotor exploratory behaviors. Over time flies habituate the arena's novelty thereby reducing exploratory behaviors. Here we report on a putative serotonergic, dorsal paired medial neuron-mushroom body circuit that modulates habituation during exploration.

The fly brain contains about 100 serotonergic (5-HT) neurons that modulate important behaviors. Our work shows that pharmacological and transgenic promotion of 5-HT signaling in the fly brain increases habituation during exploration, while inhibition of 5-HT signaling reduces habituation. Using transgenic approaches we targeted populations of 5-HT secreting neurons to identify those that may modulate habituation during exploration. Our results show that activating the 5-HT secreting dorsal paired medial neurons is sufficient for normal habituation during exploration.

Intrinsic mushroom body neurons are known postsynaptic targets of the dorsal paired medial neurons. The d5HT1A and d5HT1B receptors are broadly but differentially expressed in the α/β and γ lobes of the mushroom bodies respectively. Our results show that flies mutant for the d5HT1A and d5HT1B receptors have significant habituation deficits, which we rescued by targeted expression of the d5HT1A and d5HT1B receptors in the adult mushroom bodies. Taken together our work suggests that 5-HT signaling from the dorsal paired medial neurons to mushroom body neurons through the d5HT1A and d5HT1B receptors form a circuit that modulates habituation during exploration.

89 Secrets of the zombie fly: Determining the neurological basis of behavioral manipulation in *Drosophila*. *C. Elya*, B. de Bivort Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.

Many microbes induce striking behavioral changes in their animal hosts, but how they achieve this is poorly understood at the molecular or neural circuit level. This is due in part to the difficulty of studying non-model organisms with limited tools. As a graduate student, I discovered a strain of the fungal behavior-manipulating fly pathogen *Entomophthora muscae* infecting wild drosophilids and developed methods to propagate the fungus in lab-reared *Drosophila melanogaster*. Before sunset on their final day of life, my infected flies manifest the moribund behaviors characteristic of *E. muscae*infections: they climb to a high location, extend their proboscises, and raise their wings in a pose that permits ideal spore dispersal. In characterizing the course of infection, I discovered that *E. muscae* invades the host nervous system, which could provide a direct route for altering host behavior. As a postdoctoral researcher, I am focusing on understanding the mechanistic basis of summiting behavior, initially hypothesizing that the fly's endogenous gravitactic circuitry is manipulated by the fungus to induce upward climbing immediately before death. In developing a high-throughput assay to measure summiting behavior, I found that moribund flies consistently exhibit a burst of activity within their final three hours of life. Prior to this activity bout, flies are much more attracted to nutritive media than are healthy flies or fungus-treated flies at earlier stages of infection. During the activity bout, flies tend to move away from media, even if the media is positioned at the top of the chamber. This newly-uncovered activity element of summiting behavior suggests that the fungus may be inducing an internal arousal state in addition to or instead of manipulating gravitactic circuitry to position the fly for optimal fungal dispersal. I next aim to identify the neurons needed for fungal-induced summiting through performing a neuronal inactivation screen using my behavioral platform and to perform biochemical analysis of hemolymph from summiting animals to identify fungal molecules that elicit summiting behavior. In this way, I aim to begin defining the pathways by which *E. muscae* induces summiting behavior in its fly host.

90 Crk adaptor protein containing multiprotein signaling complexes regulate actomyosin-dependent developmental processes. *A.J. Spracklen*[1], A.N. Bonner[2], E.M. Thornton-Kolbe[2], M. Peifer[1,2] 1) Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC; 2) Department of Biology, University of North Carolina, Chapel Hill, NC.

The ability of cells to self-assemble into functional tissues is the most remarkable feature of multicellular life. Underlying this is the ability of cells to alter their adhesive properties, remodel their cytoskeletons, and generate actomyosin-dependent forces in response to a variety of cues. Context-specific multiprotein signaling complexes integrate multiple signals into appropriate and coordinated cellular responses. Disruption of this results in developmental defects, loss of adult tissue homeostasis, and can drive cancer progression. Crk is a highly conserved, small adaptor protein that uses its SH2 and SH3 domains to link upstream inputs to downstream effectors, including cytoskeletal regulators. While Crk is a well-known effector of oncogenes, like Abelson tyrosine kinase, little is known about how Crk regulates cell behavior during normal development. We are using a combination of RNAi and a powerful gene replacement platform we developed to uncover the conserved developmental roles of Crk, using *Drosophila* embryogenesis as a model. We found Crk is essential for embryonic viability and loss of Crk results in multiple defects in actomyosin-dependent morphogenic events. Loss of Crk disrupts the dynamics of interphase F-actin caps during syncytial divisions, leading to spindle collision and cell loss in the early embryo. Our current approaches are focused on asking whether Crk regulates Arp2/3-dependent actin remodeling during these early divisions. We also observe a failure to properly stabilize contractile actomyosin rings during cellularization, suggesting Crk plays an important role in regulating actomyosin localization and/or activity. In addition to these defects in early embryogenesis, Crk loss also leads to a range of central nervous system (CNS) patterning defects, ranging from subtle midline crossing defects to severe disruption of CNS architecture. We are currently asking whether Crk plays a direct role in axon guidance or whether Crk plays dual roles, participating in early events in neurulation (neuroblast delamination, survival, etc.) and also in axon guidance later in CNS patterning. These data provide important new insights into the conserved mechanisms underlying how multiprotein signaling complexes regulate cell behavior during normal development and disease.

91 Proteomic analysis of ovarian ring canals reveals the mechanism of ubiquitin-mediated regulation of the F-actin cytoskeleton. *A. Hudson*[1], K. Mannix[1], J. Gerdes[1], R. Starble[1], M. Kotteman[4], L. Cooley[1,2,3] 1) Department of Genetics, Yale University School of Medicine, New Haven, CT; 2) Department of Cell Biology, Yale University School of Medicine, New Haven CT; 3) Department of Molecular, Cellular & Developmental Biology, Yale University, New Haven, CT; 4) Laboratory of Genome Maintenance, The Rockefeller University, New York, NY.

During oogenesis, specialized intercellular bridges called ring canals form to accommodate the transfer of cytoplasm from the nurse cells to the oocyte. Ring canals grow in diameter during oogenesis, and this expansion requires a dynamic and highly organized F-actin cytoskeleton. Our previous work showed that *kelch* mutant egg chambers have highly disorganized ring canal F-actin that blocks normal oocyte growth rendering females sterile. We demonstrated that Kelch functions with Cullin 3 in a Cullin 3-RING ubiquitin ligase complex ($CRL3^{Kelch}$) to organize the cytoskeleton that drives ring canal expansion, presumably by targeting a substrate for ubiquitylation and proteolysis. We used tandem affinity purification followed by mass spectrometry to identify ring canal substrates of $CRL3^{Kelch}$, and identified HtsRC, a known ring canal protein, as a potential substrate. Furthermore, localized biotinylation of ring canal proteins with the

substrate-binding domain of Kelch fused to ascorbate peroxidase (APEX::KREP) resulted in biotinylated HtsRC. We present genetic evidence consistent with HtsRC being the single $CRL3^{Kelch}$ substrate important for the ordered expansion of the ring canal cytoskeleton, as well as biochemical evidence indicating that HtsRC is ubiquitylated and degraded by the proteasome. We also identify a short sequence motif in HtsRC that is necessary for Kelch binding. CRISPR-mediated editing of the Kelch-binding motif produced new hts alleles that phenocopy kelch: female sterility caused by ring canals nearly occluded with disorganized F-actin. Thus, control of HtsRC levels is essential for normal ring canal architecture. Finally, we determined that the N-terminal region (NTR) of Kelch is essential to for the proper regulation of $CRL3^{Kelch}$ activity. These findings uncover an unusual mechanism during development wherein a specialized cytoskeletal structure is regulated and remodeled by the ubiquitin-proteasome system.

92 The significance of sequestering H2A, H2Av and H2B on lipid droplets. *Roxan Stephenson*[1], Lili Chen[1], Jonathan Thomalla[1], Mathias Beller[2], Michael Welte[1] 1) Biology, University of Rochester, Rochester, NY; 2) Heinrich Heine University Düsseldorf.

Lipid droplets (LDs) are ubiquitous organelles with well-established roles in lipid metabolism and a poorly understood function in protein homeostasis. One of the best characterized examples of LD-protein sequestration occurs in *Drosophila* embryos where LDs recruit maternally synthesized histones H2B, H2A, and H2Av to provide a histone pool that supports embryonic development. Histones are anchored to LDs via the Jabba protein. Why histones are stored specifically on LDs rather than elsewhere is unknown.

Newly laid $Jabba^{-/-}$ embryos lack the maternal supply of H2A, H2Av and H2B proteins. To understand their absence, we first examined how the maternal histone supply normally arises. LDs are generated in nurse cells (NCs); later, NC cytoplasm, including LDs, is transferred to the oocyte. Using flies expressing endogenously regulated H2Av-RFP or H2B-mEOS, we quantified histone levels in the ooplasm via imaging. In wild type, H2Av and H2B levels increased, with a dramatic rise after transfer from NCs was completed, arguing for substantial histone synthesis in the oocyte. In $Jabba^{-/-}$, in contrast, histone levels in the ooplasm decrease as oocytes mature, suggesting that without Jabba, oocyte histones are unstable. Western analysis confirmed that stage 14 $Jabba^{-/-}$ oocytes have much less H2Av than wild type. Intriguingly, preliminary data in the wild type suggests that Jabba protein expression is dramatically upregulated as oocytes mature. We propose that the level of Jabba determines how much H2Av can accumulate at any given time point. Consistent with this hypothesis, H2Av accumulation is intermediate in oocytes expressing a single copy of Jabba.

Using deletion analysis, we identified a 32aa region in Jabba important for LD targeting and a 16aa necessary for histone binding. We then assessed if LD binding is necessary to maintain histones in the ooplasm. Although Jabba[193-320], which binds histones but not LDs, was rare to undetectable in embryos and oocytes, it was highly enriched in NC nuclei. We are currently testing the fate of the maternal histone pool in this genotype. We propose that Jabba binding to LDs retains both Jabba and histones in the cytoplasm and promotes their transport from NCs to the oocyte, where Jabba prevents histone degradation.

93 Mechanotransduction at tricellular junctions. *H. Yu*, J. Zallen HHMI/Sloan Kettering Institute, New York, NY.

The ability to sense mechanical forces is a universal property of cells. This property allows cells to detect mechanical changes in their environment and translate mechanical cues into biochemical and electrical signals that influence diverse biological processes. In particular, strengthening cell adhesion in response to mechanical force is a major outcome of mechanotransduction signaling pathways. In epithelia, forces are transmitted between cells at bicellular or tricellular junctions, where two or three cells come into contact. Although the central role of cadherin complexes in mechanotransduction at bicellular junctions has been extensively studied, it remains unknown whether or how tricellular junctions sense and respond to tension. By performing a large-scale screen for proteins that localize to tricellular junctions, we identified the adherens junction-associated component Canoe as a central player in mediating mechanotransduction pathways at tricellular junctions. The Canoe protein is enriched at tricellular junctions in a tension-dependent manner, and reducing epithelial tension using pharmacological or biophysical methods rapidly shifts Canoe localization from tricellular to bicellular junctions. In the absence of Canoe activity, tricellular junctions break apart under actomyosin-generated pulling forces. These results suggest that Canoe plays an essential role in stabilizing adhesion among three cells. We are currently investigating the upstream signaling pathways that recruit Canoe to tricellular junctions and stabilize membrane-cytoskeletal attachments at tricellular junctions under force.

94 Wash functions in the nucleus to affect Nuclear Envelope budding. *J.R. Decker*, J.M. Verboon, C.L. Prentiss, S.M. Parkhurst Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA.

Transport of macromolecular complexes from the nucleus to the cytoplasm is fundamental for all developmental processes and has been thought to occur exclusively via Nuclear Pore Complexes. Nuclear Envelope Budding (NE-budding) is an alternative pathway for nuclear exit of particularly large, developmentally-required ribonucleoprotein (megaRNP) complexes. In this recently described process, megaRNPs are encapsulated by inner nuclear membrane, cross the perinuclear space, fuse with the outer nuclear membrane, and are released into the cytoplasm, in a mechanism strikingly similar to herpesvirus nuclear egress. NE-budding is evolutionarily conserved across species and, in the case of Drosophila, is necessary for processes such as neuro-muscular junction development and mitochondrial integrity. Using biochemical characterizations of nuclear protein complexes, electron and super-resolution microscopies, and a panel of mutations which disrupt only specific activities, we show that one Wiskott Aldrich Syndrome family member, Wash, and its regulatory complex (WASH Regulatory Complex (SHRC)) are necessary for NE-budding. Wiskott-Aldrich Syndrome family proteins are known to influence membrane-cortical cytoskeleton interactions. Wash and its SHRC function upstream of the Arp2/3 complex in the cytoplasm to promote actin filament branching necessary for driving membrane deformations and generating cargo-laden vesicles. Wash and its SHRC also have diverse roles in the nucleus: *wash* mutants display disrupted nuclear morphology and aberrant organization of nuclear organelles. *wash* and SHRC mutants lack nuclear buds and display phenotypes associated with the loss of NE-budding. We find that Arp2/3-dependent Wash actin nucleation activity is required for NE-budding to occur. We are currently investigating how Wash and its SHRC drive structural changes at the interface of the nucleoskeleton and the overlying nuclear membrane to affect NE-budding.

95 Spectraplakin Shot maintains perinuclear microtubule organization in polyploid cells. *Tianhui Sun*, Yinlong Song, Mengqi Ma, Jianli Dai, Xin Liang, Jose Pastor-Pareja School of Life Sciences, Tsinghua University, Beijing, CN.

Presence of polyploid cell types in diploid organisms is widespread in nature. Polyploid cells endoreplicate their DNA through a modified cell cycle that skips mitosis as part of their differentiation programs. Upon cell cycle exit and differentiation, non-centrosomal sites govern microtubule distribution in most cells. Little is known about how polyploid cells, differentiated but cycling, organize their microtubules. Here, we show that microtubules in *Drosophila* larval adipocytes and other polyploid tissues form a dense perinuclear cortex responsible for nuclear size and position. A critical component of the perinuclear microtubule organizer (pnMTOC) is the spectraplakin Shot. Absence of Shot caused collapse of the pnMTOC into a condensed, black hole-like organizer, as plasma membrane and other structures and organelles appear to be suctioned towards its center, deeply disrupting cell organization. Both the normal pnMTOC and the ectopic organizer respond to alterations in the endocycle. Furthermore, converting normally diploid cells into polyploid cells was sufficient to induce pnMTOC formation. Also involved in pnMTOC formation are LINC/SUN nuclear membrane proteins, microtubule-binding Patronin, the motor protein Kinesin and microtubule-severing Katanin. In all, our study reveals the importance of perinuclear microtubule organization for stability of endocycling *Drosophila* cells and assigns specific functions to several pnMTOC components.

96 Epigenetic effects of transposable elements in 3D nuclear space impact genome function. *Grace Y.C. Lee*[1,2], Yuki Ogiyama[3], Nuno C. Martins[4], Brian J. Beliveau[5], C.-ting Wu[4], Giacomo Cavalli[3], Gary H. Karpen[1,2] 1) Lawrence Berkeley National Lab, Berkeley, CA; 2) Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA; 3) Institute of Human Genetics, CNRS, Montpellier, France; 4) Department of Genetics, Harvard Medical School, Boston, MA; 5) Department of Genome Sciences, University of Washington, Seattle, WA.

Transposable elements (TEs) are ubiquitous genome parasites whose evolution is tightly intertwined with the function and evolution of host genomes. They are abundant in pericentromeric heterochromatin (PCH), which is enriched for the repressive epigenetic mark H3K9me2/3 and its reader protein, HP1a. TEs are also prevalent in the gene-rich euchromatic genome and, interestingly, can lead to tens of Kb enrichment of H3K9me2/3 at flanking euchromatic sequences. Due to the biophysical properties of HP1a, PCH of different chromosomes can coalesce into a single domain within the 3D nuclear space through liquid-liquid fusion (PCH domain). This domain is enriched with silencing proteins and can significantly influence the function of genes that are recruited/brought into this phase-separated 3D space. We hypothesized that euchromatic TEs enriched for H3K9me2/3 and HP1a could also spatially interact with the main PCH domain, influencing euchromatic genome function. To investigate the spatial contacts of euchromatic loci with PCH, we developed a novel analysis method that incorporates Hi-C reads originating from repetitive PCH DNAs, which were excluded from previous Hi-C studies. Despite being far from PCH on a linear chromosome, ~14% euchromatic TEs show 3D interactions with PCH, which were validated with locus-specific FISH using Oligopaint. We leveraged polymorphic (presence/absence) TE insertions in natural populations and compared the 3D organization of homologous sequences with and without TE-induced H3K9me2/3 enrichment, which showed that spatial contacts between euchromatic loci and PCH require the presence of repressive marks. Importantly, population genetic analysis revealed that TEs spatially interacting with PCH are more strongly selected against, suggesting functional consequence of these 3D contacts with PCH. Our findings demonstrate that naturally occurring TEs could significantly influence the 3D organization of the genome, having a far-reaching impact on the function and evolution of the gene-rich euchromatic genome.

97 The polycomb silencing switch during germline development. *S.Z. DeLuca*, A.C. Spradling Embryology, Carnegie Institute, Baltimore, MD.

Gene silencing through polycomb-group (PcG) proteins regulates transcriptional programs during development and stabilizes cell fates during differentiation. While genetics and biochemistry have identified and characterized many of the proteins required for PcG silencing, much less is known about how PcG silencing is temporally controlled during development. The Drosophila ovary contains two of the longest and most experimentally tractable stem cell lineages for studying developmental timing, so we examined PcG silencing in individual cell types along both ovarian lineages. Using new genetic reporters that assay silencing at many endogenous loci in single cells, we found that while follicle cells exhibit PcG silencing throughout their development, germ cells switch on PcG silencing in nurse cells at stage 5 of oogenesis. By purifying germ cells before, during, and after the switch, we found that PcG silencing is induced by the redistribution of PRC2 activity. In early germ cells, all non-transcribed loci are trimethylated on H3K27 though Pcl, a non-specific DNA binding subunit of PRC2. As nurse cells and oocytes differentiate within an interconnected cyst, mRNA encoding Pcl is transported out of nurse cells and into the oocyte, depleting Pcl protein from nurse cells. As Pcl levels drop, PRC2 activity is relocalized to pho-bound PREs, where PRC1 is then concentrated. Residual Pcl-PRC2 then expands H3K27me3 out from PREs to seed large repressive domains. Therefore, directed mRNA transport of a PRC2 subunit functions as the primary switch to induce PcG silencing in the germline stem cell lineage. Because Pcl and asymmetric mRNA transport are critical for differentiation in other stem cell lineages, we speculate that similar switches may control PcG silencing in other developmental contexts.

98 H3K9me3-mediated gene silencing and female fate maintenance in Drosophila germ cells. Anne Smolko, Laura Shapiro-Kulnane, *Helen Salz* Dept Genetics, Case Western Reserve Univ, Cleveland, OH.

In *Drosophila,* the preservation of germ cell sexual identity is critical for gametogenesis and defects in sex-specific programming lead to both infertility and germ cell tumors. Our recent work indicates that female identity is maintained by localized deposition of H3K9me3 repressive chromatin on male identity genes to secure their silence. Classically thought of as a mark associated with the non-coding portions of the genome, H3K9me3 has only recently emerged as a player in maintaining differential gene expression.

We have identified SETDB1 as the required H3K9 methyltransferase. As expected, differential ChIP-seq analysis shows that germ cell specific loss of SETDB1 (*nos>setdb1-RNAi)* leads to a loss of H3K9me3. Interestingly, when integrated with RNA-seq data sets, we identified only 21 genes at which loss of the H3K9me3 peak correlated with ectopic expression. These SETDB1-dependent H3K9me3 domains are highly localized and do not spread into neighboring genes. Prior studies established a role for SETDB1 in germline piRNA biogenesis and transposable element (TE) silencing. However, the absence of TE sequences at these 21 loci suggests that H3K9me3 deposition is controlled by a mechanism that is different from what has been described for piRNA-guided H3K9me3 deposition on TEs. Furthermore, piRNAs are unlikely to contribute to sexual identity maintenance as mutations that specifically interfere with piRNA production, such as *rhino,* show no masculinization of the gene expression program and complete oogenesis. Thus, we have uncovered a novel function for SETDB1 unrelated to its canonical role in piRNA biogenesis and TE silencing. Our work further suggests that the primary function of SETDB1 in the sex maintenance pathway is to control transcription of *phf7,* a key regulator of male germ cell sexual fate. *phf7* encodes a testis-specific protein. Tight control is essential as ectopic expression of PHF7 in female germ cells leads to a female to male switch in transcriptional programming. *phf7* is primarily regulated by alternative transcription start site (TSS) selection. We find that in female germ cells H3K9me3 accumulates over the silenced testis-specific TSS. Loss of this peak correlates with sex-inappropriate transcription from the upstream testis-TSS and ectopic PHF7 protein expression. Thus, our findings support a novel model in repressive H3K9me3 chromatin assembles at the *phf7* testis-TSS to secure its silence thereby preventing inadvertent female-to-male reprogramming.

99 Satellite DNA Regulation in *Drosophila melanogaster*. *X. Wei*[1], D. Eickbush[2], I. Speece[2], A. Larracuente[2] 1) Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY; 2) Department of Biology, University of Rochester, Rochester, NY.

Satellite DNAs (satDNAs) are tandemly repeated DNAs found primarily near centromeres, telomeres, and on sex chromosomes. They can make up to half or more of eukaryotic genomes. Although typically thought of as junk DNAs, recent studies show that satDNAs play important roles in chromosome segregation, chromosome recognition, and maintaining genome stability. Abnormal satDNA activity is associated with chromosome missegregation, aging, and cancer. Despite its association with important phenotypes, we currently know little about satDNA maintenance at the chromatin level, or if satDNAs have specific functions. Previous studies have reported satDNA-derived transcripts. However, whether or not satDNA expression is regulated—and if it so, how—remains an open question. Using the *Drosophila* germline as a model system, we characterized the expression pattern and regulatory network of satDNAs using a combination of genomic, cytological, and molecular approaches. Our data revealed that the satDNAs are transcribed into long noncoding RNAs (lncRNAs) and then processed into small RNAs in the germline, in a way resembling piRNAs (PIWI interacting RNAs), a subset of small RNAs that function to repress transposable elements (TEs) to maintain genome stability. Moreover, we found that the satDNA piRNA production is regulated by the same piRNA pathway components as the dual-strand cluster 42AB. Taken together, our findings suggest that satDNAs are regulated by piRNAs originating from their own genomic loci, and that these piRNAs maintain heterochromatin at the satellite. This novel mechanism for satDNA regulation provides insight into general features important for understanding the roles of satDNAs in the germline.

100 Diversification and collapse of the Drosophila telomere elongation mechanism. *B. Saint-leandre*[1], S. Nguyen[2], Mia Levine[1] 1) Biology, Univeristy of Pennsylvania, Philadelphia, PA; 2) Genetics, Univeristy of Pennsylvania, Philadelphia, PA.

Virtually all eukaryotes rely on telomerase to maintain chromosome length. Drosophila is a widely studied exception. Instead of telomerase, *D. melanogaster* relies on domesticated transposable elements (TEs) that insert exclusively into telomeric DNA. This alternative mechanism of chromosome length maintenance is widely hailed as an exemplary 'genomic symbiosis' between host and mobile element. However, recent evidence that Drosophila telomere packaging proteins evolve rapidly under positive selection raises the possibility that this ostensibly stable host-TE genomic symbiosis represents instead a thinly veiled intra-genomic conflict. Here we explore the evolutionary history of the TE side of this potentially tenuous relationship, searching first for TEs derived from a classically telomere-associated subclade within the jockey family of non-LTR retrotransposons. We conducted an iterative BLAST search and *de novo* consensus-building on unassembled sequence reads from nine species that span 15 million years of Drosophila evolution. We uncovered retrotransposons related to, but phylogenetically distinct from, the canonical retrotransposon lineages known from *D. melanogaster* and its closest relatives. These PCR- and cytogenetically-validated retrotransposon lineages appear to turnover recurrently – closely related species encode non-overlapping telomere-specialized element lineages. Moreover, we discovered dramatic telomere-specialized retrotransposon copy number fluctuations and broad variation in the relative fractions full-length and partially degraded copies encoded by a host genome. In the most extreme case, *D. biarmipes,* we detected the complete loss of telomere-specialized retrotransposons of the jockey subclade. We generated a PacBio-assembly of *D. biarmipes* telomeres and found only relics of DNA transposons interspersed complex satellite repeats. Furthermore, patterns of higher order repeat unit structure at *D. biarmipes'* non-canonical telomeres suggest that this species relies instead on an ancient recombination-based mechanism to length chromosomes. Collapse and diversification of telomeric retrotransposon lineages, combined with evidence of fast evolving telomere packaging proteins, suggests that Drosophila telomeres are maintained not by a stable genomic symbiosis but instead a dynamic, constantly re-negotiated truce.

101 Chromatin reprogramming by the histone H3.3 K27M oncomutation during DNA replication. *Kami Ahmad*[1], Jay Sarthy[1], Michael Meers[1], Steven Henikoff[1,2] 1) Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Howard Hughes Medical Institute.

Diffuse midline gliomas (DMGs) are fatal pediatric brain tumors associated with mutation of the Histone H3.1 or Histone 3.3 genes. A particularly severe allele is the lysine-to-methionine substitution at residue 27 (H3K27M), a position that is a critical determinant of developmentally-silenced chromatin. Trimethylation of the H3K27 residue (H3K27me3) is a conserved feature of Polycomb-silenced domains in eukaryotes. However, how the K27M substitution interferes with chromatin silencing is unknown. We used CUT&RUN, a sensitive profiling method to detect chromatin changes induced by a mutant H3.3K27M histone in developing Drosophila tissues. Since chromatin features are disrupted during DNA replication and must then be re-established every cell cycle, we tracked changes in histone methylation, Polycomb binding, and gene silencing during cell cycle progression. We find that the mutant histone only has effects during DNA replication. Further experiments will distinguish if mutant histones act by poisoning the chromatin of Polycomb-silenced domains, or by inhibiting methylation of new histones. While DMG pediatric gliomas have greatly reduced levels of H3K27me3, we find distinctive differences in chromatin landscapes between H3.1 and H3.3 K27M pediatric tumors, and patterns of histone deposition during the cell cycle may explain oncogenic effects of the two histones.

102 A membrane transporter is required for steroid hormone uptake in *Drosophila*. N. Okamoto[1], R. Viswanatha[2], R. Bittar[1], S. Haga-Yamanaka[3], N. Perrimon[2,4], *N. Yamanaka*[1] 1) Department of Entomology, University of California, Riverside, Riverside, CA; 2) Department of Genetics, Harvard Medical School, Boston, MA; 3) Department of Molecular, Cell and Systems Biology, University of California, Riverside, Riverside, CA; 4) Howard Hughes Medical Institute, Boston, MA.

Steroid hormones are a group of lipophilic hormones that are believed to enter cells by simple diffusion to regulate diverse physiological processes through intracellular nuclear receptors. We recently challenged this model in *Drosophila* by demonstrating that a membrane transporter that we named Ecdysone Importer (EcI) is involved in cellular uptake of the steroid hormone ecdysone. *EcI* encodes an organic anion transporting polypeptide, Oatp74D, a member of the evolutionary conserved solute carrier organic anion superfamily. *In vivo*, *EcI* loss-of-function causes phenotypes indistinguishable from ecdysone- or *ecdysone receptor* (*EcR*)-deficient animals, and *EcI* knockdown inhibits cellular uptake of ecdysone. Furthermore, *EcI* regulates ecdysone signaling in a cell-autonomous manner and is both necessary and sufficient for inducing ecdysone-dependent gene expression in culture cells expressing *EcR*. Altogether, our results challenge the simple diffusion model for cellular uptake of ecdysone and may have wide implications for basic and medical aspects of steroid hormone studies.

103 Patronin regulates organ growth through Hippo signaling pathway in *Drosophila*. *Dae-Wook Yang*, Kwang-Wook Choi Korea Advanced Institute of Science and Technology, Daejeon, KR.

The Hippo signaling pathway is essential for regulating the organ size. A cascade of protein kinases, including Hippo (Hpo) and Warts (Wts), suppresses organ growth by inhibiting the Yorkie (Yki) transcriptional coactivator. Actin cytoskeleton plays a role in the regulation of Hippo signaling, but the role of microtubules in growth regulation is not well understood. Here we show that Patronin, known as a microtubule minus end protector, negatively regulates Hippo signaling in *Drosophila*. *Patronin* knockdown or mutations results in organ size reduction. *Patronin* RNAi in wing disc leads to upregulation of Yorkie (Yki) target gene expression. Growth defects by *Patronin* RNAi is suppressed by reducing the level of Hpo or Wts. Patronin is enriched in the apical region of wing discs cells and co-localizes with the Expanded (Ex)-Merlin (Mer)-Kibra complex proteins that act upstream to Hpo. Furthermore, Patronin shows physical interaction with these upstream factors. We show that growth defects caused by *Patronin* RNAi are strongly suppressed by depleting *Klp10A*, a kinesin family protein known to antagonize the Patronin function. *Patronin* RNAi phenotypes were also suppressed by reducing the microtubule destabilizing factor Spastin. Taken together, Patronin interacts with the Ex-Mer-Kibra complex to antagonize the Hippo pathway. Our data suggest that Patronin regulates Hippo signaling by stabilizing microtubule minus end.

104 Regulation of epidermal cell differentiation by the Hippo pathway. *H. Zhao*[1], C. Zhang[2], K. Moberg[2], A. Veraksa[1] 1) Department of Biology, University of Massachusetts Boston, Boston, MA; 2) Department of Cell Biology, Emory University School of Medicine, Atlanta, GA.

The Hippo pathway is a conserved growth regulation system. Its dysregulation leads to organ overgrowth and human cancers. Although the Hippo pathway has been intensively studied for its regulation of cell proliferation and organ growth, its function beyond growth control remains elusive. To explore possible novel functions of the Hippo pathway, we carried out protein affinity purification-mass spectrometry (AP-MS) analysis using transcriptional coactivator Yorkie (Yki) as bait. In addition to identifying all of the core pathway components and known accessory regulators, we found the protein Bonus (Bon) as one of the top Yki interactors. Bon is the only *Drosophila* homolog of mammalian transcriptional intermediary factor-1/tripartite motif containing (TIF1/TRIM24/TRIM33) family of proteins and has a proposed role as a cofactor of nuclear receptors, including EcR. We confirmed the Bon-Yki interaction and found that it requires the WW domains in Yki and PPxY motifs in Bon. Interestingly, Bon overexpression leads to formation of epidermal extensions (trichomes) on the surface of adult eyes, and this requires Yki and transcription factor Scalloped (Sd), as well as the interaction between Bon and Yki. We identified multiple genes that may be jointly regulated by Bon and Yki based on published ChIP-seq and RNA-seq analyses. Few, if any, of these genes are canonical Yki targets, and a subset includes genes that are controlled by ecdysone signaling. For some of these putative target genes, loss or gain of function modifies the trichome phenotype, suggesting that these genes are transcriptionally regulated by Yki and Bon and are involved in trichome fate determination. Interestingly, Yki and Sd are required for the formation of thoracic trichomes, and Warts (Wts) is required for maintaining the ommatidium vs. the epidermis cell fate. In summary, our study reveals a novel

function of the Hippo pathway in the regulation of epidermal cell differentiation. This regulation occurs through the interaction between Yki and Bon as well as their joint transcriptional control of non-classical target genes. This work broadens our understanding of the Hippo pathway beyond its role in growth control.

105 Chromatin modeling protein Hat-trick is a novel regulator of Notch signaling in *Drosophila melanogaster*. *A. Singh*[1,2], M. Mutsuddi[1], A. Mukherjee[1] 1) Banaras Hindu University, Varanasi, Uttar Pradesh, India; 2) Harvard Medical School, Boston, MA.

Notch signaling is an evolutionary conserved pathway that regulates a wide variety of developmental processes including acquisition of specific cell fates, cell proliferation, differentiation, self-renewal and cell death programs. The increasingly complex regulatory mechanisms of Notch signaling account for the multitude of functions exhibited by Notch during development. A DNA binding protein, Hat-trick (Htk), has been identified as an interacting partner of Notch intracellular domain (NICD) in a yeast two-hybrid screen and their physical interaction was further validated by co-immunoprecipitation experiments. *htk* genetically interacts with Notch pathway components in trans-heterozygous combinations. Loss-of-function and complimentary gain-of-function studies of *htk* illustrated its role in mediating Notch functions to regulate large spectrum of activities. Loss of *htk* function in *htk* mutant somatic clones showed down-regulation of Notch targets, whereas over-expression of *htk* caused ectopic expression of Notch target, without affecting the level of Notch protein. Increase in the dosage of *htk* significantly rescued the loss-of-function phenotype of *Notch* and vice-versa. Along with epistatic interactions, *Notch* and *htk* also demonstrated synergistic effects. Immunocytochemical analysis has demonstrated that Htk co-localizes with over-expressed NICD in the same nuclear compartment. We have shown here that Htk cooperates with NICD and Suppressor of Hairless to form activation complex and binds to the regulatory sequences of Notch downstream targets, *Enhancer of Split* complex genes to direct their expression. Taken together, our results suggest a novel mode of regulation of Notch signaling by a chromatin modeling protein Htk.

106 Making new connection between TOR, autophagy, and metabolism. *H.W. Tang*[1], Y.H. Hu[1], C.L. Chen[1], B.L. Xia[1], J. Zirin[1], M. Yuan[3,4], J. Asara[3,4], L. Rabinow[1], N. Perrimon[1,2] 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Howard Hughes Medical Institute, Boston, MA ; 3) Department of Medicine, Harvard Medical School, Boston, MA ; 4) Division of Signal Transduction, Beth Israel Deaconess Medical Center, Boston, MA.

Alternative mRNA processing, including alternative splicing (AS) and polyadenylation (APA), generates transcripts with different coding and UTR regions, a mechanism that regulates RNA stability and localization, protein–protein interactions, subcellular localization, and protein expression. Dysregulation of AS and APA have been linked to numerous cancers and diseases. Here, we show that the TOR signaling pathway regulates phosphorylation of the Cleavage and Polyadenylation (CPA) complex to induce AS and APA events in the Drosophila larval fat body during nutrient deprivation. CDK8 and DOA kinases are identified as links between TOR and the CPA complex. CDK8 and DOA physically interact with and directly phosphorylate CPSF6, a key component of the CPA complex, to regulate its cellular localization and RNA-binding ability. Significantly, depletion of CDK8, DOA, and the CPA complex compromises autophagy and promotes protein, energy, and lipid metabolism during starvation. These findings elucidate a regulatory mechanism linking between the TOR signaling pathway, RNA processing and metabolism.

107 The TGF-β/Activin ligand Act-β, but not Dawdle, is required for survival under chronic nutrient deprivation. *H. Bretscher*, M. O'Connor Genetics, Cell Biology and Development, University of Minnesota Twin Cities, Minneapolis, MN.

The ability to survive nutrient deprivation requires careful coordination between multiple organ systems. This coordination ensures that in the absence of nutrients, organs needed to sustain life are supplied with the required energy, while other process are slowed or halted. Many signaling pathways contribute to the process of energy storage and utilization, including the TGF-β/Activin pathway. Lack of the TGF-β/Activin ligand Dawdle, results in excess nutrient stores in the form of fat and glycogen (Ghosh and O'Connor 2013 PNAS). Despite this metabolic dis-regulation in the fed state, we find that Dawdle is not required for proper mobilization of energy stores under chronic starvation. Upon starvation, organisms preferentially burn lipid, while minimizing protein degradation. Loss of Dawdle does not alter the rate at which lipids are mobilized or protein is degraded. Furthermore, while loss of Dawdle results in elevated levels of metabolic enzymes required for the TCA cycle and Cori cycle in the fed state, down-regulation of metabolic enzymes upon starvation does not require Dawdle. This lack of requirement for Dawdle under starvation conditions suggests that TGF-β/Activin signaling mediated by Dawdle is required for proper metabolic regulation in the fed, but not starved state.

Survival under chronic nutrient deprivation is not independent of TGF-β/Activin signaling. A second ligand, Activin-β (Actβ) is required for long-term survival under nutrient deprivation. Nutrient deprivation of 72 hours results in a survival rate of just 40% in Actβ mutants compared to 80% in isogenic wild-type controls. The Actβ transcript is upregulated three fold upon nutrient deprivation, and the associated receptor isoform, Babo-B, is upregulated five-fold. Interestingly, Actβ is not required to regulate the rate at which lipids are utilized for energy or the rate at which soluble proteins are degraded. However, we find that transcript levels of 4E-BP are elevated in both fed and starved states compared to isogenic wild-type controls. We hypothesize, that loss of Actβ may alter the rates of glycogen storage and mobilization. The ability to withstand complete nutrient deprivation is a complex process requiring many organ systems. While acute nutrient deprivation is well studied, the way in which animals survive long-term nutrient deprivation remains poorly understood. Our goal is to understand the role of Actβ in survival under chronic nutrient deprivation.

108 Stabilized Acinus manages cellular stress by elevating basal levels of autophagy. *N. Nandi*[1], H. Kramer[1,2] 1) Department of Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX; 2) Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX.

Autophagy is a tightly regulated cellular process that supports survival during cellular stress. We have identified Acinus (Acn) as a key integrator of several cellular stress responses. Acn manages stress by regulating basal levels of autophagy in a TOR-independent pathway (1). Phosphorylation of the conserved serine-437 residue of Acn by Cdk5/p35 plays a critical role in adjusting basal levels of autophagy. Physiological relevance for this modification was confirmed by a phospho-mimetic mutation: Acn^{S437D} was stabilized, enhanced basal, starvation-independent autophagy and extended life span (2). Cdk5/p35-dependent phosphorylation of S437-Acn was elevated by expression of aggregation-prone polyQ-containing proteins and the resulting elevated basal autophagy efficiently reduced polyQ accumulation. By contrast, loss of *p35* compromised basal autophagy and resulted in elevated accumulation of polyQ aggregates. Acn-S437 phosphorylation is highly dynamic in developing eyes and we have identified a metal-dependent protein phosphatase (CG6036) necessary for dephosphorylating Acn at serine-437. Loss of CG6036 function drastically enhanced pS437-Acn levels and basal autophagy. N-terminal myristoylation and aspartate-231 in the metal-binding active site of this phosphatase are critical for its activity. Toxicological stress, such as cadmium poisoning, deactivates this class of phosphatases resulting in elevated levels of pS437-Acn and basal autophagy thus serving as a cytoprotective mechanism. As Acn is a subunit of the ASAP complex, a possible mechanism for Acn activity was suggested by its role in alternative splicing. Acn^{ASAP}, a mutant that interrupts its binding interface with Sap18 and RNPS1, disrupts its function in alternative splicing as predicted. However, when expressed in flies, Acn^{ASAP} enhanced basal autophagy and is primarily cytosolic. Thus, this mutant separates the regulatory functions of Acn in splicing and autophagy. Further support for a splicing-independent, non-nuclear role of Acn came from expression of myristoylated Acn that also displayed enhanced autophagy. Taken together, our data indicate that in response to several cellular stressors phosphorylated Acn shuttles out of the nucleus and promotes basal autophagy to manage stress and maintain homeostasis.

1. Haberman et al (2010) Development 137:2157-66
2. Nandi et al (2017) eLife 6:e30760

109 The sphingolipid-synthesizing enzyme *infertile crescent* engages *crumbs* for neuronal maintenance through redox signaling cascade. *Fei-Yang Tzou*, Tsu-Yi Su, Yu-Han Yeh, Chih-Chiang Chan Graduate Institute of Physiology, National Taiwan University, Taipei, Taiwan.

Reactive oxygen species (ROS) are highly reactive molecules involved in many physiological functions; therefore, it can be detrimental to cells when dysregulated. Elevated ROS levels have been observed in cells of various neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), and Retinitis Pigmentosa (RP). In RP, oxidative damage results in cone cell death while reduction of oxidative stress by overexpressing catalase, superoxide dismutase (SOD), and antioxidant treatments rescued the demise of neurons. ROS elevation is also observed in the *Drosophila* sensory neuron photoreceptor, and it leads to loss of the light-sensing structure rhabdomere, as well as progressive decline of retinal function. One of the major sources of ROS is NADPH oxidases (NOXs) enzymatic activities and the subcellular localization of NOXs underlies the spatial regulation of ROS genesis. Importantly, several studies have showed that NOXs activation are essential for activity-dependent neuronal death. Despite a wealth of knowledge of the significance of NOXs in neurodegeneration, the sites of action and the up/downstream effectors of signaling cascade remains elusive. We recently identified that *infertile crescent* (*ifc*) regulates neuronal maintenance. Loss of *ifc* leads to ROS accumulation and results in activity-dependent neurodegeneration. We have found a novel mechanism whereby the cell polarity gene *crumbs* (*crb*), orthologue human CRB1, engages *ifc* to prevent neurons from activity-dependent oxidative stress and degeneration. Specifically, we identified *ifc* as a downstream effector of *crb* by rescuing the degenerative defects of *crb* mutant with *ifc* expression in photoreceptors. Moreover, genetic and pharmacological inhibition of Rac1 and NOXs complex ameliorate the degeneration of *ifcKO* photoreceptors. Finally, the colocalization and co-immunoprecipitation studies suggest that autophagosome was the site of i*fc* regulation in Rac1-NOX activity and ROS genesis. These findings advanced our understanding in oxidative dependent neurodegeneration with detailed descriptions of cellular mechanism and disease association.

110 *Drosophila* G3BP, RASPUTIN, is sufficient but not necessary for stress granule formation in intestinal progenitor cells. *Kasun Buddika Jayawardhana Koomangodage*, Mary Hazuga, Ishara Ariyapala, Nicholas Sokol Department of Biology, Indiana University, Bloomington, IN.

Stress granules are non-membrane-enclosed foci that form in cells exposed to environmental or biotic stress and are associated with the downregulation of translation initiation. These structures have largely been characterized in tissue culture cells, but the *in vivo* roles of stress granules in adult stem cell populations remain unknown. Here we show that *Drosophila* orthologs of stress granule components identified in mammalian tissue culture cells, including AGO1, CAPRIN, eIF4E, FMRP, LIN-28, PABP, and RASPUTIN, are enriched in intestinal progenitor cells and accumulate in small cytoplasmic punctae that are reminiscent of messenger ribonucleoprotein complexes (mRNPs). Exposure of intestines to sodium arsenite or rapamycin trigger these mRNP components to aggregate specifically in progenitor cells and form a novel population of cytoplasmic granules. We therefore term these structures intestinal progenitor stress granules (IPSGs). We find by monitoring protein synthesis using O-propargyl-puromycin incorporation that IPSG formation correlates with translational downregulation and, furthermore, that polysome stabilization with cycloheximide treatment prevents IPSG formation. We also find that IPSGs are reversible assemblies and, consistently, clearance of these cytoplasmic granules leads to upregulated translation in progenitor cells. In order to probe the genetic requirement of IPSG dynamics, we analyzed the effect of loss and overexpression of individual IPSG components on IPSG formation and disassembly. From this analysis, we find that although the overexpression of *rasputin* (*rin*), the fly ortholog of core stress granule component G3BP, induces IPSG formation even in the absence of stress, loss of RIN does not eliminate IPSGs. Given that G3BP is necessary for stress granule formation in both *Drosophila* and mammalian tissue culture cells, this result is significant since it indicates that adult intestinal progenitors do not rely on G3BP for stress granule assembly. Furthermore, IPSGs induced by RIN overexpression are associated with reduced intestinal stem cell proliferation, suggesting that IPSGs ordinarily act to repress proliferation after stress. Current work is focused on ROX8 and ATX2, two additional IPSG components that preliminary analysis indicates are required for IPSG formation. In sum, this work reports the identification of a novel, progenitor-cell-specific structure and the genetic requirements for the formation of this structure.

111 Loss of Peroxisomal ACOX1 induces autoimmunity whereas a *denovo* gain of function variant induces elevated ROS and glial loss in humans and flies. *HYUNGLOK CHUNG*[1,2], Michael Wangler[1,2,3], Paul Marcogliese[1,2], Thomas Ravenscroft[1,2], Juyeon Jo[1,2], David Li-Kroeger[1,2], Shinya Yamamoto[1,2,3,4], HyunKyung Lee[2,3,4], Tiphanie Vogel[5], Hugo Bellen[1,2,3,4,6] 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, United States of America;; 2) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX 77030, United States of America;; 3) Program in Developmental Biology, Baylor College of Medicine, Houston, TX, 77030, USA; 4) Department of Neuroscience, Baylor College of Medicine, Houston, TX, 77030, USA; 5) Department of Pediatrics, Immunology Allergy and Rheumatology, Baylor College of Medicine, Center for Human Immunobiology, Texas Children's Hospital, Houston, TX, USA; 6) Howard Hughes Medical Institute, Houston TX 77030, United States of America.

ACOX1 is the first and rate-limiting enzyme of the fatty acid beta-oxidation pathway in the peroxisome. Loss of this gene results in P-ALD (Pseudoneonatal Adrenoleukodystrophy). Here, we report that patients with specific *de novo* mutations display a very different course of the disease than the patients who have an ACOX1 deficiency. To model the human disease we first created loss of function models in flies. Mutant that lack *dACOX1* display a very reduced lifespan, progressive motor deficits, and a robust auto-immune phenotype. Our data argue that loss of *dACOX1* triggers a strong cellular autoimmune response in flies that show parallels with the cellular immune response observed in children with ACOX1 deficiency. In contrast, expression of the *de novo* variant observed in probands pN237S, expression of p.N237S behaves as a strong gain-of-function mutation that stabilizes the ACOX1 dimer and produces elevated levels of ROS in specific glial cells leading to a severe dysfunction of the nervous system and death. Similarly, in mouse and one of the probands, expression of the pN250S leads to a loss of Schwann cells, motor neurons, and sensory neurons. Treatment of flies or Schwann cell cultures with an antioxidant or Catalase expression strongly suppress the phenotypes caused by ACOX1 p.N237S or p.N250S. In summary, our data indicate that the loss of ACOX1 causes an auto-immune disease and that its gain results in a ROS-driven glial death and axonal dystrophy

112 Non-canonical translation initiation factors regulate the expression of ATF4 in response to cellular stress. *Deepika Vasudevan*, Amy Yang, Ishwar Navin, Hyung Don Ryoo Dept. of Cell Biology, New York University School of Medicine, New York, NY.

Certain conditions of cellular stress impose restrictions on mRNA translation by activating the kinases PERK and GCN2 to phospho-inactivate of the α-subunit of the initiator methionine (Met-tRNA^{iMet})-carrying complex, eIF2. Such restrictive translation conditions paradoxically stimulate the synthesis of the transcription factor ATF4 to induce a stress responsive gene expression program. To discover new factors that aid in selective ATF4 translational induction during stress, we performed an *in vivo* RNAi screen in *Drosophila* larvae bearing a reporter for ATF4 transcriptional activity. Using a fat body specific driver to drive RNAi expression, we identified *eIF2D* as a candidate ATF4 regulator. We validated these observations with two independent *eIF2D* deletion alleles, both of which showed loss of the ATF4 reporter in the fat body.

The ability of *ATF4* mRNA to be synthesized despite limiting eIF2α availability is attributed to its unusual 5'UTR structure, which contains multiple upstream open reading frames (uORFs). Loss of *eIF2D* resulted in marked decrease of an ATF4 5'UTR-dsRed reporter in the fat body. Similarly, activation of GCN2 by amino acid deprivation in *eIF2D* mutant larvae showed reduced transcriptional induction of ATF4 transcriptional targets with no change in ATF4 mRNA itself. These data suggest that *eIF2D* regulates ATF4 mRNA translation via its 5'UTR. We next examined the role of *eIF2D* in the adult gut, where chronic activation of PERK is known to regulate the proliferation of intestinal stem cells (ISCs). Similar to the phenotypes observed with loss of *PERK*, ISC-specific knockdown

of *eIF2D* resulted in reduced ISC proliferation with age. *eIF2D* contains two domains: an N-terminal tRNA binding PUA domain and a C-terminal SUI domain capable of start codon recognition. Our preliminary data show that the loss of the *eIF2D* homolog complex, *DENR-MCTS1*, exerts similar effects on the ATF4 pathway. We thus propose that eIF2D/DENR-MCTS1 act as non-canonical Met-tRNA^{iMet} carrier proteins that are required for translation initiation of the ATF4 ORF under stress conditions when eIF2α availability is limited.

113 Damage to the basement membrane by ROS and JNK recruit hemocytes to overgrown tissue. *N. Diwanji*, A. Bergmann Molecular Cell & Cancer Biology, University of Massachusetts Medical School, Worcester, MA.

Apoptosis-induced compensatory proliferation **(AiP)** is a mechanism that is involved in maintaining tissue homeostasis after massive stress-induced cell death. In this phenomenon, the dying cells induce proliferation of the surviving cells to compensate for the loss, and thus restore organ size. AiP is important for wound healing, tissue regeneration and contributes to tumor repopulation following radiation or chemotherapy. Using an overgrowth tumor model (**"undead tissue"**) in *Drosophila melanogaster*, we have identified that active initiator caspase Dronc promotes generation of extracellular **Reactive Oxygen Species (ROS)**, which drive activation of the Jun-N-terminal Kinase (JNK) pathway and downstream mitogens to promote AiP. We have also observed high numbers of **hemocytes**, ***Drosophila* macrophages**, which are attracted to the undead tissue and promote its overgrowth. However, the specific mechanism of hemocyte recruitment is still unclear. Here we show that *Drosophila* epithelial undead tissue has damaged **basement membrane (BM)**. This disruption of the BM is mediated by ROS and active JNK in the undead tissue; however, these are not sufficient to cause the damage in normal tissue. We have found that **matrix metalloproteinase 2 (MMP2)** is important for disrupting the BM of undead tissue as well as normal tissue. Interestingly, both ROS and JNK regulate the expression of MMP2 in the undead tissue. Finally, the damage to the BM by MMP2 is what recruits hemocytes to the tissue. Taken together, we propose that ROS generated by the undead tissue activates JNK to upregulate MMP2, which causes damage to the BM thereby attracting hemocytes.

114 The Alary Muscles. A keystone of the heart. *Alain Vincent*, Laetitia Bataillé, Nathalie Colombié, Jean-Louis Frendo Center of Integrative Biology, CNRS/University Toulouse 3, Toulouse, FR.

A specific set of muscles, called alary muscles (AMs) due to their delta wing shape, was described as early as 1950 in *Drosophila*, as connecting the heart to the lateral skeleton in each abdominal segment. Based on morphological studies of various adult arthropods, a role of AMs in controlling heart beating was proposed, but not assessed. A first reported embryonic function of AMs was in maintaining anterior malphigian tubules in proper position during organogenesis (Weawers and Skaer, 2013). In parallel, our laboratory discovered the existence of AM lineage-related muscles in the thorax, which we called Thoracic Alary Related Muscles (TARMs). Each pair of TARMs connects the exoskeleton to a specific region of the gut (Boukhatmi, et al., 2014). Both AMs and TARMs circle specific branches of the respiratory, tracheal system. We thus proposed that AMs and TARMs could act as "abseiling ropes" in maintaining the internal anatomy of the moving larva. At ADRC 2018, we will report live-imaging of AMs and TARMs contractions/deformations during larval locomotion, using a reporter construct based on a *tailup/islet1* Cis-Regulatory Module specifically active in these muscles. The same driver was used to kill AMs in the larva, and show that they play key roles in maintaining the heart lumen and the heart and aorta in proper internal position. The control of AM/TARM development by *Drosophila* orthologs of two vertebrate Transcription Factors, Tbx1 and Islet1, expressed in the cardiopharyngeal mesoderm, and homeotic transformations of AMs into TARMs, and reciprocally, raise the question of the evolutionary history of these atypical muscles.

115 The mechanisms of dynamin-actin interaction. *R Zhang*[1], N Gerassimov[2], D Lee[1], S Kim[2], D Luvsanjav[2], J Winkelman[3], M Mettlen[1], M Abrams[1], R Kalia[4], B Ravaux[1], J Kim[2], P Keene[1], J Ditlev[1], G Zhang[5], M Rosen[1], A Frost[4], N Alto[1], S Schmid[1], M Gardel[3], E Chen[1,2] 1) UT Southwestern Medical Center, Dallas, TX; 2) Johns Hopkins University School of Medicine, Baltimore, MD; 3) University of Chicago, Chicago, IL; 4) University of California, San Francisco, CA; 5) National Institute of Biomedical Imaging and Bioengineering, Bethesda, MD.

Cell-cell fusion is necessary for embryonic development, including muscle formation. Genetic, cell biological, biochemical and structural studies are beginning to reveal mechanisms underlying cell-cell fusion. Here we show a direct, noncanonical role for dynamin, best known as a fission GTPase in endocytosis, in cell-cell fusion. We show that dynamin colocalizes within F-actin-enriched podosome-like structures at the site of fusion and is required for generating invasive protrusions. A role for dynamin, together with its interacting protein cortactin in bundling and stabilizing actin has been demonstrated; however, how dynamin bundles actin and whether GTP hydrolysis affects actin cytoskeletal dynamics remains unknown. We show that dynamin forms rings but not helices that directly bundle actin in a periodically repeated pattern. Surprisingly and in contrast to previous models, we found that dynamin interacts with actin through its proline-rich domain (PRD), which extends outward from the dynamin ring, and that the actin filaments are aligned one at a time to form a bundle. Whereas dynamin rings wrap around lipid tubule through interactions with inward facing pleckstrin homology domains, cryoEM analysis revealed that individual actin filaments are locked to the outer rim of the dynamin ring. GTP hydrolysis does not stabilize the actin-associated dynamin rings as previously suggested, but instead triggers disassembly of the rings, loosens the actin bundles, and promotes dynamic Arp2/3-mediated branched actin polymerization to create shorter actin filaments suitable for mechanical work. Thus, dynamin functions as a unique actin-bundling factor that secures actin filaments at the periphery of the dynamin ring and enables mechanical force generation by the F-actin network upon GTP hydrolysis. Our findings have general implications in understanding dynamin-actin interactions in various cellular processes.

116 Actomyosin cables prevent premature tissue internalization in the *Drosophila* embryo. *J.C. Yu*[1,2], R. Fernandez-Gonzalez[1,2,3,4] 1) Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada; 2) Translational Biology and Engineering Program, Ted Rogers Centre for Heart Research, University of Toronto, Toronto, Ontario, Canada; 3) Cell and Systems Biology, University of Toronto, Toronto, Ontario, Canada; 4) Developmental and Stem Cell Biology Program, Hospital for Sick Kids, Toronto, Ontario, Canada.

Compartment boundaries prevent mixing of cell populations during organism growth and development. In the *Drosophila* embryo, the mesectoderm, a group of glial precursors that form the ventral midline, undergo oriented divisions during axis elongation and are eventually internalized ~6 hours later. Using spinning disk confocal microscopy and image analysis, we found that the polarity factor Bazooka (Baz; Par-3 in vertebrates) localized to new junctions between mesectoderm cells after their division, becoming planar polarized to mesectoderm-mesectoderm interfaces parallel to the dorsal-ventral axis of the embryo (MM interfaces). Simultaneously, the molecular motor non-muscle myosin II and its upstream activator Rho-kinase accumulated at mesectoderm-ectoderm (ME) interfaces, forming supracellular cables flanking the mesectoderm on either side of the tissue. Thus, the planar-polarized distribution of Rho-kinase and myosin established a boundary between ectoderm and mesectoderm. Compartment boundaries often exhibit increased tension, and laser ablation revealed that ME cables sustained twofold greater tension than MM junctions. We used Fluorescence Recovery After Photobleaching to show that myosin turnover is reduced at ME cables compared to MM junctions, indicating that myosin is stabilized at the ME boundary. To determine if tension is required for myosin localization to ME cables, we used laser nanosurgery to mechanically isolate mesectoderm regions. In sham-irradiated controls, myosin fluorescence increased by 12±9%, while myosin decreased by 25±5% at isolated ME cables, suggesting a role for tension in ME boundary maintenance. Surprisingly, the width of the mesectoderm decreased rapidly in isolated regions compared to controls. Similarly, when we treated embryos with the Rho-kinase inhibitors Y-27632 or H-1152, ME cables disappeared and the mesectoderm was prematurely internalized, concomitant with the invasion of the ventral midline by ectoderm cells in which dynamic protrusions formed. Our results suggest that the redistribution of Rho-kinase following oriented cell divisions polarizes myosin and Baz within the mesectoderm to establish tissue boundaries, and that ME boundaries prevent premature internalization of the mesectoderm.

117 Photoreceptor apical domain remodeling coordinates epithelial elongation during retinal morphogenesis. *X. Sun*, N. Sanchez?Luege, I. Rebay University of Chicago, Chicago, IL.

Recent studies emphasize the importance of planar alignment and mechanical coupling of the apical domains of monolayer cells during epithelial morphogenesis. How subsequent remodeling of these specialized domains in different cell types sculpts the final 3D tissue architecture has not been characterized. We are studying *Drosophila* retinal morphogenesis, focusing on pupal stages during which the specialized apical domains of the photoreceptors involute and expand perpendicular to the epithelium surface. This dramatic restructuring establishes unique physical contacts such that the photoreceptor apical domain bridges the apical and basal domains of the surrounding non-neuronal scaffolding cells. In turn, the pigment and cone cell scaffold forms an interweaving lattice that produces a specialized basal contractile floor to support the photoreceptor clusters during the massive threefold elongation of the epithelium. To understand how the distinct contacts between the different retinal cell types organize and coordinate epithelial elongation, we have compared the rate and extent of elongation in wild type versus mutant retinas carrying cell-type specific knockout of the non-receptor tyrosine kinase and actin regulator Abelson (Abl). In wild type, we find that anchorage of the photoreceptor apical domain to the basal floor marks a transition to accelerated elongation, suggesting these connections produce the force balances needed to maintain epithelial integrity during this dramatic morphological change. Global loss of Abl disrupts both the photoreceptor apical domain and the support cell lattice. As a result, epithelial elongation and integrity is compromised, and the photoreceptor clusters fall through the basement floor. Examination of cell-type specific Abl mutant phenotypes suggests that cytoskeletal remodeling at the extending photoreceptor apical domain and an intact support cell scaffold are both crucial for retinal elongation. Further, our results suggest that extrinsic mechanical constraints imposed by the pigment and cone cell scaffold induce active remodeling of the photoreceptor apical domain. We propose a model in which remodeling of the photoreceptor apical domain coupled with real-time mechanical feedback from the pigment and cone cell scaffold drives the morphogenetic program.

118 A new member of an elite group: Clamp as a novel regulator of Zygotic Genome Activation (ZGA) in *Drosophila melanogaster* embryos. *M. Colonnetta*[1], P. Schedl[1], D. Gohl[2], G. Deshpande[1] 1) Department of Molecular Biology, Princeton University, Princeton, NJ; 2) Genomics Center, University of Minnesota, Minneapolis, MN.

Chromatin-Linked Adapter for MSL Proteins (Clamp) was identified as a key regulator of dosage compensation, a sex-specific process that results in hyperactivation of male X chromosome. Unlike other components of dosage compensation machinery, Clamp is essential for viability in both sexes. While the role of Clamp protein in the assembly of dosage compensation complex can account for male lethality, female lethality likely results from its involvement in a female-specific or sex-non-specific process. Here we have investigated sex-non-specific function(s) of *clamp* during early embryonic development. Using embryos that are zygotically compromised for *clamp* we show that Clamp activates transcription of *Sex lethal* (*Sxl*), the female sex determinant. Since *Sxl* is one of the few early-transcribed genes, we are investigating a more general role for Clamp during early zygotic transcription. Supporting the conclusion that Clamp has a broader developmental function, ChIP-seq data revealed that Clamp binds to thousands of GA-rich binding sites throughout the genome. Furthermore, embryos zygotically compromised for *clamp* display severely reduced levels (protein as well as RNA) of many segmentation genes involved in early embryonic patterning (*even-skipped*, *button-head*, *giant* etc.). These data suggested that Clamp is a novel regulator of Zygotic Genome Activation (ZGA). Consistent with this assertion, it shares several other phenotypes with known regulators of ZGA (Zelda and GAGA factor) including nuclear migration defects, centrosome aberrations and disorganized actomyosin network. Based on these data we propose that maternally deposited Clamp protein acts in concert with Zelda and GAGA factor to regulate early zygotic transcription, likely by influencing chromatin accessibility. We will explore how these regulators of early transcription together sculpt naïve unstructured chromatin into discrete functional Topologically Associated Domains or TADs. Lastly, we will examine if Clamp protein also contributes to the pre-ZGA transcription which is thought to be crucial for the establishment of early embryonic TADs.

119 Regulation of inductive signaling output by antiparallel morphogen gradients during epithelial patterning in the Drosophila ovary. *L.A. Nilson*, S. De Vito, M. Fregoso Lomas, K. Ip Department of Biology, McGill University, Montreal, QC, CA.

Epithelial patterning in the Drosophila ovary is initiated by localized activation of epidermal growth factor receptor (EGFR) signaling by a spatially-restricted ligand produced by the developing oocyte. Interestingly, this same inductive signal establishes both the anterior-posterior (AP) and dorsal-ventral (DV) axes of the tissue. In early stages, posterior EGFR signalling induces expression of the T-box transcription factors Midline and H15 (Mid/H15). In later stages, EGFR activity shifts to the anterior and instead induces expression of the homeobox transcription factor Mirror (Mirr). Which of these target genes is induced depends on the presence of opposing morphogen gradients. At the posterior, Unpaired (Upd), which activates the JAK/STAT signalling pathway, cooperates with EGFR signaling to induce expression of Mid/H15. In the anterior, Dpp cooperates with EGFR signalling to induce expression of Mirr. In addition to this positive input, we also found mutual repression between Mirr and Mid/H15. Such double negative feedback between target genes occurs in a variety of patterning contexts and presumably establishes boundaries between expression domains. In this system we identified an additional layer of negative regulation, in which each of these alternative target genes is also repressed by the opposing gradient; Upd represses Mirr while while Dpp represses Mid/H15. We propose that these data define a bistable network in which Dpp and Upd each provide both positive and negative inputs into EGFR target gene expression – activating one target while repressing the other – and the resulting choice is stabilized by mutual repression between the two alternative targets.

We are now interested in understanding how these multiple regulatory inputs are integrated. Our data suggest that *mid/H15* and *mirr* are regulated at the level of transcription, but whether the multiple inputs that we have defined impact the *mid/H15* and *mirr* regulatory elements directly remains unclear. To address this question, we have identified *cis*-regulatory modules (CRMs) that reproduce both the endogenous expression and regulation of *mid/H15* and *mirr* in this tissue. Our analysis of these elements is focused on determining 1. whether regulation by the inputs we have identified genetically is direct, 2. how Upd and Dpp can each have opposing effects on each of these targets, and 3. whether mutual repression between Mid/H15 and Mirr is necessary and/or sufficient for bistability.

120 "Survival of the fittest": Determining the mechanism by which *BenA* causes hypercompetition in the follicle stem cell niche. *Sumitra Tatapudy*, Todd Nystul UCSF, San Francisco, CA.

Although adult stem cells are often long-lived, they are not immortal, and lost or damaged stem cells must be replaced to preserve tissue homeostasis. Studies over the past decade have revealed that competition for niche occupancy between neighboring stem cell lineages is a major and well-conserved mechanism by which stem cell replacement occurs, including in Drosophila, mouse, and human tissues. In wildtype tissue, where neighboring stem cell lineages are identical, this process can be described by "neutral competition," whereas in genetically mosaic tissue, some mutations cause non-neutral or "biased" competition relative to wildtype. The identification of mutations that cause bias demonstrates that niche competition is genetically controlled, and recent studies of these mutations have begun to provide insights into the rules that govern this process. We have used the epithelial follicle stem cell (FSC) lineage in the Drosophila ovary as a model to investigate the mechanisms that underlie niche competition in an epithelial tissue. In a forward genetic screen, we discovered a mutation in *Bendless* (*Ben[A]*) that causes the strongest FSC niche competition phenotype we have seen to date. Ben is an E2 ubiquitin ligase that is a positive regulator of JNK signaling, and we confirmed that *Ben* is also required for JNK signaling in the FSC lineage. We found that JNK signaling is

constitutively active in the FSC lineage, and RNAi knockdown of *Ben* or other JNK pathway components causes severe follicle cell differentiation defects. However, we were interested to find that loss of JNK signaling alone is not sufficient to cause hypercompetition. Instead, our current data suggest that homozygosity for *Ben[A]* may cause hypercompetition by promoting an increase in the rate of proliferation. In addition, in germaria with *Ben[A]* clones, we observed a striking increase in the number of cells in the early FSC lineage that are positive for the FSC niche marker, pERK. This raises the interesting possibility that *Ben[A]* cells may alter the niche structure or allow prefollicle cells to retain at least some aspects of the FSC identity even after they have moved away from the niche. We are currently using quantitative fluorescence imaging, epistasis experiments, and mathematical modeling to understand the contribution of these phenotypes to the hypercompetition phenotype caused by *Ben[A]*.

121 Hsp83/Hsp90 physically associates with Insulin Receptor to promote neural stem cell reactivation. *J. Huang*[1], H. Wang[1,2,3] 1) Neuroscience & Behavioural Disorders, Duke-NUS Graduate Medical School, Singapore, SG; 2) NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore, SG; 3) Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, SG.

Neural stem cells (NSCs) have the ability to exit quiescence and reactivate in response to physiological stimuli. In the Drosophila brain, insulin receptor (InR)/phosphatidylinositol 3-kinase (PI3K)/Akt pathway triggers NSC reactivation. However, intrinsic mechanisms that control the InR/PI3K/Akt pathway during reactivation remain unknown. Here, we have identified heat shock protein 83 (Hsp83/Hsp90), a molecular chaperone, as an intrinsic regulator of NSC reactivation. Hsp83 is both necessary and sufficient for NSC reactivation by promoting the activation of InR pathway in larval brains in the presence of dietary amino acids. Both Hsp83 and its co-chaperone Cdc37 physically associate with InR. Finally, reactivation defects observed in brains depleted of hsp83 were rescued by over-activation of the InR/PI3K/Akt pathway, suggesting that Hsp83 functions upstream of the InR/PI3K/Akt pathway during NSC reactivation. Given the conservation of Hsp83 and the InR pathway, our finding may provide insights into the molecular mechanisms underlying mammalian NSC reactivation.

122 Shavenbaby isoforms orchestrate the proliferation *versus* differentiation switch of intestinal stem cells. *S. Al hayek* [1,2], A. Al sawadi[2], Z. Kambris[5], J.P. Boquete[4], S. Plaza[2,6], C. Polesello[2], B. Lemaitre[4], F. Payre[2], D. Osman[2,3] 1) Azm Center for Research in Biotechnology and its Applications, LBA3B, EDST, Lebanese University, Tripoli, Lebanon; 2) Centre de Biologie du Développement, Toulouse, France; 3) Faculty of Sciences III , Tripoli, Lebanon; 4) Global Health Institute, School of Life Sciences, EPFL, Lausanne, Switzerland; 5) Department of Biology, American University of Beirut, Beirut, Lebanon; 6) Laboratoire de Recherche en Sciences Végétales ,LSRV , Castanet-Tolosan,France.

The adult gut is a highly dynamic organ in charge of vital functions. Although cells that make up the gut are daily lost, intestinal homeostasis is maintained throughout adulthood due to self-renewal and differentiation properties of intestinal stem cells (ISCs). Here we show that the OvoL/Shavenbaby (Svb) transcription factor is required for adult gut homeostasis in flies. Recent work has shown that Svb is translated as a large sized repressor (Svb-REP), and then post-translationally processed in a shorter activator (Svb-ACT) in response to small peptides called Polished rice (Pri). We find that Svb is specifically expressed and processed into the activator isoform in adult gut progenitors, *i.e.* intestinal stem cells and enteroblats (ISC/EB). Svb-ACT is required to protect stem cells from apoptosis, and sufficient to promote their proliferation. Indeed, genetic assays reveal that Svb expression in ISCs is activated by two main mitogenic pathways, EGFR and Wnt. We identified an enhancer driving *svb* expression in gut progenitors and demonstrate that its activity relies on the direct binding of nuclear effectors of the EGFR and Wnt pathways. In addition, we delineated a second *svb* enhancer driving expression in differentiated enterocytes (ECs). Strikingly, we find that this is the Svb-REP isoform that is expressed and required within ECs. Svb-REP induces the differentiation of EB to EC and is required to maintain proper differentiation and survival of ECs. Finally, we show that Svb-ACT is required for the growth of ISC-derived tumors and that Svb-REP is sufficient to override deregulated signaling pathways, blocking tumorous behavior and leading to enterocyte differentiation. Taken together, our data in flies therefore demonstrate that controlled expression and maturation of the OvoL/Svb transcription factor plays a key role in the balance between stem cell maintenance/proliferation and enterocyte differentiation in the adult gut.

123 Hematopoietic "Intermediate Progenitors" represent a distinct and novel cell type that marks the transition of a true progenitor to a differentiated fate. *C. Spratford*[1,2], L. Goins[1,2], J. Girard[1,2], F. Chi[1,2], V. Ho[1], U. Banerjee[1,2] 1) Molecular, Cell, and Developmental Biology, University of California, Los Angeles, CA; 2) Broad Stem Cell Research Center, University of California, Los Angeles, CA.

The transition from a multi-potent hemoctye progenitor into different types of mature, functional blood cells is a widely studied process in both *Drosophila* and vertebrates, yet the cellular mechanisms that underlie this transition are not well understood. Previous analysis has hinted that blood progenitors likely transition to the differentiated fate through an intermediate cell with characteristics of both, rather than by a direct single-step differentiation process. However, such studies are limited by the lack of markers or drivers that directly identify the proposed intermediate progenitor fate. Here we utilize the *Drosophila* hematopoietic organ, the larval lymph gland, to investigate the molecular mechanisms regulating the progression from progenitor into mature hemocyte. We generated a driver that is activated by the co-expression of two halves of GAL4, one driven by a progenitor gene and the other by a differentiation gene (i.e. a "Split"-*GAL4* driver system), which exclusively labels cells that express both genes. This system allows us to genetically manipulate these intermediate cells to determine the molecular characteristics of the transition state. Our analysis reveals that the fluorescently labeled cells do indeed constitute a distinct cell type that makes up a defined "Intermediate Zone" located between the previously established progenitor and mature hemocyte zones. By genetically manipulating these cells, we demonstrate that this intermediate zone can be altered in size under differing genetic conditions, and therefore its cells constitute a distinct population during the process of lymph gland hematopoiesis. We present evidence that the cell cycle profile of the Intermediate Zone is unique compared to the progenitor zone, and lineage tracing reveals that the mature hemocyte population is largely derived from these intermediate progenitors. Furthermore, our data show that an interplay between JNK and Wnt signaling pathways is responsible for the transition from progenitor into the Intermediate Zone, while the Ras/Raf/MAPK pathway promotes exit to the differentiated state. These are amongst the critical molecular mechanisms regulating the transition from progenitor into mature blood cell that we will present at this meeting. It will also be interesting to discuss if such intermediate states might be identifiable in the well-established stem cell systems of the germline and intestine using the described strategy.

124 Local role for steroids in regenerative growth in *Drosophila*. *D.E. Terry*, J Wardwell-Ozgo, P Byun, C Zhang, K.H. Moberg Cell Biology, Emory University, Atlanta, GA.

A decrease in regenerative capacity with increasing developmental age is a common feature of wound healing conserved across many species. For example, perinatal humans and mice are capable of regenerating the distal portions of amputated digits, but this ability is quickly lost thereafter. Likewise, Drosophila larval imaginal discs have high regenerative capability that declines as they approach pupation. Genetic analysis of Drosophila wing disc regeneration using a temperature-controlled ablation system developed by Smith-Bolton and Hariharan (2009) has demonstrated that a number of conserved pathways contribute to wing regrowth (e.g. Wnt, JAK/STAT, Hippo). Based on our previous work linking the Hippo and ecdysone (Ec) pathways in disc cells (Zhang et al. 2015), we investigated Ec roles in regenerating wing discs. Although injury lowers systemic Ec levels through the relaxin and insulin-like peptide dILP8 (Colombani et al. 2015), we find evidence that transcriptional activity of the Ec receptor (EcR) is upregulated at the site of injury in larval wing discs and required for efficient wing regrowth in adults. Moreover, local depletion of the active form of Ec hormone (20E) using a "20E sponge" or local knockdown of the P450 enzyme Shade, which converts Ec to 20E, inhibits wing regeneration. Our data suggest that local synthesis of 20E and induction of the Ec hormone pathway may be a required element of the imaginal disc regenerative program in Drosophila. This may help explain an older body of clinical literature showing that topical application of

estrogen can speed epidermal wound repair in mice. Furthermore, the implication that wounds generate their own supply of 20E to activate the Ec pathway during regeneration may be analogous to the ability of some late stage prostate cancers to acquire castration resistance due to autonomous production of hormone. These data will be discussed in the context of tissue homeostasis and cancer.

125 Evolutionarily conserved Wingless signaling pathway is regulated by newly identified *Newt* genes to trigger regeneration response in *Drosophila*. *A.S. Mehta*[1], A Luz-Madrigal[2], PA Tsonis[1], A Singh[1] 1) Department of Biology, University of Dayton, Dayton, OH; 2) Department of Biology, Miami University , Oxford, USA.

Notophthalmus viridescens possess amazing regeneration capabilities but due to lack of available genetic tools the mechanism driving such regeneration has not been well understood. Here we used *Drosophila* imaginal discs to study regenerative role of five newly identified newt proteins that have new sequence motifs. These proteins were identified by *denovo* assembly of newt transcriptome combined with proteomic validation. Using transgenic approach these genes were misexpressed in developing eye field of early as well as late eye *Drosophila* mutants where cell death was induced in photoreceptor cells during early 2nd instar (in early eye mutants) and late 3rd instar (in late eye mutants) stage, respectively. The penetrance of mutant phenotype was 0%. Strikingly, Newt genes when expressed in the background of such mutants show significant regeneration of missing eye tissue. Even more, these genes having signal peptides, exhibited non-autonomous regeneration as shown by flip out clones. Using Retinal determination (RD) fate markers, we demonstrated that regeneration response was restricted only to fly eye field. These regenerated tissues (eyes) showed 6 ± 1.5 folds increase in mitotic index as compared to the mutants. In comparison, there was only 1-fold downregulation of cell death, suggesting that newt regeneration genes employ cell proliferation function to promote regeneration. Additionally, downregulation in apoptosis is preventing regenerated tissue from further death. Using RNA sequencing, we identified the role of signaling pathway that has been perturbed by newt genes to induce regeneration in *Drosophila.* We found that member of evolutionarily conserved Wnt/Wingless (Wg) pathway exhibit 4-fold downregulation of expression. Additionally, the genes related to the molecular class of development, apoptosis and cell cycle were highly enriched. Using QPCR and gene expression analysis in developing eye we verified RNA seq results and found that Wg is significantly downregulated by these newt genes to promote regeneration. Perturbing positive and negative regulators of Wg signaling pathway and blocking Wg transport revealed that newt genes regulate Wg/Wnt pathway in regenerative response. Our results demonstrate a unique class of genes present in Newts which employ conserved pathways to trigger regeneration response, and also provided a novel platform to bridge the gap of unraveling the mechanism behind regeneration tool kit from newts.

126 Functional analysis of *de novo* evolved genes in male *Drosophila* reproduction. *B. Kelly*, G. Mascha, P. Rumde, P. Patel, G. Findlay Department of Biology, College of the Holy Cross, Worcester, MA.

De novo evolved genes arise from previously non-coding genomic material and have potential to develop integral functions within a relatively short evolutionary time-frame. Many *de novo* genes in *Drosophila melanogaster* are expressed predominantly in male reproductive organs, suggesting roles in improving male fertility. Our lab is performing an RNAi screen to identify testis-expressed *de novo* genes that impact male fertility. To date, five such candidates have been identified. One gene, *saturn,* originated early in the evolutionary history of the *Drosophila* genus and has since been duplicated and lost in various species. Fertility assays using both RNAi-mediated knockdown (KD) and CRISPR-mediated knockout flies showed that *saturn* is required for full male fertility. Western blots confirmed that *saturn* encodes a protein, and preliminary immunofluorescence experiments suggest that the protein may be enriched in the post-meiotic testis. We also identified two distinct roles for *saturn*, as its loss both reduces sperm production in males and prevents efficient localization of sperm to the storage organs in females. *Redstone* is another newly evolved gene with enriched expression in the testis. *Redstone* KD and null males exhibit normal sperm production. However, females mated with *redstone* mutant males show deficits in both egg-laying and egg-to-adult viability. We are currently investigating fertilization efficiency and the events of early embryonic development, and we are developing an antibody against redstone in order to perform protein localization assays. Together, these results suggest that *de novo* genes have evolved to influence multiple steps of *Drosophila* spermatogenesis. As we continue to construct and screen RNAi lines for over 50 additional putative *de novo* genes, we expect to identify more novel regulators of male fertility.

127 Save our sons: Surprising roles for RNAi to resolve intragenomic sex chromomsome conflict. *Chun-Ming Lai*[1], Jeffrey Vedanayagam[1], Ching-Jung Lin[1,2], Raphaelle Dubruille[3], Benjamin Loppin[3], Eric C. Lai[1] 1) Department of Developmental Biology, Sloan-Kettering Institute, 1275 York Ave, Box 252, New York, NY 10065; 2) Weill Graduate School of Medical Sciences, Weill Cornell Medical College, New York, NY 10065; 3) Laboratoire de Biome ? trie et Biologie Evolutive - UMR5558, Universite ? Claude Bernard Lyon I, 16, rue R. Dubois - Baˆ t. G. Mendel, 69622 Villeurbanne Cedex, France.

Since its discovery ~20 years ago, RNAi was rapidly adopted as a revolutionary technique for gene silencing. Nevertheless, understanding its endogenous biological utilities, especially in animals, has proven more elusive. We and others revealed endogenous siRNAs in D. melanogaster some time ago, but only recently did we realize their major endogenous utility is to control gene expression in the testis. In particular, our studies of "hpRNA" siRNAs showed that while the entire class is poorly conserved, each has a highly complementary target mRNA(s). But what is the point of an entirely non-conserved regulatory network? We now appreciate hpRNA/RNAi function is especially critical in the context of suprising capacities of de novo genes that are emerging in the testis meiotic landscape. By studying a non-model species, D. simulans, we found that specific hpRNAs and the RNAi pathway suppress emergent selfish genes that wage intragenomic conflict between the sex chromosomes. In the absence of these hpRNAs, X-chromosome loci become derepressed and deplete the Y-sperm pool, resulting in near-complete son loss. In our recent study, we focused on the X-chromosome distorters Dox and MDox, which are suppressed by the autosomal hpRNAs Nmy and Tmy. However, a fundamental question remained that individual nmy mutants, as well as now-lost tmy introgression mutants, are male fertile but exhibit highly biased sex-ratio of their progeny. By contrast, D. simulans RNAi mutants are completely male sterile. We therefore wonder whether nmy, tmy double mutants may have stronger phenotypes than the single mutants, whether other distorter loci are suppressed by these hpRNAs, and/or whether other hpRNAs controlling other emergent intragenomic conflicts exist. Our studies thus far provide evidence for several of these notions. First, Tmy specifically targets a novel, recently-emerged X-chromosome target that we provisionally call "UDox", and second, our transcriptome studies of D. simulans RNAi mutants reveal an array of de novo hpRNAs that were born in the simulans clade and preferentially target X-chromosome genes. All of these genes emerged recently, in which the X may be using these de novo genes as distorter loci, and subsequent emergence of hpRNAs defend against these distorters by RNAi mediated silencing. The rapid emergence of distorters and their suppressors conforms to an evolutionary “arms-race” where continuous cycles of sex chromosome conflict are resolved by RNAi to maintain population fitness. We seek a functional understanding of some of these X-chromsome loci. Altogether, these findings strongly support a widespread and recent sex chromosome conflicts that are actively being silenced by the RNAi/hpRNA pathway, and whose resolution may have contributed to speciation within the simulans clade.

128 Diapause-associated SNPs vary clinally but not seasonally in natural populations of *D. melanogaster*. *P.A. Erickson*[1], H.M. Stone[1], D.Y. Song[1], P.S. Schmidt[2], A.O. Bergland[1] 1) Biology, University of Virginia, Charlottesville, VA; 2) Biology, University of Pennsylvania, Philadelphia, PA.

Organisms living in temperate environments utilize seasonal changes in environmental cues such as light and temperature to exploit the favorable seasons and avoid the unfavorable ones. While the diversity of physiological and behavioral strategies to life in seasonal environments has long been appreciated, the

genes underlying seasonal responses are elusive, particularly for insects. Many insects, including some *Drosophila melanogaster,* undergo a genetically determined arrest of reproductive development (diapause) in response to unfavorable environmental conditions. In *D. melanogaster*, the ability to enter diapause is more common in northen populations, where flies endure harsher winters, and in the spring, when flies have recently overwintered. Using a novel hybrid swarm-based genome wide association study, we examined the genetic basis and evolutionary history of ovarian diapause. We generated two outbred populations representing eastern North American clinal diversity by intercrossing 68 sequenced, inbred lines for several generations. We exposed hybrid females to different temperatures and day lengths, characterized ovarian development for 2800 flies, and reconstructed their full phased genomes using low-coverage Illumina sequencing data. We identified hundreds of SNPs associated with diapause, each with a relatively small effect. These loci are enriched for SNPs that vary in allele frequency clinally but are depleted for those SNPs that vary seasonally, suggesting a distinct genetic architecture underlying adaptation to spatially and temporally varying selection pressures. Nonetheless, we show that pro-diapause alleles tend to be relatively common in the spring and in northern latitudes. Finally, we placed the outbred populations in outdoor mesocosms to track how diapause and its underlying alleles change over seasonal time in our focal populations experiencing natural conditions. These experiments, integrating functional, evolutionary and quantitative genetics, will shed light on the genes and variants underlying seasonal responses. Further, our results suggest that while alleles associated with seasonal responses vary predictably across clines, their behavior is in fact less predictable across seasons.

129 Genome-wide signatures of non-random mating suggest extreme micro-environment population structure in *Drosophila santomea* and other species. C. Han[2], P. Reilly[2], K. Deitz[1], D. Matute[3], *P. Andolfatto*[1] 1) Biological Sciences, Columbia University, New York, NY; 2) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ; 3) Biology, UNC-Chapel Hill, Chapel Hill, NC.

Population genomic analysis of whole genome sequencing (WGS) data promises to shed light on fundamental questions in biology and evolution. For Drosophila, population samples are usually established as descendent populations of a single wild-caught female ("isofemale lines"). These females are typically multiply-mated and the lines become partly inbred rendering them unsuitable for population genomic analysis as they are. As a result, WGS data has generally been collected either from isogenized versions of isofemale lines (by a variety of methods) or from pools of wild-caught individuals. Both methods are a departure from the approach used in other organisms, such as humans, that sequence outbred diploid individuals. In a population genomic survey of *Drosophila santomea*, an island endemic sister-species of *D. yakuba*, we sequenced the genomes of 34 wild-caught individuals widely distributed across the island of Saõ Tomé. Plots of population-level genome-wide diversity reveal nothing particularly remarkable. Despite this, on closer inspection, we identified unusually long tracts of identity-by-descent (IBD) within individuals, with a substantial proportion of these individuals (>75%) harboring more IBD than expected for offspring of matings at the level of 1st cousins or closer. The level of diversity between individuals greatly exceeds that within individuals (FST = 0.194), strongly suggesting non-random mating. We show that a likely demographic model is one of a large number of extremely small populations (resembling isofemale lines) with limited migration between them. This is particularly surprising given the small geographic scale over which these samples were collected and indicates the existence of a large number of extremely small micro-environments on the island. This observation presents two key questions: How did such a strong IBD pattern go as yet undetected, and is this phenomenon restricted to *D. santomea*? To our knowledge, there is no WGS of outbred diploid individuals available in Drosophila, thereby preventing the easy identification of such IBD patterns. We thus analyzed previous data for isogenized individuals by creating "synthetic diploid" individuals and detect similar patterns of extended IBD tracts in some samples of Drosophila species, but not in others. This finding has profound implications for downstream population genomic inference of demographic and selection parameters.

130 X-chromosome meiotic drive in *Drosophila simulans*: Genetic basis drive suppression. *Cécile Courret*[1], Quentin Helleu[3], Pierre Gérard[2], David Ogereau[1], Matthieu Falque[2], Laurence Moreau[2], Catherine Montchamp-Moreau[1] 1) Genomics, EGEvolution Génome Comportement et Ecologie, CNRS, Université Paris-Sud, IRD, Université Paris-Saclay, 91190 Gif-sur-Yvette, France.CE, CNRS, Gif sur Yvette, FR; 2) Génétique Quantitative et Evolution-Le Moulon, INRA, CNRS, Université Paris-Sud, AgroParisTech, Université Paris-Saclay, 91190 Gif-sur-Yvette, France; 3) Department of Ecology and Evolution, University of Lausanne, Biophore Building, 1015 Lausanne, Switzerland.

Meiotic drivers are selfish genetic elements that promote their own transmission into the gametes, thus triggering intragenomic conflicts. In the Paris *sex-ratio* system of *Drosophila simulans*, X-linked drivers prevent the segregation of the heterochromatic Y chromosome during meiosis II, and hence the production of Y-bearing sperm. These drivers, which cause a bias toward females in the progeny of carrier males, are currently spreading across the species range. Natural selection, which tends to restore equal sex ratio, has favored the emergence of resistant Y chromosomes and autosomal suppressors, thus making the distorters cryptic or nearly in the wild. We present here recent findings on drive suppression.

First, we investigated gene variation among 351 Y chromosomes from 29 population samples collected over more than 20 years and showing a wide continuum of phenotypes, from sensitivity to complete resistance. We identified only three haplotypes. One of them is associated with resistance and proved able to replace sensitive Ys within an handful of years in populations invaded by the drivers, showing that intragenomic conflicts can drive astonishingly rapidly the evolution of Y chromosomes. *In situ* hybridization with satellite DNA probes revealed extensive structural variation, suggesting that repeated sequences are rapidly evolving and may account for the continuum of resistant phenotypes.

Second, in order to characterize autosomal suppression, we used Quantitative Trait Locus (QTL) mapping with recombinant inbred lines (RILs). The RILs derived from a parental line carrying suppressors and another devoid of them. We found two QTL on chromosome 2 and three on chromosome 3, with strong epistatic interactions. This highlight the multiplicity of actors involved in this intragenomic battle over the sex ratio.

131 Male recombination created geographically distributed haplotypes of the young neo-Y chromosome of Drosophila albomicans. *K.H.C. Wei*, D. Bachtrog Integrative Biology, University of California Berkeley, Berkeley, CA.

Within the Drosophila genus, neo-sex chromosomes often arise through chromosomal fusions between autosomes and sex chromosomes. Because of the absence of male recombination, a neo-Y, at its inception, is immediately subject to various population genetic pressures like Muller's Ratchet and reduced effective population size causing it to degenerate through irreversible accumulation of deleterious mutations. *Drosophila albomicans*, found predominantly in Asia Pacific, has a pair of young neo-sex chromosomes formed from two separate Robertsonian fusions involving the ancestral X and Y with the same autosome; this coincided with the split from its sister species, *D. nasuta* around 200,000 years ago. Recent findings revealed that, surprisingly, recombination occurs in male *D. nasuta* which has resulted in unexpected amounts of diversity on the neo-Y of *D. albomicans*. To further characterize the effect male recombination had on the evolution of the neo-Y, we sampled inbred strains collected at different regions of Asia. Phylogenetic analyses revealed three distinct types of neo-Ys, each of which is specific to one of the three geographical region: Okinawa, mainland Japan, and mainland southeast Asia. Inconsistent with a single origin of the neo-Y, average nucleotide diversity across the chromosome (π = 0.00132) is not drastically lower than that of the neo-X (π = 0.00183). When comparing neo-Y types to the neo-Xs, we identify large segments with low amounts of differentiation as evidenced by reduced FST. Maximum likelihood trees generated from sliding windows reveal that regions of reduced FST are associated with polyphyly of the neo-Ys, whereby the neo-Y types are scattered among the neo-Xs within the trees. However, all neo-Ys form one deep monophyletic group near the fused centromere where nucleotide diversity also drops precipitously (π = 0.00036). These results indicate that recombination with the neo-X introduced at least three haplotypes after a single Y fusion. Interestingly, the neo-Y appears to have a pre-existing introgression from *D. nasuta* as the monophyletic branch falls within the *D. nasuta* clade in trees near the fusion. This

segment is subsequently interrupted by different neo-Y haplotypes due to recombination in males, leaving ~3 and ~5 Mb remnants of the introgression. Surprisingly, we find no evidence of systematic down regulation of genes on the neo-Ys using allele-specific RNA-seq and ChIP-seq, which raises the interesting possibility that male recombination may have delayed the onset of degeneration on the neo-Y.

132 Host-virus co-evolution in Drosophila innubila highlights non-RNAi pathways as key to antiviral response. *T. Hill*, B. Koseva, R. Unckless Molecular Biosciences, University of Kansas, Lawrence, KS.

Hosts and viruses coevolve with each other in an evolutionary arms race at a time scale so rapid that it can often be observed almost in real time. Isolated geographical populations have the potential to observe this coevolutionary arms race in real time across "replicate" populations. *Drosophila innubila* is often infected with the Drosophila innubila Nudivirus and inhabits mountain woodlands in the southwestern USA. These mountains are often separated by hundreds of kilometers of desert which represent a formidable barrier to migration. To study host/virus coevolution in these distinct populations, we sequenced wild-caught individuals from each of four populations. Strikingly, the host population shows very little population structure across the genome aside from the Muller B chromosome where population structure appears to be driven by segregating inversions. The virus, on the other hand, is quite structured with most viruses fitting into one of three clusters almost perfectly. Given the genome-wide lack of structure, we focused on genes involved in the host immune response to viruses. Surprisingly, the canonical antiviral pathway, antiviral RNAi, shows little evidence of adaptive evolution. However, the JAK-STAT and Toll pathways, both involved in antiviral defense, showed rapid adaptive evolution. Finally, we performed genome-wide association studies in both host and virus to find variants associated with viral titre in the wild. We found no associated host variants, but several tightly linked viral polymorphisms were significantly associated with viral titre. Our results suggest that, asymmetry in gene flow may influence the coevolutionary arms race either with the host able to utilize mutations occurring throughout the species range or the virus able to specialize to a particular region.

133 Precise regulation of RhoA promotes proper tissue curvature. *A.C. Martin*[1], Marlis Denk-Lobnig[1], Joern Dunkel[2], Pearson Miller[2] 1) Biology, Massachusetts Institute of Technology, Cambridge, MA; 2) Mathematics, Massachusetts Institute of Technology, Cambridge, MA.

Bending and folding epithelial tissues is critical for morphogenesis. However, the mechanisms that specify the proper tissue curvature are not well understood. Here, we investigated how precise regulation of the RhoA GTPase promotes the curvature of the Drosophila ventral furrow. Ventral furrow formation is promoted by a multicellular Myosin 2 gradient, in which cells closest to the ventral midline have more Myosin 2. We found that there is a multicellular gradient of RhoA activation, by RhoGEF2, across the ventral furrow tissue. In addition, we found that C-GAP (RhoGAP71E) inhibits RhoA throughout the ventral furrow, which positions the multicellular Myosin 2 gradient and influences the tissue curvature. We developed a mechanical model of ventral furrow formation that further support the importance of a contractile gradient in promoting proper tissue curvature. In summary, our work supports a model in which precise regulation of the RhoA GTPase determines tissue curvature.

134 Crumbs-complex directed apical membrane dynamics controls epithelial cell ingression. *S. Simoes*[1], P. Giannatou[1], D. Ter Stall[1], K. Al Kakouni[1], M. Pellikka[1], T. Lam[1], D. Kim[1], R. Fernandez-Gonzalez[1,2], U. Tepass[1] 1) Cell and Systems Biology, University of Toronto, Ontario, CA; 2) Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, CA.

Epithelial to mesenchyme transitions (EMT) are ubiquitous during animal development and are responsible for cell escape during epithelial tumor progression. EMT involves loss of apical-basal polarity and cell-cell adhesion and the acquisition of migratory and/or proliferative ability. Using a novel in vivomodel – the ingressing neuroblasts (NB) in the Drosophila embryo – and applying quantitative live imaging, we are addressing the cellular mechanisms involved in the removal of apical membrane and apical determinants. We found that depletion of genes involved in endocytosis, apical protein degradation or recycling significantly impact on ingression kinetics. Loss of apical membrane and cell-cell contacts are counteracted by the apical determinant Crumbs, which is endocytosed and targeted for degradation from the apical cortex into the Hrs/ESCRT 0 and PtdIns(3)P positive compartment of sorting endosomes. We demonstrate that internalization of Crumbs regulates the loss of apical membrane and relies on ubiquitination of the Crumbs cytoplasmic domain. Completion of ingression also depends on the interaction between the Crumbs binding partner Stardust/PALS1 and the E3 Ubiquitin ligase Neuralized. Together, our results suggest that temporally regulated ubiquitination of multiple members of the Crumbs complex mediates its internalization and degradation in the endolysosomal system, driving the loss of the apical domain during EMT.

135 The LRR receptor Tartan establishes polarity at tissue compartment boundaries during convergent extension. *A.C. Pare*[1,2], J. Zallen[1] 1) HHMI/Sloan Kettering Institute; 2) Department of Biological Sciences, University of Arkansas, Fayetteville, AR.

A fundamental aspect of animal development is the reorganization of homogenous epithelial sheets into complex tissues with spatially defined groups of cells. To properly coordinate cell movements and changes in cell shape, individual epithelial cells must be able to sense their orientation with respect to the global body axes. Therefore, determining how axial information is encoded at the molecular level is a central question of developmental biology. Using the *Drosophila* germband as a model for studying epithelial elongation, we showed that actomyosin planar polarity and cell movements are coordinated using a dense, high-resolution map of cell-surface receptors that are expressed in overlapping, repeating stripes. This map consists, in part, of three members of the Toll receptor family, which is well known for its conserved role in detecting pathogenic molecules in the immune system. Here, we show that Toll receptors primarily direct planar polarity at cell-cell interfaces within future embryonic compartments (parasegments). By contrast, cell-cell interfaces between compartments do not require Toll receptors to become polarized – indicating that other polarity cues remain to be identified. Using a candidate gene approach, we chose to focus on the cell surface receptor Tartan, which, like the Toll receptors, contains an extended extracellular array of leucine-rich repeats (LRRs). Tartan is expressed in alternating embryonic compartments, with sharp boundaries of expression coinciding with compartment boundaries. We show that *tartan* loss-of-function and Tartan overexpression affect actomyosin and junctional polarity specifically at compartmental boundaries, but not at cell-cell interfaces within compartments. These findings demonstrate that there are two overlapping yet independent polarity systems at work in the germband: one consists of three Toll receptors and functions to orient cell intercalation within compartments, and a second, controlled by Tartan, mediates polarity at compartment boundaries. We propose that differences in downstream signaling between these two systems may underlie the distinct morphological characteristics of compartmental boundaries.

136 Linking tissue morphogenesis and patterning to the data mining framework: a proposal and a proof of concept. *T. Stern*[1,3], S. Shvartsman[2], E. Wieschaus[1,2,4] 1) Department of Molecular Biology, Princeton University, Princeton, NJ; 2) Lewis-Sigler institute for integrative genomics, Princeton university, Princeton, NJ; 3) European Molecular Biology Organization, Heidelberg, Germany ; 4) Howard Hughes Medical Institute, Chevy Chase, MD.

Research over the last decades has identified an increasing repertoire of conserved cellular behaviors, or "motifs", that act as building blocks of tissue morphogenesis. However, a comprehensive framework for the exploration and analysis of these motifs, similar to the frameworks used to map and discover

motifs in sequence data, is yet to be established. Here, we take the first step towards this goal by developing a generic algorithm that can learn to recognize any sub- to multi-cellular behavior from user provided examples and map its appearances in live imaging data. This algorithm allows us to recover the spatiotemporal combinations of multicellular protocols and to characterize their temporal dynamics. Our strategy relies on the transformation of the intricate geometry, topology and molecular expression profiles in a developing tissue into time-series data, thereby allowing to address the problem using robust data-mining strategies established in the Machine Learning community as a subsequence matching task. To illustrate this strategy, we used it to map intercalary behaviors, namely T1-transitions and rosettes, during *Drosophila* germband extension in wild type embryos and embryos lacking the AP patterning information, showing differences in motif distribution over the tissue as well as in the dynamics of the individual motif. Moreover, we identified a novel multicellular intercalation motif, referred to as "rosette-hub", which coordinates iterative cell transport from the DV to the AP axis of the embryo. Our work paves the way to the systematic discovery and statistical analysis of morphogenetic processes and for the identification of aberrant multicellular behaviors in developmental abnormalities.

137 Septate junction proteins maintain tissue integrity during dorsal closure. *C.A. Rice*[1], R. Fernandez-Gonzalez[2,3,4], T. Zulueta-Coarasa[3,4], R. Ward[1] 1) Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA; 2) Department of Cell & Systems, University of Toronto, Toronto, ON, CA; 3) Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, CA; 4) Translational Biology and Engineering Program, Ted Rogers Centre for Heart Research, University of Toronto, Toronto, ON, CA.

The process of dorsal closure in *Drosophila* involves the dorsalward movement of the lateral epidermis to cover a gap temporarily occupied by the extraembryonic amnioserosa and requires continuous contact between the two tissues to coordinate morphogenetic movements and maintain the integrity of the embryo. The interface between these two tissues is maintained by both integrins and adherens junctions. Although components of the occluding septate junction such as Coracle (Cora) and Macroglobulin complement-related (Mcr) do not appear to be localized to this region, they are required for efficient completion of dorsal closure. Here, we are studying the role of these proteins during closure, including through live imaging of septate junction mutants. Despite their absence in the amnioserosa and at the leading edge, we find these proteins are critical for maintaining interaction between the lateral epidermis and amnioserosa, and separation of these tissues along the leading edge is responsible for the closure phenotypes in *cora, Mcr,* and *Neurexin-IV* mutants. Dorsal closure progresses normally in these mutants until the later stages of closure (fast phase). At this point, the DE-cadherin protein appears to become depleted within the amnioserosa and along the leading edge and tears occur between the two tissues. Laser ablation studies show that tension and the viscosity-elasticity ratio are not affected in these mutants, suggesting the tearing is caused by adhesion defects. These results suggest a role for septate junction proteins in adhesion, but the absence of these proteins along the leading edge suggests an indirect role, possibly by regulating levels of DE-cadherin and/or integrins.

138 Septate junctions coordinate epithelial integration with growth of stem cell progeny during intestinal turnover. *Paola Moreno-Roman*[1], Irina Kolotueva[2], Elsa Su[3], Lucy Erin O'Brien[3] 1) Department of Biology, Stanford University, CA, USA; 2) University of Lausanne, Switzerland; 3) Department of Molecular and Cellular Physiology, Stanford University, CA, USA.

The intestinal epithelium forms a barrier between the external environment and the interior body. This barrier is created by a closed network of septate junctions, which must be dynamically maintained during organ turnover. How new stem cell progeny integrate into this junctional network is poorly understood. Examining the intestinal epithelium of the adult Drosophila midgut, we find that new progeny achieve seamless integration by coordinating growth, polarization, and differentiation with biogenesis of septate junctions de novo. The core septate junction proteins snakeskin, mesh, and tetraspanin2a are missing in stem cells but activated in ternimal enteroblast daughters. As an enteroblast differentiates, these proteins localize to the apical-most tip of the growing cell, forming a nascent plaque that induces remodeling of and coalesces with the mature junctional network. When we prevent enteroblasts from forming septate junctions, cells grow but cannot integrate or generate apical polarity. Instead, they become squamous and accumulate under the basal epithelium. Conversely, when we block TOR-dependent enteroblast growth, junction biogenesis is partially inhibited. By using de novo biogenesis of septate junctions to drive integration of stem cell progeny, the midgut epithelium incorporates new replacement cells without compromising barrier function.

139 A quantitative analysis of EGFR dynamics during early *Drosophila* development. *N. Revaitis*[1], R. Marmion[1], N. Yakoby[1,2] 1) CCIB, Rutgers University, Camden, NJ; 2) Biology, Rutgers University, Camden, NJ.

Organogenesis requires the coordination among multiple cell signaling pathways to develop tissues into functional organs. While the overall impact of ligands and their associated signaling pathways has been studied by many labs, the mechanisms behind the distribution of ligands remains widely unknown. During development, ligand-receptor binding is causal to tissue patterning and morphogenesis. Receptor dynamics throughout development often remains elusive throughout this process. In the *Drosophila* follicular epithelium, a uniformly distributed epidermal growth factor receptor (EGFR) is activated by a localized ligand source to set the anterior-posterior and dorsal ventral axes of the fly. However, the quantitative changes in EGFR localization during this developmental time remain mostly unknown. The activation of EGFR by the oocyte-secreted TGF-α-like ligand Gurken (GRK) varies during the transition from a posterior to a dorsal fate determination. Using CRISPR-Cas9, we generated a fully functional endogenously labeled EGFR with EGFP. This fly enabled us to determine the quantitative changes in receptor levels and localizations. Using ELISA, we determined the levels of EGFR from stages 8 of oogenesis to stage 5 of embryogenesis. As far as we know, this is the first quantitative analyses of EGFR in these tissues. Also, we used this fly to trace the localization of the receptor throughout oogenesis. Interestingly, we detected dramatic changes in EGFR localization in regions of high GRK. At stage 8, EGFR is restricted to the apical side of the follicle cells. This localization is lost during later stages of development, where EGFR is found in the apical and basolateral sides of the cells. Using endosomal markers, we determined the localization of EGFR and EGFR-GRK complexes throughout the developmental stages. Using these data, we aim to better understand how GRK shapes the distribution of EGFR activation throughout oogenesis.

140 The Dynamics of Germline Mutations and DNA Repair in Single-cell RNA-seq of Adult *Drosophila T*estis. *E. Witt*, S. Benjamin-Hong, N. Svetec, L. Zhao The Rockefeller University, New York, NY.

The testis expresses a highly complex and variable set of genes, but it is unclear what cell types and biological processes underlie these transcriptional patterns. The spatial and temporal patterns of germline mutations, DNA repair and expression regulation during spermatogenesis is an important aspect of reproduction and evolution. To better understand this subject, we performed whole-tissue single-cell RNA-sequencing of adult *Drosophila* testis. We used unsupervised clustering and marker genes to identify 6 major cell types in the male germline lineage and 3 types of somatic cells. We constructed a developmental trajectory and confirmed that spermatogenesis occurs along a single-branched lineage. We found many putative germline mutations within the sperm lineage, most of which probably originate in early spermatogenesis. We observed no germline mutations in mature spermatids. This finding fits our observation of upregulated DNA repair genes in the earliest stages of spermatogenesis. We propose that germline mutations tend to occur at all stages except spermatid maturation, with DNA repair occurring throughout spermatogenesis. Our results suggest a relationship between mutation rates and DNA repair machinery at the single cell level, and highlight the high mutational stress of spermatogenesis.

141 GCNA preserves genome integrity and fertility across species. *Courtney Goldstein*[1], Varsha Bhargava[1], James Amatruda[1,2], Kim Orth[1], Judith Yanowitz[3], Michael Buszczak[1] 1) Molecular Biology, UT Southwestern Medical Center, Dallas, TX; 2) Department of Internal Medicine, University of Texas

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Germ cells transfer genetic information from parents to their progeny, and any change in germline DNA is inherited by succeeding generations. Therefore, germ cell DNA must be protected from potentially deleterious internal and external assaults. While these cells employ several known repair pathways, specialized mechanisms that ensure high-fidelity replication, chromosome segregation and repair of germ cell genomes remain poorly understood. Here we identify an uncharacterized but highly conserved protein called Germ Cell Nuclear Acidic Peptidase (GCNA) responsible for maintaining genome integrity. GCNA contains a large acidic intrinsically disorder region (IDR) and a SprT metalloprotease domain. *GCNA* mutants exhibit chromosomal instability, replication stress, and an accumulation of DNA-protein crosslinks (DPCs). While the SprT domain is required to limit replication stress and limit meiotic induced damage, most of GCNA's function maps to the IDR. This work shows GCNA protects germ cells from various sources of damage, providing novel insights into conserved mechanisms that promote genome integrity across generations.

142 Centromere clustering promotes meiotic homolog pairing. *T. Hatkevich*[1], V. Boudreau[2], P. Maddox[2], T. Rubin[3], J.R. Huyhn[3], J. Sekelsky[2,4] 1) Curriculum of Genetics and Molecular Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC; 2) Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC; 3) Collège de France, CIRB, Paris, France; 4) Integrative Program for Biological and Genome Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC.

The central function of meiosis is to segregate chromosomes at each of its two divisions, which is dependent on crossover (CO) formation between homologs at the end of meiosis I (MI). To ensure CO formation in late MI, unpaired homologous chromosomes must identify one another and stably pair during early MI. *How this chromosomal identification process occurs is of high research interest*. It is posited that the initial step of homologous recognition is tethering chromosomal structures, such as centromeres and telomeres, to the nuclear envelope, and through nuclear oscillations, the envelope-anchored structures rotate and eventually cluster within one nuclear region to ultimately facilitate homology search. While centromere clustering in early MI is nearly ubiquitous throughout sexually reproducing organisms, directly testing if centromere clustering promotes homolog pairing has been difficult due to a lack of genetic tools. Through a meiotic genetic screen, a separation-of-function mutation within the *Mcm5* gene, $Mcm5^{A7}$, has been identified in *Drosophila,*which exhibits high chromosomal mis-segregation, or nondisjunction (NDJ), due to a severe decrease in crossovers between homologs. While investigating additional mutant phenotypes, we found that the $Mcm5^{A7}$ mutation disrupts meiotic centromere clustering but does not affect the formation of meiotic chromosomal structures, such as the synaptonemal complex (SC), and does not perturb meiotic progression. Thus, we utilized $Mcm5^{A7}$ as a genetic tool to examine the function of centromere clustering in early MI. We found that defects in centromere clustering, which we show is due to a lack of pericentric Smc1-localization, is associated with defects in homologous chromosome pairing. When we partially rescue centromere clustering through ectopic Smc1 overexpression in $Mcm5^{A7}$ mutants, homologous pairing significantly increases, leading to partial rescue of homologous crossing over and of meiotic NDJ. *These results conclusively show that centromere clustering directly promotes homolog pairing.* Interestingly, we observe full, stable SC formation between heterologous sequences in these mutants, suggesting that centromere clustering also functions to prohibit non-homologous synapsis. Lastly, we show that centromere movements still occur at the nuclear envelope in $Mcm5^{A7}$ mutants, indicating that meiotic nuclear oscillations are not sufficient for centromere clustering.

143 Neuropeptide Dh31 signaling regulates early germline cyst survival during adult *Drosophila* oogenesis. *Tianlu Ma*, Metabel Markwei, Daniela Drummond-Barbosa Department of Biochemistry and Molecular Biology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD.

Neuropeptides are evolutionarily conserved signaling molecules that lie at the intersection between the environment and our physiology and regulate many aspects of biology, including reproduction. In mammals, multiple neuropeptides regulate the levels of the key reproductive hormone gonadotropin-releasing hormone. The levels of these regulatory neuropeptides in turn respond to physiological changes such as changes in systemic leptin and insulin levels. However, much remains unknown about the identity and mechanisms of action of other neuropeptides regulating reproduction. In particular, the *Drosophila* system has been underexplored to advance these questions. We therefore conducted an RNAi-based screen aimed at identifying neuropeptides and neuropeptide receptors with roles in adult *Drosophila* oogenesis. We identified the neuropeptide diuretic hormone 31 (Dh31) and its cognate receptor Dh31-R as potential regulators of oogenesis. Specifically, adult-specific ubiquitous somatic knockdown of Dh31-R caused a decrease in egg production compared to controls. Similarly, Dh31 global mutant flies laid fewer eggs than their sibling controls, confirming that Dh31 signaling is required for normal oogenesis. Furthermore, adult-specific knockdown of Dh31 produced in neurons (as opposed to enteroendocrine cells, another site of Dh31 expression) is required for oogenesis. We next investigated the specific steps of oogenesis controlled by Dh31 signaling. In Dh31- or Dh31-R ubiquitous-knockdown females, we found a statistically significant decrease in the number of early germline cysts, along with a significant increase in the number of Dcp-1-positive germaria, indicating that early germline cysts die under conditions of low Dh31 signaling. Using tissue-specific drivers, we have determined that Dh31-R is required specifically in neurons to promote cyst survival, suggesting the involvement of a neuronal circuit in the control of early cyst survival. We are currently identifying the specific neurons involved and the relevant downstream G proteins mediating the effects of Dh31-R. We also plan to determine the environmental or physiological inputs that are conveyed by Dh31 signaling to modulate the ovary.

144 Epithelial cell gene expression and function during developmental nurse cell clearance in *Drosophila melanogaster* ovaries. *Diane Lebo*, Victoria Jenkins, Albert Mondragon, Alexandra Lion, Kim McCall Boston University, Boston, MA.

Every day, billions of cells die to maintain homeostasis within the human body. If a cell corpse isn't cleared away, it can proceed to a secondary necrotic state in which the cell membrane ruptures thus releasing its intracellular contents. To avoid the associated damage and disease, two classes of cells clear away the dead and dying cells – professional and nonprofessional phagocytes.

For professional phagocytes, such as macrophages, clearance is their primary function. Nonprofessional phagocytes, as the name implies, have a separate primary function and thus must transition to a clearance state. Although nonprofessional phagocytes are less efficient at clearance than professionals, due to their number and proximity, nonprofessional phagocytes may be more accessible to dying cells than professional phagocytes. Additionally, immunoprivileged tissues rely solely on nonprofessional phagocytes. The overall aim of our study is to determine what genes are differentially regulated in nonprofessional phagocytes to increase their capacity for clearance.

The ovary of *Drosophila melanogaster*is an ideal tissue for studying nonprofessional phagocytes as it is both immunoprivileged and each egg chamber contains fifteen nurse cells that undergo programmed cell death as part of development or in response to environmental cues. The follicle cells that form a single epithelial layer surrounding the developing egg and nurse cells are the nonprofessional phagocytes that respond to death in the germline. Once an egg chamber reaches the final stages of development, the nurse cells transfer their contents to the oocyte in a process called dumping and subsequently die. Simultaneously, a subset of follicle cells, known as stretch follicle cells, transition to a clearance state in which they remove the nurse cells. It is this particular transition that our study seeks to define.

To obtain a genome-level view of the changes within follicle cells, we obtained the translatome of epithelial and clearance state follicle cells. Translatomes were isolated by Translating Ribosome Affinity Purification (TRAP) where GFP-tagged ribosomes were immunoprecipitated and the associated mRNA was sequenced. We identified over eight hundred genes in clearance-state follicle cells that demonstrate statistically significant differential expression including genes related to calcium binding and metabolism. Progress on the characterization of these differentially expressed genes will be presented.

145 Sex-specific specification of the follicle stem cells in the developing *Drosophila* ovary. *A. Dove*, N. Murphy, M. Van Doren Biology Department, Johns Hopkins University, Baltimore, MD.

Sexual dimorphism is crucial for the propagation of a sexually reproducing species and the formation of an oocyte vs. sperm. We are interested in how the sex determination pathway controls sexual dimorphism, including how the conserved transcription factor Doublesex (Dsx) regulates sex-specific development of the somatic gonad. Follicle cells are female-specific cells that surround and nurture the developing oocyte and are found in diverse animals, including flies and mammals. The germarium of the *Drosophila* ovary contains two different types of somatic cells, the escort cells (which nurture the germline early in differentiation) and follicle cells (which nurture the germline later) and arise from a stem cell population, the follicle stem cells (FSCs). We are investigating the development of the FSCs and their relationship to the escort cells to determine if they come from a group of common precursor cells. The best-known marker for FSCs is the transcription factor Castor, which labels female FSCs in addition to pre-follicle cells, and stalk cells. We have conducted a time-course immunostaining of pupal ovaries examining Castor expression as a readout for FSC specification. Castor is not observed at 2 hrs through 7 hrs after pupal formation (APF). The earliest Castor expression can be seen at 8 hrs APF in cells intermingled with the germ cells in the middle of the developing ovarioles. At 24 hrs APF, Castor expression is seen primarily in the basal stalk cells posterior to the germline. We are currently using lineage analysis to study the origins of the FSCs and if they are related to basal stalk cells. Through our imaging of the pupal ovary during its formation, we are gaining a basic understanding of the developmental coordination of the different cells types. We are determining what is required during development for the specification of the FSCs and we believe that the escort cells precede FSC specification. Additionally, we have determined that FSC specification is dependent on the JAK/STAT pathway, similar to what is thought for male somatic cell specification. Knockdown the JAK/STAT pathway in the somatic cells of the ovary results in a loss of follicle cells and Castor expression. We also see an expansion of the escort cell population. We are investigating the mechanisms that control female FSC specification and how the JAK/STAT pathway may intersect with information about sexual identity regulated by Dsx

146 Adaptive evolution of piRNA pathway proteins affects piRNA biogenesis but not TE transcripts. *L. Wang*[1], D. Barbash[2], E. Kelleher[1] 1) Department of Biology & Biochemistry, University of Houston, Houston, TX, USA; 2) Department of Molecular Biology & Genetics, Cornell University, Ithaca, NY, USA.

In metazoan germlines, the piRNA pathway acts as a genomic immune system: employing small-RNA (piRNA) mediated silencing to defend host genome from the harmful effects of transposable elements (TEs). In response to dynamic changes in genomic TE content, host genome is proposed to alter the piRNAs that they produce to silence the most active TEs. However, piRNA pathway proteins, which execute piRNA biogenesis and enforce targeted sequence silencing, also evolve rapidly and adaptively. If TE silencing evolves through changes in piRNAs, what necessitates the adaptive changes among piRNA pathway proteins? To address this question, we performed interspecific complementation on three adaptively evolving piRNA pathway proteins: Armitage, Aubergine and Spindle-E. We compared the ability of *Drosophila melanogaster* and *D. simulans* wild-type alleles to complement a *D. melanogaster* mutant background for TE transcript regulation and piRNA biogenesis. Phenotypes for which the *D. simulans* alleles fail to fully complement the mutant, or differ between the alleles of the two species, point to diverged functions that are potential targets of adaptive evolution among piRNA pathway proteins.

Surprisingly, we observed that *D. simulans* alleles exhibited minimal functional divergence in TE transcript regulation as compared to the *D. melanogaster* alleles. Rather, *D. simulans armitage* and *aubergine* exhibited defects in piRNA biogenesis. Defects associated with *armitage* were particularly severe; decreases in both ping-pong biogenesis and phasing biogenesis were linked to the global reduction of piRNA abundance in the presence of the *D. simulans* allele. The reduced piRNA abundance associated with *D. simulans aubergine* was also accompanied by decreased ping-pong biogenesis. Our results suggest that piRNA biogenesis, rather than TE transcript regulation, is the primary target of adaptive evolution among these adaptively evolving piRNA pathway proteins. Furthermore, the absence of downstream effects on TE transcripts resulting from changes in the piRNA pool suggests complex and unknown feedback controls exist between piRNA processing, transcriptional and post-transcriptional TE silencing.

147 New Tools and Methods for Neuronal Circuit Analysis in Drosophila. *G.M. Rubin*, FlyEM & FlyLight Project Teams Janelia Research Campus, HHMI, Ashburn, VA.

I will describe new resources for circuit analysis that are being developed by the Janelia FlyEM and FlyLight Project Teams. The FlyEM and FlyLight Team Projects have synergistic goals: proving a complete wiring diagram of the adult Drosophila central nervous system along with the genetic tools needed to measure and manipulate the activity of well-defined groups of cells within this neuronal network.

For example, the FlyEM project recently reported a dense reconstruction of the vertical lobe of the adult mushroom body (Takemura *et al*. eLife, 2017). In a dense reconstruction, the intent is to reconstruct all cells in the volume, rather than trace the processes and connections of a selected set of neurons (for an example of this latter approach, see Zheng *et al.* Cell 2018). Most of the effort in generating a connectome based on electron microscopic images occurs after image collection: reconstructing the shapes of all neurons and mapping the number and locations of chemical synapses between them. Over the past two years, there has been 50X increase in the rate at which such dense reconstructions can be generated; most of this increase resulted from improvements in machine vision segmentation algorithms provided by our collaborators at Google (Janueszewski *et al*. https://arxiv.org/abs/1611.00421), but improved sample preparation and imaging methods have also been important. In the next two years we anticipate a further 4X improvement from a combination of better sample preparation, improved imaging methods, further advancements in segmentation and better software tools for the final step of computer-assisted human proof reading. We are optimistic that this 200X increase in efficiency will enable the FlyEM team and collaborators to generate the first full fly connectome—a circuit map of the entire adult brain and ventral nerve cord—within three years.

To complement the connectome, highly specific genetic drivers to individually manipulate the several thousand cell types that constitute the fly's brain will be needed to probe circuit function. Generating these tools is the primary goal of FlyLight. By the time the connectome is complete, FlyLight and a set of collaborating laboratories expect to have generated such split-GAL4 lines for approximately half of the roughly 5,000 cell types in the fly central nervous system.

148 Spying on the dynamics of acetylcholine, dopamine, octopamine, and 5-HT in fly's brain by constructing new genetically-encoded GRAB sensors. *Y. Li*[1,2,3] 1) State Key Laboratory of Membrane Biology, Peking University School of Life Sciences, Beijing, China; 2) PKU-IDG/McGovern Institute for Brain Research, Beijing, China; 3) Peking-Tsinghua Center for Life Sciences, Beijing, China.

Neuronal communication in the brain relies on the release of neurotransmitters. In both flies and mammals, conserved chemical neurotransmitters such as acetylcholine (ACh), dopamine (DA) and 5-HT play critical roles in a plethora of important physiological processes, including sensory information processing, sleep-wake control and learning & memory. Despite important roles of these neurotransmitters, their dynamics in the brain is surprisingly largely unknown, in

part due to technical gaps: we currently lack of sensitive molecular sensors capable of reporting neurotransmitters' dynamics with good spatiotemporal resolution as well with chemical- and cell-type specificity. To fill this technical gap, our group tap into human and fly's GPCRs, engineer them to fuse with a conformation-sensitive GFP, and successfully create a family of genetically-encoded GRAB (GPCR activation based) sensors. These GRAB sensors harness the ligand induced conformational change of their cognate GPCRs and produce a large fluorescent signal output from the fused GFP. We subsequently made transgenic flies expressing GRAB-ACh, DA, octopamine (OA), and 5-HT in various neurons using Gal4/UAS, LexA/LexAop and QF/QUAS systems. By imaging live flies under two-photon microscope, we found these GRAB sensors capable of reporting triggered ACh, DA, OA and 5-HT release elicited by direct electric stimulation, with as few as one single stimulus. We also observed that GRAB sensors enabled detection of evoked endogenous ACh, DA, OA and 5-HT release elicited by physiological relevant stimuli, i.e. application of fruity odor or punishment related stimuli (abdomen shocks), even with single cell resolution. Importantly, ectopic expression of GRAB sensors *in vivo* does not appear to alter the cell physiology, measured by a concurring expressed red calcium sensor or a red cAMP sensor. Thus, GRAB sensors for ACh, DA, OA and 5-HT provide broadly-applicable molecular tools to understand the function and regulation of neurotransmitters in diverse physiological processes of flies *in vivo*.

149 Selectable, drug-based genetics and transgenesis in *Drosophila melanogaster*. *N. Matinyan*[1,2], A. Sarrion-Perdigones[2], Y. Gonzalez[2], H. Dierick[3,4], K. Venken[1,2,5,6,7] 1) Integrative Molecular Biomedical Sciences Graduate Program, Baylor College of Medicine, Houston, TX; 2) Verna and Marrs Mclean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX; 3) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 4) Department of Neuroscience, Baylor College of Medicine, Houston, TX; 5) Department of Pharmacology, Baylor College of Medicine, Houston, TX; 6) Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX; 7) Mcnair Medical Institute, Baylor College of Medicine, Houston, TX.

We have developed a drug-based, selection strategy for the generation and genetic manipulation of transgenic fruit flies using commonly available antibiotics and other drugs. Our method couples either an antibiotic resistance marker or a drug sensitivity marker to desired genetics changes to select rather than screen for modified animals. Exposure of developing larvae to drug-laced food eliminates non-modified, sensitive animals while selecting for modified larvae expressing the resistance marker. Conversely, transgenic larvae expressing a drug sensitivity marker are selected against via drug exposure without affecting non-modified, non-sensitive animals. We are able to select flies expressing the resistance marker for the antibiotics: G418, puromycin, blasticidin, or hygromycin. Moreover, utilizing drug sensitivity markers we are able to select against sensitive, modified larvae using either the fungicide 5-fluorocytosine or the antiviral drug ganciclovir. Drug treatment is largely cost-effective and all drugs are commonly available from most, if not all, vendors. Selectable markers are dominantly expressed and unlike physical markers have no endogenous analogues allowing use in any genetic background. In addition, drug-based selection markers, unlike fluorescent markers, do not interfere with downstream analysis or application. Marker conferred drug resistance or sensitivity is specific to the corresponding drug, allowing multiple markers to be together in pairs or in multiples for complex genome engineering involving several, concurrent genetic changes currently extremely difficult with conventional physical or fluorescent-based screening. We have utilized this system for single-step visualization of protein co-localization via paired co-injection of fluorescently tagged, selectable genomic BAC clones into double docking site fly lines followed by dual selection of injected animals. Selection-based genetics eliminates screening for transgenic individuals, can be used in any genetic background, and allows for rapid multiplexed genome modification in a single step. Selection-based genetics should be easily adaptable for use in other insect species such as mosquitos and would be especially helpful in non-model organisms and those with few currently available genetic tools.

150 Bellymount: A novel, method for longitudinal, intravital imaging of abdominal organs in adult Drosophila. *L.A.J. Koyama*[1,2], YH Su[2], A. Aranda-Diaz[3], J. Martin[2], S. Balachandra[2], W. Ludington[4], K.C. Huang[3], L.E. O'Brien[2] 1) Department of Developmental Biology, Stanford University, Stanford, CA; 2) Department of Molecular and Cellular Physiology, Stanford University, Stanford, CA; 3) Department of Bioengineering, Stanford University, Stanford, CA; 4) Department of Embryology, Carnegie Institution for Science, Bethesda, MD.

In adult Drosophila, many cell- and tissue-level processes such as clonal evolution, bacterial colonization, and ageing take place over days or weeks. However, there is a paucity of approaches to monitor the same cells and organs in live animals over multiday timescales. Here we present Bellymount, an approach for intravital, longitudinal imaging of Drosophila abdominal organs for periods up to 4 weeks. Bellymount is compatible with standard imaging platforms and comprises a simple, 3D-printed chamber and a microprocessor. Within this chamber, up to 5 adults are adhered to a coverslip while microcontrolled pulsing of CO2 applies tunable anesthesia. Bellymount yields multichannel, micron-resolution images of abdominal tissues including the midgut, crop, ovaries, fat body, Malpighian tubules, and hemocytes—all within animals that are live and intact. Animals are released after imaging and remain viable, which allows the same tissues to be re-imaged in subsequent sessions. To demonstrate the potential of Bellymount, we traced the evolution of GFP-labeled stem cell clones in the midgut. Over 6 days of periodic imaging, some clones increased in cell number whereas others decreased, and adjacent clones occasionally merged together. In other experiments, we tracked colonization of the digestive tract by the commensal bacterium Lactobacillus plantarum. Following oral administration, mCherry-expressing Lactobacillus filled the crop and midgut lumen; however after 3 days, Lactobacillus were lost from the midgut but maintained in the crop. Overall, Bellymount opens the door to longitudinal imaging of multi-day processes within the Drosophila abdomen, enabling deeper understanding of cell- and tissue-level dynamics.

151 GAL4s, LEGOs, and 3D-printers: the genetic toolbox of the 21st century Drosophilist. *G.F. Gilestro* Department of Life Sciences, Imperial College London, London, UK.

For many years, starting from Seymour Benzer's seminal work, the fruit fly *Drosophila melanogaster* has been considered the model organisms of choice to dissect the genetics of behaviour. In the past decade, *Drosophila* has also emerged as an excellent model for studying not only the genes but the neuronal circuitry of behaviour too: the combination of a rapidly delineating connectome together with an unrivalled repertoire of genetic tools have established fruit flies as one of the most promising animal models to study neuronal circuits. The limiting factor for ethomics – the high-throughput approach to behavioural studies – is therefore not the availability of genetic tools, but rather the access to an objective, reproducible and scalable system to detect and classify behaviour.

Historically, *Drosophila* neuroscientists have often shown a high degree of ingenuity in devising paradigms and creating apparatus able to capture relatively simple behaviours in a high-throughput fashion, usually driven by the desire to perform genetic screens. Analysis of phototaxis, geotaxis, response to ethanol inebriation, olfactory learning and habituation, and biology of circadian rhythms are all successful examples of clever paradigms that have allowed high-throughput screenings of specific behaviours. Here we argue that modern technologies – such as 3D-printing, Open Source Software, inexpensive micro-computers, Machine Learning dedicated chips, crowdsourcing – have the potential to build on this tradition and to greatly expand the repertoire of a *Drosophila* scientist. We will present techniques for inexpensive and rapid construction of behavioural paradigms and analytical tools; we will discuss how the landscape of neurogenetics can thrive from partnering with the so-called Maker culture.

152 Techniques and computational methods for single-cell regulatory genomics in Drosophila. Sara Aibar Santos, Carmen Bravo Gonzalez-Blas, Jasper Janssens, Dafni Papasokrati, Suresh Poovathingal, Ibrahim Taskiran, *Stein Aerts* Center for Brain and Disease Research, VIB-KULeuven, Leuven, BE.

Single-cell transcriptomics and single-cell epigenomics allow building cell atlases of any tissue, which can provide unprecedented insight into the dynamics of

cellular state transitions during developmental or disease trajectories. In the first part of my talk I will describe recent single-cell technologies as well as computational methods to identify transcription factors, gene networks, and cell states from single-cell data. These methods include *SCENIC* for the inference of gene networks from scRNA-seq data; *cisTopic* for the prediction of co-regulatory enhancers from scATAC-seq data; and *SCope* for the visualisation of single-cell atlases. To integrate single-cell epigenome and transcriptome data we exploit cis-regulatory sequence analysis, using deep learning and large databases of transcription factor recognition motifs. In the second part of my talk I will present several data sets from the Fly Cell Atlas, with a focus on the central and peripheral nervous system, where we aim to trace genomic regulatory programs of neuronal identity at single-cell resolution.

153 FlyBase updates presentation. *S. J. Marygold*[1], N. Perrimon[2], The FlyBase Consortium 1) Department of Physiology, Development and Neuroscience, University of Cambridge, UK; 2) Dept. of Genetics, Harvard Medical School, Boston, MA.

Updates at FlyBase.org will be presented. Learn how to make the best use of the new FlyBase tools and features at FlyBase.org for your research and teaching.

154 A Gene Disruption Project (GDP) update: using CRISPR with PCR-generated homology donors to knock-in Swappable Integration Cassettes in introns of genes in flies and in S2 cells. *O. Kanca*[1], S.M. Knight[2], G.O. Amador[2], D. Yang-Zhou[2], J. Zirin[2], S. Mohr[2], D. Li-Kroeger[1], K.L Schulze[1,4], Y. He[1,4], W. Lin[1], J.L. Salazar[1], S. Yamamoto[1], M.F. Wangler[1], C.Y. Hu[3], R.W. Lewis[3], A.C. Spradling[3,4], N. Perrimon[2,4], H.J. Bellen[1,4] 1) M & H Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Genetics, Harvard Medical School, Boston, MA; 3) Department of Embryology, Carnegie Institution for Science, Baltimore, MD; 4) Howard Hughes Medical Institute.

Comprehensive gene functional annotation remains one of the greatest challenges in the post genomic era. Versatile genetic tools are invaluable to gain information about the function of genes. The GDP integrates Swappable Integration Cassettes (SIC) in introns of genes through MiMIC and CRIMIC (CRISPR-Mediated Integration Cassette) strategies. SICs typically contain a mutagenic region (e.g. Splice Acceptor-T2A-GAL4-polyA) and a dominant marker (e.g. 3xP3-GFP) flanked by attP sites for Recombination Mediated Cassette Exchange (RMCE). To date the GDP has integrated SICs in introns of more than 2000 genes and generated tools to convert these SICs into diverse genetic tools. These include Double Header to make GFP protein traps or T2A-GAL4 gene traps, and Flip-Flop to create conditional knock out alleles.

Currently GDP uses CRISPR-mediated homologous recombination with double-stranded DNA homology donor plasmids to insert the SICs. We are currently developing new methods that use single stranded DNA (ssDNA) homology donors that can be produced with PCR-based methods on numerous templates. ssDNA use significantly decreases the effort and cost of generating homology donors. So far, we targeted 20 genes in flies with ssDNA with the success rate of ~60% for the selected genes. Moreover, we tested the use of ssDNA donors on S2 cells for homologous recombination. We find that the ssDNA donor-based pipeline is more efficient than using a standard double-stranded donor in S2 cells. We have successfully tagged proteins that are restricted to specific cellular compartments, including nuclear envelope and membrane-bound organelles, with artificial exons encoding GFP. Altogether, we have developed a knock-in strategy for fly transgenesis and S2 cell culture that will be useful for many applications.

155 p53 genes and the game of transposons. *J. Abrams*, A. Wylie, A. Jones, P. Kurtz, B. Tiwari, C. Caillet, S. Das, S. Royer, P. Chen UT Southwestern Medical Center, Dallas, TX.

Throughout the animal kingdom, p53 genes govern stress response networks by specifying adaptive transcriptional responses. The human member of this gene family is mutated in most cancers, but precisely how p53 prevents tumor development is not well understood. We found that *Drosophila* p53 acts to restrict retrotransposon activity. Furthermore, transposon eruptions occurring in the p53^{-}germline were incited by meiotic recombination and transcripts produced from these mobile elements accumulated in the germ plasm. We also found that p53 genetically interacts with components of the piRNA (piwi-interacting RNA) pathway and, in gene complementation studies, normal human p53 alleles suppressed transposons, but mutant p53 alleles from cancer patients could not. Consistent with these observations, we identified patterns of unrestrained retrotransposons in p53-driven mouse and human cancers. Furthermore, p53 status correlated with repressive chromatin marks in the 5' sequence of a synthetic human retroelement. Together, these observations indicate that ancestral functions of p53 operate through conserved mechanisms to contain retrotransposons. Since human p53 mutants are disabled for this activity, our findings raise the possibility that p53 mitigates oncogenic disease in part by restricting transposon mobility.

156 Neural mechanisms for dynamic acoustic communication. *Mala Murthy* Princeton University, Neuroscience Institute, Princeton, NJ.

Social interactions require continually adjusting behavior in response to sensory feedback. For example, when having a conversation, sensory cues from our partner (e.g., sounds or facial expressions) affect our speech patterns in real time. Our speech signals, in turn, are the sensory cues that modify our partner's actions. What are the underlying computations and neural mechanisms that govern these interactions? To address these questions, my lab studies the acoustic communication system of *Drosophila*. To our advantage, the fly nervous system is relatively simple, with a wealth of genetic tools to interrogate it. Importantly, *Drosophila* acoustic behaviors are highly quantifiable and robust. During courtship, males produce time-varying songs via wing vibration, while females arbitrate mating decisions. We discovered that, rather than being a stereotyped fixed action sequence, male song structure and intensity are continually sculpted by interactions with the female, over timescales ranging from tens of milliseconds to minutes – and we are mapping the underlying circuits and computations. We have also developed methods to relate song representations in the female brain to changes in her behavior, across multiple timescales. Our focus on natural acoustic signals, either as the output of the male nervous system or as the input to the female nervous system, provides a powerful, quantitative handle for studying the basic building blocks of communication.

157 Y chromosome evolution in 400 *Drosophila* species. *Bernardo Carvalho*[1], Eduardo Dupim[2] 1) Departamento de Genetica, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, BR; 2) Departamento de Genetica, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, BR.

Y chromosomes are widely believed to evolve from a normal autosome through a process of massive gene loss (with preservation of some male genes), shaped by sex-antagonistic selection and complemented by occasional gains of male-related genes. The net result of these processes is a male-specialized chromosome. This might be expected to be an irreversible process, but it was found in 2005 that the *Drosophila pseudoobscura* Y chromosome was incorporated into an autosome. Y chromosome incorporations have important consequences: a formerly male-restricted chromosome reverts to autosomal inheritance, and the species may shift from an XY/XX to X0/XX sex-chromosome system. In order to assess the frequency and causes of this phenomenon we searched for Y chromosome incorporations in 400 species from *Drosophila* and related genera. We found one additional large scale event of Y chromosome incorporation, affecting the whole *montium* subgroup (40 species in our sample); overall 13% of the sampled species (52/400) have Y incorporations. While previous data indicated that after the Y incorporation the ancestral Y disappeared as a free chromosome, the much larger data set analyzed here indicates that a copy of the Y survived as a free chromosome both in *montium* and *pseudoobscura* species, and that the current Y of the *pseudoobscura* lineage results from a fusion between this free Y and the neoY. The 400 species sample also showed that the previously suggested causal connection between X-autosome fusions and Y incorporations is, at best, weak: the new case of Y incorporation (*montium*) does not have X-autosome fusion, whereas nine independent cases of X-autosome fusions were not followed by Y incorporations. Y incorporation is an underappreciated mechanism affecting Y chromosome evolution; our results show that at least in *Drosophila* it plays a relevant role and highlight the need of similar studies in other groups.

158 Waking up "Sleeping" Neural Stem Cells. *Hongyan Wang* Duke-NUS Medical School, Singapore, SG.

Neural stem cells have the potential to regenerate and repair brain damage. In the mammalian adult brain, the majority of neural stem cells (NSCs) are in a mitotically inactive, quiescent state with little regenerative ability. Interestingly, in response to extrinsic stimuli, these quiescent NSCs can exit from quiescence and resume proliferation (reactivation) to generate new neurons. How NSCs switch between quiescence and proliferation remains poorly understood. Recently, *Drosophila* NSCs, also known as neuroblasts, have emerged as a powerful model to study the mechanisms underlying NSC quiescence and reactivation *in vivo*. *Drosophila* neural stem cells enter into quiescence at the end of embryogenesis and they subsequently reactivate in early larval stages. In response to dietary amino acids, *Drosophila* NSCs exit from quiescence by activation of an evolutionarily conserved insulin/IGF signaling pathway. Here, we focus on elucidating intrinsic mechanisms during NSC reactivation. We show that the spindle matrix complex containing Chromator (Chro) functions as a key intrinsic regulator of NSC reactivation downstream of extrinsic Insulin/IGF signalling. Chro also prevents NSCs from re-entering quiescence at later stages. NSC-specific *in vivo* profiling have identified many downstream targets of Chro, including a temporal transcription factor Grainy head (Grh) and a NSC quiescence-inducing factor Prospero (Pros). Spindle matrix proteins promote the expression of Grh and repress that of Pros in NSCs to govern their reactivation. Our data demonstrate that nuclear Chro critically regulates gene expression in NSCs at the transition from quiescence to proliferation. We will also discuss other novel intrinsic mechanisms by which NSC are reactivated.

159 Towards a brain architecture for visual behavior selection. *Gwyneth Card* HHMI Janelia Research Campus.

Selecting the right behavior at the right time is critical for animal survival. Animals rely on their senses to deliver information about the environment to sensory processing areas in the brain that extract relevant features and form the perceptual representations that guide behavior. We aim to uncover the organization of this feature space and the neural mechanisms by which these cues are translated into dynamic motor activity.

Our current focus is visually-driven behaviors of the fly. In particular, those driven by visual looming cues produced by an approaching predator or an imminent collision. The same looming stimulus can evoke a wide range of different behaviors, including a rapid escape jump, a slower, more stable takeoff sequence, or a landing response. We use whole-cell patch clamp physiology in behaving flies, calcium imaging, high-throughput/high-resolution behavioral assays, and genetic tools to examine the transformation of information from sensory to motor. I will discuss our recent work investigating the representation of ethologically-relevant visual features in the fly optic glomeruli and the mechanisms by which descending neurons read out this feature information to produce an appropriate behavioral choice.

160 Upstream regulation of Hippo signaling in epithelial cells. *Rick Fehon* Molec. Gen. & Cell Biology, University of Chicago, Chicago, IL.

The regulation of tissue growth is a critical aspect of normal development. Systematic screens in Drosophila have revealed the Hippo growth control pathway, which subsequent studies have shown to be highly conserved and an important target for understanding tumorigenesis in humans. We focus on understanding how upstream Hippo pathway regulators are organized and function at the cortex in polarized epithelial cells using endogenously-expressed, fluorescently-tagged forms of pathway proteins coupled with high-resolution confocal microscopy in living tissues. Upstream regulators include three membrane-associated proteins, Kibra, Merlin, and Expanded, together with the transmembrane polarity component, Crumbs. We have shown that while Crumbs and Expanded localize and function exclusively at the junctional cortex, Kibra and Merlin function in parallel at the medial cortex. Rather than promoting Kibra activity, our results indicate that Crumbs functions to repress Kibra, possibly by sequestering it at the junctional cortex. In addition, in a separate line of work we have made the surprising discovery that Yorkie, the transcriptional coactivator that mediates pathway output, has a second 'life' at the cell cortex where it functions in a positive feedback loop that regulates pathway activity. Although the significance of multiple inputs from separate cellular compartments is not yet clear, these results highlight the complexity of Hippo pathway regulation and suggest intriguing future directions for understanding the cellular mechanisms that regulate tissue growth.

161 Anti-apoptotic function of ecdysone signaling in Drosophila. *Jae H Park*, Gyunghee Lee, Ritika Sehgal, Zixing Wang BCMB, Univ Tennessee, Knoxville, TN.

There are nearly 10,000 neurons in the Drosophila larval central nervous system (CNS). During metamorphic development, these neurons have distinct fates, and a significant number of larval neurons in the CNS undergo programmed cell death. Although ecdysone and its cognate ecdysone receptors (EcRs) is known to orchestrate the neuronal apoptosis, doomed neurons are eliminated at different metamorphic stages. How EcR signaling induces such diverse neuronal fates is not well understood. In our study, we have employed Crustacean cardioactive peptide (CCAP)-peptidergic neurons and vCrz neurons as a model system as they undergo apoptotic death at different stages during pupal development. Interestingly, we found that expression of EcR dominant negative (EcR^{DN}) caused precocious death of the CCAP neurons in a caspase-dependent manner and such earlier death was fully rescued by co-expression of EcR-B isoforms and partially by EcR-A, suggesting that EcR isoforms function redundantly to protect CCAP neurons from their premature death. Moreover, the protection of CCAP neurons does not seem to involve Ultraspiracle (Usp), a conventional dimeric EcR partner, as expression of Usp-RNAi or putative Usp dominant negative did not cause CCAP cell death. As a downstream, a cell death promoter *grim* is essential for the EcR^{DN}-mediated CCAP neuron death. From these results, signaling through EcR is likely to protect CCAP neurons from their premature death via repression of *grim* until their natural apoptosis at post-emergence. These data suggest differential aspects of EcR action for orchestrating the opposite fates (death or survival) of larval neurons during metamorphosis.

162 Using whole genome sequencing as a tool to identify novel regulators of apoptosis. *A. Shields*, R. Li, H. Liu, L.J. Zhu, M. Conti, A. Bergmann Department of Molecular, Cell & Cancer Biology, University of Massachusetts Medical School, Worcester, MA.

For years, the backbone of the *Drosophila* apoptotic pathway has been known. However, many important players have yet to be identified. Known regulators of apoptosis in *Drosophila*, including caspases Dronc and drICE, and the apoptosome component dArk, are encoded by recessive genes. To identify novel recessive genes that participate in apoptotic cell death, we expressed the proapoptotic factor, *hid*, in the *Drosophila* eye using the *GMR* promoter (*GMR-hid*) to generate a strong eye-ablation phenotype, induced genetic mosaics using the *ey-FLP/FRT* system, and generated point mutations using the chemical ethyl methanesulfonate (EMS). Mutations on chromosome arms 2R, 3L, and 3R that suppressed the *GMR-hid*-induced, eye-ablation phenotype were selected for further study. Our results suggest that these mutations play a role in developmental apoptosis and likely act in parallel or downstream of caspases in the apoptotic pathway. Whole genome sequencing of the mutant flies followed by comparison to the published *Drosophila* genome and *FRT* controls provided gene-level identification of candidates. Using specific RNAi and mutant lines, candidate genes are currently being tested for their involvement in developmental apoptosis.

163 Evaluation of *spitz* in cell survival after telomere loss. *M. Brakhane*, C. Bearden, R. Kurzhals Biology, Southeast Missouri State University, Cape Girardeau, MO.

Telomeres are found at the ends of chromosomes and protect the DNA from being seen as a double strand break. Attrition of telomeres during DNA replication can lead to genomic instability and cancer development in mammalian cells. This is analogous to telomere loss in fly cells. We cause telomere loss by first inducing formation of a dicentric chromosome that breaks during mitosis delivering a chromosome without a telomere to each daughter cell. Typically,

cells die in response to telomere loss, but a small percentage survives. To understand how cells can survive in the presence of a single broken end we did a misexpression screen to identify modifiers of the cell death phenotype. We identified *spitz* as a suppressor of cell death after telomere loss. We want to understand the mechanism by which *spitz* is affecting cell survival in those cells that are lacking a single telomere. In order to do this, we are examining other genes in the *spitz* pathway, including *Star*, *rhomboid*, *argos*, and *Egfr*. We are also examining *spitz* paralogs, *vein*, *keren*, and *gurken*, and different *spitz* chimeras to further characterize *spitz* suppression of cell death after telomere loss. Our preliminary results show misexpression of *Star* leads to suppression of the cell death phenotype, similar to misexpression of *spitz*, and reduction of *rhomboid* leads to an enhanced cell death phenotype, consistent with the roles these genes play in the *spitz* pathway. Other preliminary data suggests that the level of *spitz* is important for determining if cells will proliferate or go through apoptosis, which is consistent with studies by other groups. In addition, we are examining the level of apoptosis and cell proliferation in these genotypes after telomere loss.

164 Analyzing the importance of ubiquitin-dependent selective protein aggrephagy in Drosophila. *G. Juhasz*, A. Bhattacharjee, A. Urmosi Institute of Genetics, Biological Research Centre, HAS, Szeged, Csongrad, HU.

During autophagy, selected intracellular components are degraded in lysosomes to recycle damaged or obsolete proteins, complexes, and organelles. Autophagy was shown to be critical for neuronal function, supposedly via the continuous selective turnover of ubiquitinated cargo. Accordingly, Atg mutant flies, mice and humans show developmental delay, increased stress sensitivity, ataxia, neurodegeneration, and a consequently shortened lifespan. Ubiquitination is a common degradation signal for proteins and organelles such as mitochondria. Selective autophagy receptors can recognize ubiquitinated cargo and bind to Atg8/LC3 family proteins to ensure their capture into autophagosomes. In Drosophila, only one autophagy receptor for ubiquitinated protein aggregates is known: p62/Ref(2)P, the ortholog of human SQSTM1 whose mutations are found in amyotrophic lateral sclerosis, frontotemporal dementia and Paget disease. P62 possesses a C-terminal ubiquitin-binding domain (UBA), an N-terminal PB1 domain to mediate aggregate formation, and a short LIR (LC3-interacting region) motif in an unstructured region, which is responsible for LC3/Atg8a binding on autophagic membranes.
To investigate the autophagy-specific role of p62/Ref(2)P in Drosophila, we replaced two previously characterized key amino acids within the LIR motif: a Tryptophan and an Isoleucine to Alanines by editing the endogenous gene using CRISPR. The emerging Drosophila line carries these p62 LIR mutations, disrupting the autophagic degradation of p62. Large-scale accumulation of p62 and ubiquitinated protein aggregates are observed in various tissues of flies homozygous for the p62 LIR mutation, while animals are viable and fertile. Surprisingly, we find that the p62 LIR mutation has no effect on fly development, neurodegeneration and lifespan, indicating that the large-scale build-up of ubiquitinated protein aggregates is not detrimental to neurons or other cell types. Interestingly, p62 LIR mutant animals show increased resistance to oxidative stress provoked by feeding flies paraquat, a Parkinsonian drug, likely due to induction of antioxidant responses by p62 accumulation.

165 The Fbox protein CG6758 regulates Xbp1-induced cell death in the *Drosophila*eye. C Santos[1], N Schweizer[1], C Gaspar[1,2], V Rasheva[1], F Cairrão[1], J Sánchez[3,4], U Mayor[3,4,5], C Adrain[2], *P Domingos*[1] 1) Instituto de Tecnologia Química e Biológica (ITQB), Universidade Nova de Lisboa, Oeiras, Portugal; 2) Instituto Gulbenkian de Ciência (IGC), Oeiras, Portugal; 3) Department of Biochemistry and Molecular Biology, University of the Basque Country (UPV/EHU), Leioa, Bizkaia, Spain; 4) Functional Genomics Unit, CIC bioGUNE, Derio, Spain; 5) Ikerbasque, Basque Foundation for Science, Bilbao, Bizkaia, Spain.

The Unfolded Protein Response (UPR) is composed by homeostatic signaling pathways that are activated by excessive protein misfolding in the Endoplasmic Reticulum (ER). Prolonged ER stress and UPR activation may lead to cell death and photoreceptor degeneration. We found that over-expression of the transcription factor Xbp1 spliced (Xbp1s) induces retinal degeneration in Drosophila and we performed a genetic screen to identify genes that, downstream of Xbp1s, mediate the induction of retinal degeneration. In this genetic screen, we used the FLPase/FRT technique and looked for EMS induced mutations that suppress Xbp1s induced retinal degeneration. So far, we identified mutations in 3 independent genes: Xpd, Eaf and CG6758. We are pursuing CG6758, a gene encoding an Fbox protein with unknown biological function. F-box proteins form complexes with Skp, Cullin-1 and E2 ubiquitin ligases (SCF complexes) to mediate the ubiquitination of specific substrates, and leading to the degradation of these substrates by the proteasome. We did two proteomic screens to identify binding partners and ubiquitylated substrates of Fbox/CG6758 and identified LaminB and Ataxin-2 as possible candidate substrates of Fbox/CG6758. We are currently investigating how regulation of LaminB and Ataxin-2 protein levels by Fbox/CG6758 leads to the suppression of the retinal degeneration process induced by Xbp1s.

166 Loss of the ER metalloprotease CG14516 rescues retinal degeneration by reducing ER stress-induced apoptosis in a *Drosophila* model of retinitis pigmentosa. *Rebecca Palu*, Clement Chow Human Genetics, University of Utah, Salt Lake City, UT.

An important goal of the Precision Medicine Initiative is to address the phenotypic heterogeneity that impedes diagnosis and treatment in both Mendelian and complex genetic diseases. Cryptic genetic variation is a key contributor to this heterogeneity, but the underlying genetic architecture and modifiers are largely unknown. Understanding the roles of genetic modifiers in disease processes will enable the development of individualized therapeutic approaches. One process that commonly contributes to phenotypic heterogeneity is the ER stress response. In a previous study, we utilized the *Drosophila* Genetic Reference Panel (DGRP) to study the effects of natural genetic variation on a model of ER stress-associated retinal degeneration. Overexpression of a mutant, misfolded rhodopsin protein ($Rh1^{G69D}$) induces ER stress in the developing eye, ultimately resulting in apoptosis and a small, degenerate adult eye that is incredibly variable among DGRP strains. Using genome-wide association methods, we identified 84 conserved candidate modifiers, many of which are associated with ER stress and apoptosis. We went on to characterize a number of these as suppressor modifiers, knock-down of which leads to reduced degeneration in our model of ER stress. One of these suppressor modifiers is the uncharacterized ER-associated metalloprotease *CG14516*. Loss of this enzyme in the $Rh1^{G69D}$ model rescues the degeneration, resulting in a larger, healthier eye. This rescue is a result of reduced ER stress-induced apoptosis and reduced activation of JNK signaling through the atypical cyclin Cdk5. Strikingly, loss of CG14516 activity does not alter canonical ER stress signaling through IRE1, PERK, or ATF6. This suggests that loss of *CG14516* reduces ER stress-induced apoptosis without altering the benefits of pro-survival pathways induced by the Unfolded Protein Response. Finally, we found that loss of *CG14516* can reduce ER stress-associated JNK signaling and apoptosis induced through several methods and across multiple tissues, making this a general modifier of the ER stress response. This metalloprotease and its human orthologues are good candidate targets for therapeutic development in a number of diseases where stress-induced apoptosis is a primary cause of degeneration.

167 Regulation of the Unfolded Protein Response by Fic-mediated AMPylation and deAMPylation of BiP protects photoreceptors from light-dependent degeneration. *A. Casey*[1], A. Moehlman[2], K. Servage[1,3], H. Krämer[2], K. Orth[1,3,4] 1) Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX; 2) Department of Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX, USA; 3) Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX, USA; 4) Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX, USA.

In response to environmental, developmental, and pathological stressors, cells engage homeostatic pathways to maintain their function. Among these pathways, the Unfolded Protein Response (UPR) protects cells from the accumulation of misfolded proteins in the ER. The activity of the major endoplasmic reticulum chaperone and UPR master regulator BiP is regulated by Fic-mediated AMPylation during resting states and Fic-mediated deAMPylation during times of stress. In this study, we aimed to understand the consequences of this regulation of BiP in the *Drosophila* visual system. After 72 hours of constant light,

photoreceptors of *fic*-null and AMPylation-resistant *BiP*^{T366A} mutants, but not wild-type flies, display loss of synaptic function, disintegration of rhabdomeres, and excessive activation of ER stress reporters. Strikingly, this phenotype is reversible: photoreceptors regain their structure and function within 72 hours once returned to a standard light:dark cycle. These findings show that Fic-mediated AMPylation of BiP is required for neurons to adapt to transient stress demands. In addition, we show that excessive AMPylation by a constitutively-AMPylating Fic^{E247G} mutant is lethal in Drosophila. This lethality is cell autonomous as directed expression of the mutant Fic^{E247G} to the fly eye does not kill the fly but rather results in a rough and reduced eye. Lethality and eye phenotypes are rescued by the deAMPylation activity of wild-type Fic, consistent with Fic acting as a deAMPylation enzyme. Furthermore, lethality is also rescued by the expression of AMPylation-resistant *BiP*^{T366A}, indicating unregulated AMPylation of BiP is lethal. This work elucidates the importance of the reversible AMPylation of BiP in maintaining the *Drosophila* visual system in response to stress.

168 Analysis of Gp210 function during ER stress responses in *Drosophila melanogaster*. Brian Jenkins, Brad Darwin, Alex Chang, Cole Lambo, Grace Walker-Stevenson, *Sean Speese* Department of Neurology, OHSU - Jungers Center for Neuroscience Research, Portland, OR.

Although Gp210 was the first nuclear pore complex (NPC) protein identified 36 years ago, a role in supporting nucleocytoplasmic transfer remains elusive. A number of studies suggest that Gp210 is not required for the assembly, localization or function of the NPC, consistent with observations that not all cells express Gp210. Recent inquiries into Gp210 function have uncovered roles in a variety of cellular processes including regulation of gene specific transcription in muscle cells, maintenance of ER homeostasis, cellular differentiation, and regulation of T-cells. Despite the intriguing nature of this protein and the fact that it is overexpressed in numerous types of cancers, few *in-vivo* studies have been conducted.

We have undertaken an in-depth study of Gp210 function in *Drosophila melanogaster,* investigating *gp210* mutant phenotypes in multiple tissues and cell types during various stages of the *Drosophila*life cycle. Similar to recent studies in zebrafish and mice, we have found that strong mutations in *gp210* result in viable and fertile animals with a normal lifespan and very few pronounced phenotypes.

Importantly, we do find that *gp210* mutants have elevated levels of ER stress in some tissues, as assessed by Grp78/Bip immunostaining. We are currently utilizing additional stress sensors to determine what tissues are sensitive to the loss of Gp210. Preliminary results suggest that *gp210* mutants are unable to activate the expression of a subset of genes in response to ER stress induction via tunicamycin challenge. Interestingly, this subset of genes are known to be regulated by the ER stress responsive transcription factor, ATF4, suggesting that Gp210 may function together with ATF4 to regulate gene expression downstream of ER stress induction. We are currently conducting tests to determine if we can observe a genetic and/or biochemical interaction between Gp210 and ATF4. Interestingly, overexpression of Gp210 increases the lifespan of flies being challenged with ER stress via tunicamycin feeding. These findings are intriguing in light of the fact that many tumor types overexpress Gp210 and they suggest that Gp210 may be functioning to mitigate ER stress in tumor cells, thereby making it a possible thearapuetic target. Ultimately, these *in-vivo* studies will add to our understanding of this enigmatic "NPC" protein.

169 Deciphering the physiological role of IRE1 signaling in Drosophila eye development. *S. Mitra* Skirball Institute, New York University, New York, NY.

In eukaryotes starting from yeast to human, the presence of unfolded or misfolded protein inside the endoplasmic reticulum (ER) lumen activates signaling cascades, commonly referred as Unfolded Protein Response (UPR). One of the major component of UPR is IRE1 signaling pathway. IRE1 (inositol – requiring enzyme) is an ER-membrane based protein consisting of two different domains, Luminal domain (sensor of misfolded proteins inside the ER lumen) and RNase domain (cleaves specific sets of mRNAs). In presence of misfolded proteins, a conserved histidine residue in the RNase domain (Histidine 890 in Drosophila) is responsible to degrade a specific sets of mRNAs including XBP1 in the cytosol. Many of the previous studies have conducted cell culture-based experiments where UPR signaling is activated in presence of either chemicals or proteins. However, certain UPR- related genes are required for the normal development of the organism including flies, fish and Drosophila, strongly suggesting that there is physiological stress during development that requires UPR pathways to be resolved. In our present study, we examined the role of IRE1 signaling in Drosophila eye development. Fly eye is composed of eight photoreceptors (R1-R8) and the apical part of each photoreceptor is known as rhabdomere. Proper trafficking and maturation of Rhopdopsin-1 (Rh1) protein is responsible to generate rhabdomere in fly eye. Previous study has established that loss of IRE1 in eye cells leads to the reduction in rhabdomere morphogenesis, whereas the mechanistic details about the exact role of IRE1 in drosophila eye development is still not clear. Using Drosophila as a genetic tool, our initial findings suggest that mutation in dIRE1 RNase domain (H890A) shows a significant rough eye phenotype. Further analysis suggests that IRE1 is involved in fate determination in Drosophila eye tissue and this function of IRE1 is dependent of its conventional RNase activity. In a nutshell, our data represents the evidence about the importance of IRE1 pathway in tissue-specific development of Drosophila.

170 The GATOR2 complex regulates the dynamic recruitment of TSC to lysosomes. *Y. Zhang*, S. Yang, C. Ting, K. Kim, L. Bettedi, E. Ghaniam, M. Lilly NICHD, NIH, Bethesda, MD.

The nutrient sensitive Target of Rapamycin Complex 1 (TORC1) is a master controller of metabolism in eukaryotes that regulates physiology, metabolism and aging. In the presence of amino acids, the Rag GTPase complex, comprised of the heterodimer of RagA and RagC, recruits TORC1 to lysosomes where it is activated by Rheb. The Gap Activity Towards Rags (GATOR) complex is a highly conserved upstream regulatory of TORC1 activity which is comprised of two subcomplexes, GATOR1 and GATOR2. The GATOR1 complex inhibits TORC1 activity while the GATOR2 complex restrains the activity of GATOR1. In response to amino acid starvation, the GATOR1 complex acts as a GTPase activating protein towards RagA, converting RagA to its GDP bound form which is incapable of recruiting TORC1 to lysosomes. The GATOR2 complex is comprised of five proteins, Mio, Wdr24, Wdr59, Sec13 and Seh1. In *Drosophila*, mutations in *seh1* and *mio* cause the deregulation of the GATOR1 complex and the constitutive inhibition of TORC1 activity in the female germline. Reduced TORC1 activity in *mio* and *seh1* mutants results in a block to oocyte growth and development. To identify upstream regulators and downstream effectors of the GATOR1 complex, We screened 3472 TRiP collection lines, representing 3103 target genes, to identify genes that when co-depleted with *seh1* in the female germline, rescue the *seh1*^{RNAi} ovarian growth phenotype. From the screen, we identify, 67 genes,in which co-expression in the female germline fully suppressed the *seh1* ovarian growth deficit. Notably, we identified multiple genes that negatively regulate growth and metabolism including negative regulators of the TORC1/PI3K/Insulin signaling such as *pten*, *Ampk*, *gigas*, *mapk* (*rl*), *ras* and *p22A*^{B55} (*twins*), and *pp2A-C* (*mts*). Based on their proposed role in the activation of TORC1 we were surprised to find that RagA and RagC depletions rescued the *seh1* small ovary phenotype and dramatically increased TORC1 activity. These data suggest that RagA and RagC facilitate the inhibition of TORC1 activity in the *seh1*mutant background. Using a combination of genetics and lysosomal fluorescence recovery after photobleaching (FRAP), we determined that the GATOR2 complex regulates the dynamic recruitment of the potent TORC1 inhibitor TSC with lysosomes. Thus, our data are consistent with the model that the GATOR2 complex, thru the regulation of GATOR1, impacts the recruitment of both TORC1 and TSC to lysosomes.

171 Identifying the Secretome and Transmembrane Proteins of Non-Professional Phagocytes. *A. Calikyan*, S. Serizier, A. Mondragon, M. Silvestrini, J. Peterson, A. Emili, J. Kwan, K. McCall Biology, Boston University, Boston, MA.

Apoptosis and the engulfment of dead cells are essential processes for the proper development and maintenance of an organism. While apoptosis is well-characterized, many modulatory signals that control apoptotic cell engulfment have not been well described. We use the *Drosophila* ovary as a model for cell

death and clearance, where follicle cells act as non-professional phagocytes and engulf the dying germline. In this system, several transmembrane receptors, such as Draper, have been found to be essential for nurse cell clearance by follicle cells. However, large-scale approaches have not been used to determine the signaling molecules of follicle cells that promote cell clearance. We hypothesized that many of the proteins involved in this network of signaling are secreted or are transmembrane proteins. This project, thus, focuses on identifying the "secretome" and transmembrane proteins of engulfing follicle cells by labeling proteins in the endoplasmic reticulum (ER) with a biotin phenol tag. Horseradish peroxidase (HRP) catalyzes the biotinylation of all proximal proteins. When fused to an ER retention signal (KDEL) the expression of HRP is targeted to the ER. We generated flies carrying this HRP-KDEL construct under UAS control, and then expressed it in desired follicle cell populations. We demonstrated that HRPKDEL localizes around the follicle cell nucleus with immunohistochemistry, suggesting it is expressed appropriately in the endoplasmic reticulum. Moreover, the stains probing for biotinylated proteins and the HRPKDEL construct, respectively, co-localize, indicative of proximal protein biotinylation. Protein biotinylation was also confirmed by western blot analysis. Ongoing experiments will identify and characterize the biotin-labeled proteins by mass spectrometry to determine the secretome of follicle cells. By characterizing the secretome of the follicle cell population, we can identify new proteins that regulate the engulfment of apoptotic cells.

172 Follicle cell actin dynamics and calcium bursts during nurse cell death. *P.G. Candelas*, A. Mondragon, K. McCall Boston University, Boston, MA.

Cell death is a key component in development and for the continued renewal of tissues.Phagoptosis is a process in which phagocytes directly lead to the death of other cells. This process of cell death is significantly less characterized when compared to other mechanisms of cell death, such as apoptosis. In the Drosophila ovary, phagoptosis appears to play a key role in the developmental process of oogenesis. Recent studies have shown that genes associated with phagocytosis are required for the programmed death of nurse cells in the Drosophila ovary (Timmons et al. 2016). Ovaries are made up of 15 nurse cells, a single oocyte, and a layer of follicle cells bordering them. During the process of egg chamber development, all of the nurse cells undergo programmed cell death. During late oogenesis, each nurse cell is surrounded by a group of follicle cells referred to as stretch follicle cells. These stretch follicle cells have recently been implicated as a main promotor of nurse cell phagoptosis. However, an exact mechanism to explain how these stretch follicle cell induce nurse cell death is not fully classified. To achieve a more detailed understanding of this mechanisms, we are examining the function of the cytoskeleton in this process via live imaging. We hypothesize that the follicle cell cytoskeleton plays a significant role in nurse death due to the importance of actin during phagocytosis. Further, we intend to use these live imaging studies to investigate the role of calcium before, during, and after clearance of the nurse cells. Previous studies have shown that calcium bursts within the cell are associated with the initiation of phagocytosis in macrophages, as well as other phagocytic cell types (Weaver et al. 2016). Pilot studies done by our lab utilizing live imaging have shown dynamic changes in follicle cell actin before and during the death of nurse cells. These videos have revealed that follicle cell actin polymerizes towards the nurse cell immediately before acidification. Following acidification of the nurse cell, the follicle cell actin changes direction, moving towards the phagocytic follicle cell. Additionally, through live imaging we have been able to successfully observe calcium bursts in the follicle cells immediately before nurse cell death. Overall, this work is providing a more detailed understanding of nurse cell death.

173 Establishment of an Adult Onset Model of Defective Phagocytosis to Study Neurodegeneration. *H. Gandevia*[1], J. Elguero[1], J. Etchegaray[1], M. Feany[2], K. Kaplan[1], K. McCall[1] 1) Department of Biology, Boston University, Boston, MA; 2) Department of Pathology,Brigham and Women's Hospital, Boston, MA.

Cell death and cell clearance are important regulatory events that constitute development in multicellular organisms. Programmed neuronal death is critical for normal development. Disruptions in the mechanisms of dead cell (referred to as corpse) clearance can lead to deleterious consequences such as neurodegeneration. *Draper* is the primary receptor involved in processing and clearance in the central nervous system and other tissues. Previous work in the lab has shown that when *draper* is knocked down specifically in glia, phagocytosis is impaired. This results in decreased dead cell clearance and subsequent accumulation of corpses. This impaired receptor function results in age-dependent vacuolization, which is the formation of holes in the brain and a characteristic of neurodegeneration. However, how loss of *draper* or persistent corpse accumulation leads to vacuolization is unclear. To determine if the persistence of corpses or loss of phagocytic capacity in adulthood promotes vacuolization, we are creating an adult onset model. This is achieved by knocking down *draper* only in adulthood to lead to impaired phagocytic processes in the absence of developmental defects. We are using the Q binary expression system to drive glial-specific knockdown of *draper* via RNAi. We tested this system by knocking down *draper* throughout development and observed corpses. This expression can be suppressed throughout larval and pupal stages so the fly develops with a fully functional Draper receptor. This suppression is achieved by the use of QS, which inhibits the QF-drprRNAi driver. Once the flies reach adulthood, they are fed quinic acid to inhibit the suppressor. This allows us to temporally regulate when *draper* is functioning or knocked down. We will then analyze brains with immunocytochemistry and sectioning to quantify the degree of vacuolization. From this we will determine how the loss of *draper* and/or accumulation of corpses leads to neurodegeneration.

174 Ionizing radiation induces regenerative properties in a caspase-dependent manner in *Drosophila*. *Tin Tin Su*[1], Shilpi Verghese[1,2] 1) MCD Biology, University of Colorado, Boulder, CO; 2) Emory University, Atlanta, Ga.

Cancer treatments including ionizing radiation (IR) can induce cancer stem cell-like properties in non-stem cancer cells, an outcome that can interfere with therapeutic success. Yet, we understand little about what consequences of IR induces stem cell like properties and why some cancer cells show this response but not others. In previous studies, we identified a pool of epithelial cells in *Drosophila*larval wing discs that display IR-induced regenerative properties. These cells are resistant to killing by IR and, after radiation damage, change fate and translocate to regenerate parts of the disc that suffered more cell death. Here, we report the identification of two new pools of cells with IR-induced regenerative capability. We addressed how IR exposure results in the induction of stem cell-like behavior, and found a requirement for IR-induced caspase activity and for Zfh2, a transcription factor and an effector in the JAK/STAT pathway. Unexpectedly, the requirement for caspase activity was cell-autonomous within cell populations that display regenerative behavior. We propose a model in which the requirement for caspase activity and Zfh2 can be explained by apoptotic and non-apoptotic functions of caspases in the induction of stem cell-like behavior.

175 Death by Splicing: Alternative splicing regulated by DOA kinase induces cell death. *L. Rabinow*[1], Y. Zhao[2], D. Sturgill[3], M-L Samson[1], B. Oliver[3], N. Perrimon[1] 1) Genetics, Harvard Medical School, NRB 337, Boston, MA; 2) Stockholm University Department of Molecular Biosciences, The Wenner-Gren Institute Stockholm Sweden; 3) NIDDK, NIH, Bethesda, MD.

Alternative splicing leads to transcripts encoding proteins with differential and even opposing functions. DOA kinase substrates include SR and SR-"like" proteins, globally regulating numerous cellular and developmental processes through the regulation of alternative splicing and other steps in mRNA maturation and usage. The kinase itself is expressed as multiple isoforms via use of alternative promoters, leading to at least 7 different proteins. Mutant *Doa* alleles suppress cell death due to ectopic expression of *hid* or *rpr* in the eye disc, and mutants possess excess pigment cells in the adult eye, a characteristic of blocked cell death. Over-expression of any of 3 DOA isoforms in the developing eye imaginal disc leads to varying degrees of cell death and caspase activation, whereas RNAi directed against 2 other isoforms also induces it, demonstrating opposing roles for the alternative proteins. Cell-death is *p53*-independent but necessitates protein kinase activity, since expression of an inactive kinase provokes no phenotype. RNA-Seq analysis in *Doa* mutants reveals changes in the alternative splicing or alternative promoter utilization of multiple transcripts affecting cell death, including, but not limited to *th*, *Atg1* and *drpr*.

Reduction of function by mutation or RNAi of no single RNA-binding protein or cell-death effector tested significantly suppressed *Doa*-induced cell death in the eye, suggesting that multiple targets may contribute to the observed phenotypes.

176 Decoupling developmental apoptosis and neuroblast proliferation in *Drosophila*. *K. Harding*[1,2], K. White[1,2] 1) Cutaneous Biology Research Center - Massachusetts General Hospital, Harvard University, Boston, MA; 2) Developmental and Regenerative Biology Program, Harvard University, Boston, MA.

Tissue growth and cell death are opposing forces that must be balanced for an organism to develop properly. Our lab studies upstream regulators of stem cell fate decisions by focusing on developmental apoptosis of the embryonic abdominal neuroblasts in the *Drosophila* ventral nerve cord. Apoptosis of *Drosophila* abdominal neuroblasts is tightly coordinated in time and space to ensure that the cell death program is activated only in a specific subset of stem cells. At the same time, proliferation of neuroblasts is also controlled by temporal and spatial signals. As some of the same upstream factors regulate both cell death and cell division, it has been unclear whether embryonic neuroblast apoptosis requires cell proliferation. We have determined that ectopic cell cycle arrests are insufficient to inhibit developmental apoptosis. In addition, we have observed that neuroblast cell death is initiated and executed independent of cell cycle phase. Therefore, while some upstream temporal and spatial factors regulate both neuroblast cell division and cell death, our work suggests that these effects represent independent downstream pathways. We are investigating the regulatory divergence between cell proliferation and cell death to determine how the same upstream factors can lead to fundamentally different cell fate decisions in different contexts.

177 The Dark Side of Light: Effects of Light Exposure on Aging Phenotypes. *J.M. Giebultowicz*[1], E.S. Chow[1], T. Nash[1], S. Fu[1], D. Kretzschmar[2], R. Fey[3], D.A. Hendrix[3] 1) Dept Integrative Biology, Oregon State Univ, Corvallis, OR, USA; 2) Oregon Health & Science University, CROET, Portland, OR, USA; 3) Department of Biochemistry and Biophysics, Oregon State Univ, Corvallis, OR, USA.

Light is necessary for life, but the lifelong daily exposure to artificial light is a matter of increasing health concerns. With prevalent use of LED lights, humans are exposed to increased amount of light in the blue spectrum, which is also known to cause retinal damage. Devices emitting blue-enriched LED light are relatively new; therefore, the long-term effects of exposure to blue light across lifespan are not known. We measured effects of light on *Drosophila* and determined that flies exposed daily to 12h of white light (WL) had significantly reduced longevity compared to flies in constant darkness (DD). Compromised longevity was associated with damage of retinal cells and neurodegeneration in the brain. Exposure of adult flies to 12 h of blue LED light (BL) per day accelerated neurodegeneration in retina and brain and reduced longevity more dramatically than WL. These effects are not mediated by damaged photoreceptors, because mutants do not develop eyes (eyes absent, eya) showed shortened lifespan and brain neurodegeneration after blue light exposure. BL also changed locomotor activity patterns in flies. Based on these data, we hypothesize that life-long exposure to blue-enriched light has adverse photo-toxic effects on the nervous system causing neurodegeneration and premature death. We have conducted RNA-seq to identify transcriptome changes in response to BL exposure in dark-reared flies to identify early molecular effectors of blue light exposure that may help to understand the mechanism of phototoxicity.

178 Influences on developmental homeostasis of eye facet number using DGRP sequenced strains. *J.N. Thompson*, D.M. Tinney, T. Holy Biology Department, Univ. of Oklahoma, Norman, OK.

Genetic effects of modifiers of cell death are being analyzed using variation in expression of the dominant *Bar* mutation as a model. Recently our focus has been on mechanisms influencing symmetry of cell death. Even though average eye facet number in *Bar* varies as a function of background genetic modifiers from different Drosophila Genetic Reference Panel (DGRP) genomes, symmetry is largely retained. In pairs of eyes from genetically identical individuals, symmetry is high in spite of fly-to-fly variation in total facet number. Our approach focuses on heterozygous effects of genetic backgrounds from the DGRP series of sequenced strains. When *Bar* (*Basc*) females are mated to males from each DGRP strain, the effect of modifiers of cell death can be quantified by counting eye facets using SEM images of the eyes. Out of 92 DGRP lines assayed for modifiers of cell death, symmetry measures could be derived from 88 lines. Developmental homeostasis was quantified as a function of fluctuating asymmetry, $FA = |S_1 - S_2| / ((S_1 + S_2)/2)$. This is the scaled average difference between the two sides; we did not detect asymmetrical handedness bias. Only 18 genes were identified by association mapping to have a highly significant association with symmetry (probability of $p < 10^{-7}$ or better). Of these, several were linked to nervous system development. One of the most significant associations ($p < 10^{-8}$) was with a pathway that might explain how symmetry can be maintained over significant distances relatively late in development. That association is being explored by more directly targeting that pathway in *Bar* heterozygotes. The expression of symmetry was independent of total facet number. Different DGRP genomes caused a wide range of degrees of cell death. But the association of facet number and expression of symmetry was zero (regression = -7.0×10^{-5}). In summary, sequenced genetic backgrounds differ significantly in their effect on eye facet cell death. But symmetry within individuals is maintained. A mechanism that can explain symmetry of structures forming late in development is suggested by genes identified in an association analysis of sequenced strains.

179 Regulation of Hemocyte Activation by Reactive Oxygen Species. A. Myers, C. Harris, *C. Brennan* Department of Biological Science, Cal State University Fullerton, Fullerton, CA.

The production of reactive oxygen species (ROS) by macrophages and other white blood cells as a response to infection was described over fifty years ago, along with the important microbicidal activity effected by the ROS. More recently, it has been appreciated that phagocyte ROS have additional non-killing roles, including intra- and intercellular signalling, and regulation of degradative hydrolytic activities in the phagosome. ROS are generated both as byproducts of mitochondrial electron transport chain respiration, as well as by dedicated enzymes, such as NADPH oxidase (NOX). The hemocytes of Drosophila are a good system to dissect both the mechanisms regulating ROS production, as well as the roles of ROS, both microbicidal and regulatory. We have reported a biphasic production of ROS in hemocytes following bacterial infection, and identified a role for ROS in the inflammatory activation of hemocytes, including plasmatocyte spreading and adhesion, and crystal cell rupture. Here we report newer findings, including the roles for NOX and Duox (dual oxidase) in the hemocyte ROS response.

180 Role of Lysosome in Immune Priming of Hemocytes. Y Chao[1], M Rousseau[1], K Venkatachalam[1,2], *C Wong*[1,2] 1) Department of Integrative Biology and Pharmacology, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, TX; 2) Graduate Program in Biochemistry and Cell Biology, MD Anderson Cancer Center and UTHealth Graduate School of Biomedical Sciences, Houston, TX.

Immune priming confers better host defense against future microbial infections in organisms that lack classic adaptive immune machinery. In mammals, immune priming of innate immune cells, such as resident macrophage, contributes to altered metabolism and tissue homeostasis of local environment. Understanding the molecular basis of how immune priming is established in innate immune cells could inform better strategies for treating recurrent infections and inflammatory disorders. Using a *Drosophila* priming paradigm, we found that wild-type flies possess heightened capacities to clear injected gram-negative *Escherichia coli* (*E. coli*) for more than 2 weeks after priming with heat-killed *E. coli*. Priming with latex beads, heat-killed gram-positive *Enterococcus faecalis*, or mammalian cells did not result in the same primed response. We also found that flies primed at different ages show different capacities in clearing infecting bacteria. Since lysosomal processing of microbes generates molecular patterns for intracellular recognition, we examined the role of lysosome in the primed immune response. By manipulating gene expression levels in hemocytes, we found that lysosomal ion homeostasis is a critical determinant of immune priming. Furthermore, while the lysosome—NF-κB/Relish signaling axis is required for acute clearance of bacteria, sustained activation

of NF-κB/Relish diminishes the primed immune response. These findings suggest that modulation of NF-κB/Relish activity after initial phagocytic clearance is necessary for establishing priming in hemocytes. Our preliminary data also indicate several candidate molecules that could mediate this dynamic relationship between lysosome and NF-κB signaling. In conclusion, lysosomal processing of bacteria and resolution of NF-κB/Relish signaling are necessary for priming hemocytes with an elevated immune response to future bacterial infection.

181 A tissue communication network coordinating innate immune response during muscle stress. *E.R. Geisbrecht*, N. Green, J. Walker, A. Bontrager, M. Zych Kansas State University, Manhattan, KS.

Complex tissue communication networks function throughout an organism's lifespan to maintain tissue homeostasis. Using the genetic model *Drosophila melanogaster*, we have defined a network of immune responses activated following the induction of muscle stresses, including hypercontraction, detachment, and oxidative stress. Of these stressors, loss of genes causing muscle detachment produce the strongest levels of JAK-STAT activation. In one of these mutants, *fondue (fon)*, we also observe hemocyte recruitment and the accumulation of melanin at muscle attachment sites (MASs), indicating a broad involvement of innate immune responses upon muscle detachment. Loss of *fon* results in pathogen-independent Toll signaling in the fat body and increased expression of the Toll-dependent antimicrobial peptide (AMP) Drosomycin. Interestingly, genetic interactions between *fon* and various Toll pathway components enhance muscle detachment. Finally, we show that JAK-STAT and Toll signaling are capable of reciprocal activation in larval tissues. We propose a model of tissue communication for the integration of immune responses at the local and systemic level in response to altered muscle physiology.

182 Dissecting the impact of chronic infection on tolerance towards secondary infection in *Drosophila melanogaster*. *F.P. Satriale*, M.C. Chambers Bucknell University, Lewisburg, PA.

The use of non-mammalian infection models like *Drosophila melanogaster* has made it easier to study bacterial pathogenesis. *D. melanogaster* proves to be an excellent model in that the innate immunity pathways and tissue physiology of this organism are very similar to those of mammals. Bacterial pathogenesis can prove to be lethal when the microbe is injected into the thorax of the fly, but our lab has found that microbes lingering from a previous pathogenic infection can provide significant protection against future acute infection.

The aim of this project was to distinguish whether this protection was due primarily to an increase in tolerance or resistance to infection. Resistance to infection is a measure of how well the host inhibits bacterial growth, while tolerance is a measure of how well the host survives while carrying higher loads of the pathogen. For this experiment, we chronically infected flies with either *Serratia marcescens* or *Enterococcus faecalis* and then hit them with a secondary infection of *Providencia rettgeri* at a range of doses. To distinguish between resistance and tolerance, we monitored both fly survival over the first week of infection and the bacterial load ten hours after infection. The ten hour time-point was selected because it is far enough into the infection to detect significant differences in bacterial load that would be indicative of altered resistance, but before any mortality has occurred. Impact on tolerance and resistance was assessed using both linear and logistic modeling. While linear modeling is more commonly used to assess changes in tolerance, logistic modeling has been shown by other labs to allow more precise conclusions with regards to changes in tolerance.

Our results suggest that the protective benefit is due to increased tolerance for most combinations of chronic and secondary infection, specifically that flies with chronic infections exhibit increased vigor and potentially decreased disease severity. Flies that had *E. faecalis* and *S. marcescens* as the chronic infection showed a general increase in survival of *P. rettgeri* at every bacterial load. Ongoing research is focused on determining the mechanism behind this improved tolerance. This includes experiments to determine whether localization of the secondary infection is altered by chronic infection using fluorescence microscopy and work in mutant lines with known tolerance defects to see if they are still protected by chronic infection.

183 Identification and characterisation of molecularly-distinct *Drosophila* macrophage subpopulations with enhanced inflammatory responses to injury. *J.A. Coates*[1], A Brittle[2], E Armitage[2], M Zeidler[1], I. R. Evans[2] 1) Department of Biomedical Science and the Bateson Centre, University of Sheffield, Sheffield, UK; 2) Department of Infection, Immunity and Cardiovascular Disease and the Bateson Centre, University of Sheffield, Sheffield, UK.

Macrophage heterogeneity is a firmly established principle in vertebrates, encompassing tissue-resident subpopulations and polarised activation states. However, there is much discussion over the exact types and functions of these different macrophage populations, presenting the need for a simpler model system. *Drosophila melanogaster* possess cells that are functionally equivalent to macrophages (plasmatocytes of the hemocyte lineage), but to date these cells have been largely considered to inhabit homogeneous populations, with only limited evidence suggesting macrophage heterogeneity might exist in flies.

However, our work and the work of others indicates that *Drosophila* embryonic macrophages, much like their vertebrate counterparts, do not respond uniformly to a variety of immune stimuli, including phagocytic challenge and tissue damage, leading us to hypothesise that macrophage heterogeneity is an evolutionarily conserved facet of cellular innate immunity that is present in *Drosophila*. By screening the Vienna Tilling array library, we have identified enhancer-GAL4 lines that label molecularly-distinct subpopulations of *Drosophila* macrophages *in vivo*. We have extensively evaluated the functions of these subpopulations, identifying enhancer lines that label subpopulations of macrophages. Importantly, some of these subpopulations of macrophages exhibit enhanced inflammatory responses to injury within the developing embryo. Furthermore, several subpopulations migrate at faster rates and/or display reduced rates of apoptotic cell clearance *in vivo*. Moreover, we have recently begun to uncover the underlying mechanisms behind these behaviours.

We have definitively demonstrated macrophage heterogeneity in *Drosophila* for the first time and developed novel tools in which to examine macrophage subpopulations. This work extends the capacity of *Drosophila* as a model organism for studying macrophage behaviour in vivo and shows that macrophage heterogeneity is a key feature of cellular innate immune systems that is conserved across evolution.

184 Using the *Drosophila* Genetics Reference Panel to Identify Host Factors Associated with *Coxiella burnetii* Infection. *Z.P. Howard*, R.M. Guzman, Z. Liu, S.N. White, A.G. Goodman School of Molecular Biosciences, Washington State University, Pullman, WA.

Coxiella burnetii is a Gram-negative, obligate intracellular, macrophage-tropic bacterium and the causative agent of the zoonotic disease Q fever. Association between host genetic background and the development of *Coxiella* infection has been demonstrated both in humans and animals; however, specific genes associated with susceptibility to infection remain largely unknown. In this study, we used the *Drosophila* Genetics Reference Panel (DGRP) to identify host genetic variants that affect host susceptibility to *C. burnetii* infection. Following infection of each DGRP line with *C. burnetii*, we monitored mortality rates and calculated hazard ratios with respect to mock (PBS) infection. We used hazard ratios from both male and female flies as the input phenotype for a genome-wide association study (GWAS). Two genome-wide suggestive variants were located at intergenic regions between CG2574 and PKCd, both of which have been shown to control microbial infection. On chromosome 3L, we identified a sequence variation upstream of the known Gram-negative bacterial recognition gene for peptidoglycans (PGRP-LC/LA). Further experiments validated that functional PGRP-LC protects *Drosophila* from *C. burnetii* infection. Finally, we found a region on chromosome 3L (6164795:6194790) that contained a high density of suggestive variants ($P < 10^{-5}$). Two of the variants in this region were missense mutations in CG7376, an E3-ubiquitin ligase homologous to human SHPRH. Future work will ask how orthologous genes identified in our screen function during *Coxiella* infection in mammalian models. Additionally, a phenotype transcriptome association will be performed to identify transcript levels of the genes containing significant variants, to support the impact of the variant during *C. burnetii* infection. Our findings will have broad-ranging impacts by identifying host

factors that when dysfunctional confer susceptibility to *Coxiella* infection. These results will be important for better prediction of pathogen spread in domestic ruminant reservoirs and disease progression in humans.

185 Exploiting a cyclic dinucleotide-mediated immune response to reduce the burden of *Coxiella burnetii* infection. *R. Marena. Guzman*, Alan Goodman Molecular Biosciences, Washington State University, Pullman, WA.

The Gram-negative bacterium *Coxiella burnetii* is the causative agent of Query (Q) fever in humans and coxiellosis in livestock. Importantly, tick species are also a natural reservoir for *C. burnetii* yet little is known about their role in arthropod-livestock-human transmission. There is currently no effective vaccine against Q fever available in the United States, therefore new therapeutic approaches are needed to reduce infection in reservoir animals and control the spread of *C. burnetii* to humans. Our project addresses the use of cyclic dinucleotides (CDNs) as a novel therapeutic approach to stimulate insect host immune responses to reduce bacterial load. CDNs are central in bacterial metabolism and regulatory processes. The major cytosolic sensor for CDNs in mammalian hosts is STING (STimulator of Interferon Genes), which upon activation, induces an innate immune response through the induction of type I interferons (IFNs) and pro-inflammatory cytokines. *Drosophila* contain a functional STING ortholog (dmSTING) that binds CDNs and mediates an NFkB/Relish-specific immune response. We show that *C. burnetii* infection in *Drosophila* S2 cells results in the production of immunostimulatory CDNs. We also show that CRISPR/Cas-9 deleted dmSTING flies exhibit higher mortality and reduced induction of antimicrobial peptides (AMPs) following *C. burnetii* infection as compared to control flies. Finally, priming the immune response with CDNs prior to *C. burnetii* infection results in the reduction of bacterial load in a STING-dependent manner. Since arthropods are an important reservoir of *C. burnetii,* our use of *Drosophila* as an animal model to study pathogenesis has long-lasting implications where CDNs can be used to reduce bacterial load in insect species.

186 Immunity divergence in *D. simulans* and *D. mauritiana*. *M. Nabors*, R. Unckless University of Kansas, Lawrence, KS.

There is ample evidence for rapid evolution of genes involved in immune defense in many species. It is still relatively rare, however, that we have examples that tie this genetic divergence to phenotypic divergence in the ability to fight infectious disease. This is important because we presume that this rapid evolutionary divergence is due to a host-pathogen arms race, but we have little direct evidence that this is true. Our work aimed to understand the molecular basis of divergence in immune defense and determine the extent to which fast evolving genes lead to this phenotypic divergence. To determine phenotypic divergence in immune defense, we performed systemic infection on *D. simulans* and *D. mauritiana*, and measured survival five days post infection. Interestingly, we found that the species with greater resistance depended on the pathogen used. *D. simulans* mounts a more effective immune defense against infection with the Gram-positive *E. faecalis (50% survival for D. simulans, 38% survival for D. mauritiana, $P_{[logistic\ regression]}$=0.09). D. mauritiana mounts a stronger immune defense against infection with the Gram-negative P. rettgeri (50% survival for D. mauritiana, 3% survival for D. simulans, $P_{[logistic\ regression]}$<0.0001, Figure 4).* This suggests that there is specificity to the evolution in immune defense in both species. It is not the case that one species has a universally more effective immune response, but that each has likely evolved to combat the specific suite of pathogens it is most likely to encounter. To better understand the evolution of immune defense between species, we performed interspecific genetic mapping to determine genomic regions associated with the divergence in immune defense between species. We tested for variation in immune defense within and between *D. mauritiana* and *D. simulans*. We then used a backcross design (hybrid males are infertile) through females to map this divergence in both pathogens to the chromosome and performed an RNA-seq to identify potential candidate genes.

187 Profiling sex dimorphism of immune gene expression in Drosophila. *MD Mursalin Khan*, Blake Rochester, Bayley Atkins, Isabella Blair, Rita M. Graze Biological Sciences, Auburn University, Auburn, AL.

There are sex differences in immune function in both vertebrates and invertebrates, including in *Drosophila melanogaster*. A better understanding of sexual dimorphism of the immune response will contribute to our overall understanding of immunity, as well as our understanding of variation in immune function, physiology and lifespan. To understand whether sex dimorphism observed in *D. melanogaster* at the level of immune gene expression may be conserved, we examined expression of the immune genes *Deptiricin B(DptB), Defensin (Def), Drosocin (Dro), Toll-9,* and *immune deficiency (imd)* in adult males and females of four species of the *Drosophila melanogaster* subgroup: *D. melanogaster*, *D. simulans*, *D. sechellia,* and *D. yakuba* after treatment with gram negative bacterial antigen Peptidoglycan-EK (PGN-EK) and gram positive heat-killed methicillin-resistant *Staphylococcus aureus* (MRSA). Gene expression differences between sexes and species were measured by qPCR. Species differed in the degree to which immune genes were upregulated in response to PGN-EK or heat-killed MRSA. Sex dimorphism was observed in expression of some of the immune genes assayed and the degree of sex dimorphism differed between species. Overall, we found that expression of immune genes in response to these treatments is not conserved. There are differences between species both in the overall response to treatment and in the degree of sexual dimorphism for expression.

188 Balancing selection in Drosophila AMPs may be maintained via functional diversity amongst alleles. *Joanne Chapman*, Mason Wilkinson, Robert Unckless Molecular Biosciences, University of Kansas, Lawrence, KS.

Antimicrobial peptides (AMPs) are key components of the innate immune repertoire of insects, providing direct microbicidal activity against pathogens. While the majority of *Drosophila* immune genes are fast evolving, this does not seem to be the case with AMPs. Instead, balancing selection appears to be a driving force in AMP evolution. Balancing selection could act to maintain functional diversity in AMPs if the selective advantage of specific alleles is context dependent, thereby promoting maintenance of multiple alleles over evolutionary timescales. Here, we specifically set out to test whether AMP alleles show functional differences in-vitro and in-vivo.

In-vitro assays (MIC and ZOI) revealed clear differences in activity amongst naturally occurring AMP alleles. These differences were context dependent, for example alleles varied in their efficaciousness against different bacterial species. We then tested whether possession of specific AMP alleles influenced infection outcomes in flies in-vivo. We found that both bacterial load and survival were correlated with the specific AMP repertoire of the fly. Furthermore, these functional tests revealed that differences could be attributed to single amino acid changes in the peptide.

Taken together, these results strongly suggest that AMP alleles segregating within *Drosophila* are functionally divergent, and that the maintenance of multiple AMP alleles in populations is adaptive. Furthermore, single amino acid changes in AMPs can have profound effects on antimicrobial activity. Maintenance of adaptive polymorphism in AMPs may provide host populations flexibility to respond to a rapidly changing and diverse pathogen fauna, as predicted by host-pathogen co-evolutionary dynamics. Alternately, it may allow host populations to adaptively trade off immune requirements with other life-history and fitness traits.

189 Determining the causes and consequences of genetic variation in Diptericin, a *Drosophila* antimicrobial peptide. *S. R. Mullinax*, R. L. Unckless Molecular Biosciences, University of Kansas, Lawrence, KS.

The innate immune system provides hosts with a crucial first line of defense against pathogens. It is activated rapidly (within minutes or hours of infection) and is broad spectrum, recognizing and destroying a large variety of pathogens. In general, immune genes are some of the most rapidly evolving genes in genomes. However, there is evidence that one important category of immune genes, the antimicrobial peptides, are not rapidly evolving in *Drosophila.* Instead

they are under the influence of balancing selection such that multiple alleles are maintained in populations over the evolutionary timescale. Antimicrobial peptides (AMPs) are important effector genes involved in innate immunity. Diptericin, a *Drosophila* AMP, contains a segregating serine (S)/arginine (R) polymorphism in *D. melanogaster* and *D. simulans* populations that strongly influences survival upon infection with the Gram-negative bacterium *Providencia rettgeri.* Here, we assessed the survival of CRISPR genome edited *D. melanogaster* flies with different genotypes (SS, SR, RR) after systemic infection by various bacteria. Flies with the SS genotype had a higher survival rate 5 days after a systemic infection with either a Gram-positive or Gram-negative bacteria. These results support the earlier work in natural *D. melanogaster* lines, whereby *diptericin* genotype influences immune defense effectiveness *P. rettgeri.* As well, we analyzed differences in microbiome composition between the genotypes by spiral plating whole flies and comparing colony forming units and bacterial species found within each genotype. This revealed a role for Diptericin in maintenance of the microbiome, with some bacterial species only present in one genotype (SS or RR) or the other. This result is further characterized through the use of 16S sequencing on co-reared SS, RR, and SR flies. These results will help identify the effects of maintenance of *diptericin* variation, and how this can influence the fitness of the organism through life history tradeoffs.

190 Regulation of post-mating immune response in female *Drosophila melanogaster*. *K.E. Gordon*[1], M.F. Wolfner[1], B.P. Lazzaro[2] 1) Molecular Biology and Genetics, Cornell University, Ithaca, NY; 2) Cornell Institute for Host-Microbe Interactions and Disease, Cornell University, Ithaca, NY.

In *D. melanogaster* and many other species, female reproductive investment comes at a cost to immunity and resistance to infection. Within hours of mating, *D. melanogaster* females exhibit inhibited immune responses and become more susceptible to bacterial infection. Previous studies showed that females were less resistant to bacterial infection at 2.5 and 26.5 hours after mating, but did not test whether a mated female would eventually recover virgin levels of immunity. To investigate the permanence of mating-induced reduction in immune capacity, we tested whether mated females could recover virgin levels of immunity when infected at 2, 4, 7, or 10 days after mating. We observed no recovery of immune capacity in mated females over time, and the magnitude of the survivorship difference between mated females and virgins remained the same at each mating time point. We conclude that mating has a permanent suppressive effect on the female immune system. Knowing that females mate multiply, we tested whether a second mating further affected immune performance. We hypothesized that females who mated twice might become more susceptible to infection than females mated once. Instead, we found that females mated either once or twice before infection survived at equal proportions and both significantly lower than virgin females. This indicates that effects of a single mating are sufficient to suppress the immune response and a second mating does not compound the effect. During mating, the male transfers seminal fluid proteins that change female physiology and behavior. One such seminal fluid protein, Sex Peptide, induces the female to produce Juvenile Hormone (JH). The role of JH in promoting egg development is well established. We and others have previously shown that JH is immunosuppressive and decreases resistance to bacterial infection. We thus hypothesize that JH signaling might control resource allocation between reproduction and immunity. Future experiments will seek to understand whether JH titers in mated and virgin females correlate with our understanding of the dynamics of the post-mating immune response and whether limiting investment in reproduction can improve immune capacity.

191 Parasitic nematode FAR proteins play a key role in modulating host immunity. *Sophia C. Parks*[1], Shyon Nasrolahi[1], Anna Buchman[2], Raghavendran Ramaswamy[3], Naoki Yamanaka[4], Omar Akbari[2], Martin Boulanger[3], Adler R. Dillman[1] 1) Department of Nematology, University of California, Riverside; 2) Division of Biological Sciences, Section of Cell and Developmental Biology, University of California, San Diego; 3) Department of Biochemistry & Microbiology, University of Victoria, British Columbia, Canada; 4) Department of Entomology, University of California, Riverside.

Parasitic nematodes continue to cause disease throughout the world. However, parasitic disease processes are only superficially understood, and little is known about how they evade host immune systems. One possibility is that proteins and small molecules released by these parasites modulate host immunity. Among the many secreted proteins, fatty acid- and retinol-binding proteins (FARs), have been suggested to affect host immunity. The functional role of FAR proteins in animal hosts has yet to be described. We tested two FAR proteins that are released in high abundance during a *Steinernema carpocasae* infection. *S. carpocapsae* is a generalist insect-parasitic nematode, capable of infecting a variety of insect hosts. We chose to study two different FAR proteins released by activated *S. carpocapae* infective juveniles. By generating transgenic *Drosophila melanogaster* flies that express one of two different FAR proteins, and by exposing flies to recombinant proteins, we were able to measure expression- and dose-dependent effects of these FAR proteins, in the context of bacterial infection. We have used several different promoters to drive FAR expression in transgenic flies, and have investigated the effects of expressing FAR proteins in the fat body, hemocytes, and salivary glands, as well as the effects of ubiquitous expression. We measured resistance to a bacterial infection by injecting the LD_{30} (a dose that kills ~30% of the infected wild-type flies) into our experimental and wild type adult flies and found a dose dependent immunomodulatory effect, where a low dose improves the outcome of infection and a higher dose reduces host resistance, that is distinctive between the two FAR proteins we tested. Now that the significant effect of FAR proteins has been demonstrated for the first time in an active infection in animals, we are currently seeking to understand the biochemical mechanism by which FAR proteins modulate host immunity.

192 The role of intestinal TOR signaling following pathogenic bacterial infection in Drosophila. *Rujuta Deshpande*[1,2,3], Savraj Grewal[1,2,3] 1) Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta, CA; 2) Alberta Children's Hospital Research Institute, Calgary, Alberta, CA; 3) Department of Biochemistry and Molecular Biology Calgary, University of Calgary, Calgary, Alberta, CA.

Upon oral infection with pathogenic bacteria, Drosophila adults mount organism-wide immune and physiological responses in order to counteract the infection and promote survival. The intestine plays a central role in mediating these effects. For example, upon infection, damaged intestinal epithelial cells trigger a local cytokine response to promote stem cell-mediated regeneration in order to promote repair and maintain tissue integrity. In addition, the intestine functions as an endocrine organ to signal to other tissues such as the fat body and brain in order to promote innate immune responses and adaptive physiological changes. Understanding how the intestine senses infection and coordinates these various physiological processes is an area of intense investigation.

We have begun exploring the role for TOR kinase signaling in these infection responses. TOR kinase is a central regulator of cell, tissue and body metabolism. In particular, different stressors such as nutrient starvation, oxidative damage and infection can suppress TOR kinase signaling, which is important for modulating metabolism and promoting survival. We began by examining how TOR signaling changes in response to infection with *P. entomophila (P.e)*. Surprisingly, instead of seeing inhibition, we found that *P.e.* induced a rapid (within 4hrs) increase in TOR kinase signaling as measured by increased levels of phosphorylated S6 kinase (a direct TOR target). These effects were seen in both male and female flies. Moreover, immunostaining experiments indicated that these increases appeared to occur largely in mature polyploid epithelial enterocyte cells, rather than dividing intestinal stem cells or their differentiating daughter cells, the enteroblasts. We also found that these increases in TOR signaling were mimicked by feeding animals paraquat in order to trigger reactive oxygen species, which are known effectors of bacterial infection. One key effector of TOR kinase signaling is the stimulation of protein synthesis, in part via upregulation of dMyc transcription factor activity. We found that *P.e.* infection induced expression of tRNAs and several dMyc target genes involved in ribosome biogenesis. These data suggest that upregulation of TOR and protein synthesis within the intestinal epithelium is an acute response to bacterial infection. We are currently using both gain- and loss-of-function genetics to explore how TOR activity within the epithelial cells of the intestine can modulate both the local and systemic effects of bacterial infection on adult physiology.

193 The nematode-associated bacterium *Xenorhabdus innexi* has increased virulence when co-injected with secreted nematode protein. *V. Alonso*[1], S. Parks[1], S. Nasrolahi[1], S. Aryal[2] 1) Nematology , University of California, Riverside, Riverside, CA; 2) Marrone Bio Innovations, Davis, CA.

Xenorhabdus innexi, efficiently kill the southern mole cricket *Neoscapteriscus borellii.* However, the roles of nematode and bacteria in this tritrophic interaction are not completely understood. One hypothesis is that the nematode serves as a vector in transmitting highly pathogenic bacteria, and that the bacteria facilitate killing of the host. Another hypothesis is that the nematode and bacteria act together to kill the host, where the nematode contributes to pathogenicity through its excreted/secreted products. Here, we evaluated the relative contributions of *X. innexi* and *S. scapterisci* to pathogenicity during infections of the fruitfly *D. melanogaster* and the sand cricket *Gryllus firmus* to better understand the process of infection by entomopathogenic nematodes. We found that *G. firmus* is resistant to *X. innexi* infection, and that the addition of a nematode fatty acid- and retinol-binding protein (FAR) has an immunomodulatory effect on the insect host, such that *X. innexi* is more virulent when co-injected with recombinant FAR protein.

194 A survey of the microorganisms colonizing three *Drosophila* species in the wild. *Emma Pagella*, Joanne Chapman, Robert Unckless Molecular Biosciences, Univeristy of Kansas, Lawrence, KS.

Drosophila is commonly used as a model in studies of infection and immunity. Yet, few studies have quantified the pathogen pressure that flies face in the wild. Here, we set out to catalogue the microorganisms colonizing several species of *Drosophila*. In particular, we focused on culturable bacteria, fungi and yeast isolated from wild-caught *D. buskii, D. tripunctata,* and *D. hydei.* 16S Sanger sequencing was used to confirm genus identity. Subsequently, experimental infections of 50 males from each of five *D. melanogaster* lines, as well as *D. busckii*, were used to determine pathogenicity of the isolated strains. To this end, we used three wild type lines (W^{1118}, OregonR, CantonS) and two lines with lowered immune functioning (IMD- targeting the IMD pathway and Spz- targeting the Toll pathway). Using these methods, we have identified a variety of microorganisms, and confirmed the pathogenicity of a subset of these. As such, we have not only developed a catalogue of natural pathogens to use in the study of *Drosophila* infection, but we have also confirmed that the pathogen burden faced by flies in the wild is not trivial.

195 Effects of spaceflight and simulated microgravity on a host-pathogen system. *Rachel Gilbert*[1], Sharmila Bhattacharya[2] 1) USRA/Space Biosciences, NASA Ames Research Center, Moffett Field, CA; 2) Space Biosciences, NASA Ames Research Center, Moffett Field, CA.

While it has been shown that decades of astronauts and cosmonauts suffer from immune disorders both during and after spaceflight, the underlying causes are still poorly understood, due in part to the fact that there are so many variables to consider when investigating the human immune system in a complex environment. Invertebrates have become popular models for studying human disease because they are cheap, highly amenable to experimental manipulation, and have immune systems with a high genetic similarity to humans. Fruit flies (*Drosophila melanogaster*) have been shown to experience a dramatic shift in immune gene expression following spaceflight, but are still able to fight off infections when exposed to bacteria. Furthermore, a recent spaceflight mission showed that flies are more susceptible to infection following exposure to microgravity conditions, compared to ground-reared flies form the same population. Additionally, the common bacterial pathogen *Serratia marcescens* was shown to become more lethal to fruit flies (both space- and ground-reared) after being cultured in space, suggesting that not only do we need to consider host changes in susceptibility, but also changes in the pathogen itself after spaceflight conditions. Being able to simulate spaceflight conditions in a controlled environment on the ground gives us the ability to not only evaluate the effects of microgravity on the host immune system, but also how the microorganisms that cause immune disorders are being affected by these drastic environmental shifts. In this study, I use both spaceflight and ground-based (simulated microgravity) environments to examine the genetic changes associated with increased *S. marcescens* virulence in order to understand how microgravity is affecting this pathogen, as well as how these genetic changes influence and interact with the host immune system. This study will provide us with more directed approaches to studying the effects of spaceflight on human beings, with the ultimate goal of being able to prevent human immune dysfunction in future space exploration.

196 Male-Killing *Spiroplasma* Densities in *Drosophila* Exposed to Resistant Parasitoid Wasps. *A.V. Stankov*, M. Mateos Texas A&M University , College Station, TX.

Heritable endosymbionts are commonly associated with arthropods and can have influential and diverse effects on their hosts, including manipulation of host reproduction and protection against natural enemies. Bacteria in the genus *Spiroplasma* (class Mollicutes) associate with several species of *Drosophila*, including *Drosophila melanogaster.* Strains of *Spiroplasma* naturally associated with *D. melanogaster* have been shown to kill the sons of infected females and to confer protection against several parasitic wasps. Nonetheless a few wasp species are unaffected in the presence of *Spiroplasma* in *Drosophila*. One such "resistant" wasp is *Leptopilina guineaensis.* Bacterial endosymbiont density has been shown to be important in the expression of certain phenotypes and for vertical transmission. This study aimed to determine whether the "resistance" of *Leptopilina guineaensis* to *Spiroplasma* involves a reduction in *Spiroplasma* densities estimated via quantitative PCR, (q)PCR, over the time course of seven days post-wasp attack. Our initial results suggest that infection densities of *Spiroplasma* in the presence of the wasp are lower at the later developmental stages. It is therefore possible that the mechanism by which the wasp is resistant to *Spiroplasma* involves inhibition of *Spiroplasma* growth.

197 A gut filling: The kinetics of the *Wolbachia* colonization in *Drosophila* guts. *N. Vaisman*[1,2], M. Massaad[1], H. Frydman[1] 1) Biology, Boston University, Boston, MA; 2) CAPES Foundation, Ministry of Education of Brazil, Brasília.

About half of the world population are at risk of contracting human diseases transmitted by mosquitos. *Wolbachia*, an intracellular bacterium vertically inherited, is emerging as a novel way to block disease transmission. Introduction of *Wolbachia* from *Drosophila* into mosquitoes, suppresses the growth of several viruses, including Dengue and Zika. Although *Wolbachia* infects primarily the female germline, several somatic cell types are also infected. We have recently shown that *Wolbachia* also colonizes the *Drosophila* gut epithelium (Simhadri *et al.,* 2017). Therefore, the gut is potentially the first site of *Wolbachia* suppression of ingested viruses. However, our understanding of *Wolbachia's* colonization of the gut and its role on pathogen blocking is limited. This work aims to characterize the *Wolbachia* colonization in *Drosophila* guts and their potential effects on this tissue.

Imaging analysis reveals that *Wolbachia* are homogeneously spread throughout larval guts, but colonize adult midguts as isolated patches of infected cells. We hypothesize that the different patterns of infection between larvae and adult reflect the infection status of the gut progenitor cells, with limited lateral transmission between differentiated cells. According to our model, most progenitor cells of the larval gut are infected, generating a gut with a widespread population of infected cells. In contrast, only a subset of the adult midgut progenitors (AMP) and intestinal stem cells (ISCs) are infected, resulting in clusters of infected cells in the differentiated progeny. Utilizing genetic markers of AMPs and ISCs, we confirmed our hypothesis: the infection status of differentiated cell reflects the infection status of the closest progenitor cell. These data indicate *Wolbachia* vertical inheritance not only across generations, but also at the cellular level. Lineage labelling experiments are currently underway to confirm this model. In addition, they will reveal if the presence of *Wolbachia* in a progenitor cell affects the intestinal stem cell division rate and patterns of stem cell differentiation. Lastly, studies with viral infections will give us insights on the role of *Wolbachia* presence in the gut in pathogen blocking, knowledge relevant for *Wolbachia* based approaches to block human diseases transmission.

198 The gut microbiome as a driver of host dietary preference in *Drosophila melanogaster*. *T.B. Call*, J.M. Chaston Brigham Young University, UT.

The "gut microbiome", or the combination of microorganisms that colonize the interior of the GI tract of all macro-organisms, plays a significant role in host health and physiology. For example, host feeding behaviors are manipulated by microbiota. In a recent report, *Drosophila melanogaster* were shown to forage

microbes from their diet based on prior exposure. One gap is that it is not known if the gut microbiota alter fly preferences for diets of different composition. Here we quantified *D. melanogaster* dietary preferences using an automated feeding assay. We manipulated the fly microbiota in four treatments by inoculating bleach-sterilized *D. melanogaster* eggs with single, representative species of the two major bacterial groups found in flies: acetic acid and lactic acid bacteria; with both species together; or by leaving the flies to develop bacteria-free. Then, to test if the microbiota impacts feeding preferences based on nutritional content of the diet, we compared the dietary preferences of flies for a 10% yeast 10% glucose diet versus a yeast- or glucose-restricted diet (75%, 50%, or 25% of the normal amount of either yeast or glucose, for a total of 6 diet conditions). Differences in feeding preference with bacterial treatment or diet condition were assessed by combining three quantitative measures of feeding rate - frequency, duration, and interval between eating periods – into a single preference index. Preference indices of flies (10 flies per condition in each of three separate experiments) were compared using linear models and ANOVA to define the impact of the gut microbiota on host feeding preference. These results will help us understand how different members of the microbiota can influence animal feeding behaviors.

199 The influence of natural diet and microbiota community on metabolic phenotype of *Drosophila melanogaster*. *Andrei Bombin*, Owen Cunneely, Kira Eickman, Abigail Ruesy, Ryan O'Rourke, Laura Reed The University of Alabama.

Obesity is an increasing worldwide epidemic and contributes to physical and mental health losses. The development of obesity is caused by multiple factors including genotype, hormonal misregulation, psychological stress, and gut microbiota. Our project investigates the influence of microbiota community acquired from environment and horizontal transfer on traits related to obesity. Such traits include weight gain, fat storage, and blood (hemolymph) sugar. The study applies the novel approach of raising *D. melanogaster* from ten, wild-derived, genetic lines (DGRP) on naturally rotten peaches, thereby preserving genuine microbial conditions. In order to control for the effect produced only by live microorganisms, we use autoclaved rotten peach diet as a control for the natural diets. The preliminary results have shown that microbial composition of lab and peach food types differs and that consumption of the natural diet leads to decreased weight and triglyceride concentrations. On the diets deprived of the original living microbiota flies gained significantly more weight. Thus, our results suggest that live microbial community plays an active role in shaping metabolic phenotype. Our results also indicated that inheritance of parental microbiota might partially rescue the phenotype induced by a lack of the natural microbial community in the food. In addition, interactions between parental microbiota, diet, and genotype significantly influenced the metabolic profile.

200 Investigating the microbiome's role in female *Drosophila melanogaster* post-mating gene expression changes. *S. Delbare*, Y. Ahmed-Braimah, M. Wolfner, A. Clark Cornell University, Ithaca, NY.

Correlative studies in humans and experiments in model organisms have demonstrated that the microbiome has a major impact on a wide range of host traits. Specifically, in *Drosophila melanogaster*, the absence of the microbiome affects gut physiology, immunity, expression of genes related to growth and developmental pathways, behaviors such as locomotion, and, depending on the study, reproduction. Here, we investigate whether the presence or absence of a microbiome in either the female and/ or her mate, affects the female transcriptome post-mating. This is in part motivated by the observation that following mating, females induce expression of many genes involved in the innate immune response. Matings with axenic and conventional flies were set up in a 2x2 full factorial design and 3' RNA-seq was performed on pooled samples of whole mated females at six hours post-mating, and on axenic and conventional virgin females as well. Between axenic and conventional virgin females, 160 genes were differentially expressed. This set included genes with immune functions and genes involved in sterol, carbohydrate and lipid metabolism. When comparing mated axenic females with mated conventional females, only eight genes were differentially expressed. Among these were three antimicrobial peptides which were strongly upregulated in axenic females, relative to conventional mated females, regardless of whether these females had mated to an axenic or conventional male. Male microbiome status affected the female's expression of only one gene (*painless*), whose transcripts were upregulated more strongly if a female mated to an axenic male. These preliminary results suggest that the presence or absence of a microbiome has no major effect on the female's ability to induce typical post-mating transcriptional changes. Our results also demonstrate that activation of the female immune response by mating is not simply driven by microbial exposure.

201 Effect of Nora virus infection on native gut bacterial communities and lifespan of *Drosophila melanogaster*. *M. Nemecek*, R. Best, S. Liesemeyer, D. Carlson, J. Shaffer, K. Carlson Biology, University of Nebraska at Kearney, Kearney, NE.

Gastrointestinal microflora is a key component in the maintenance of health and longevity in both vertebrate and invertebrate species. In humans and mice, non-pathogenic viruses present in the gastrointestinal tract ability to enhance the effects of the native bacterial flora. However, it is unclear whether non-pathogenic gastrointestinal viruses, such as Nora virus, that infect *Drosophila melanogaster* lead to similar observations as in humans or mice. We conducted a longevity study on Nora virus infected (NV+) and uninfected (NV-) *D. melanogaster* in relationship to presence (B+) or absence (B-) of the native gut bacteria. Four different treatment groups were generated, NV+/B+, NV+/B-, NV-/B+, and NV-/B-. Sixty virgin female flies from each treatment group was put into each of three cages. Dead flies were collected every three days until the cages were empty. The longevity results were analyzed via Kaplan-Meier survivorship analysis and demonstrate that Nora virus may be detrimental to the longevity of the organism, whereas bacterial infection is beneficial and necessary. This data led us to hypothesize that Nora virus infection may be affecting the makeup of the gut bacterial community present. To test this, NV+ and NV- virgin female flies were collected and allowed to age for 4 days. Surface sterilization followed by gut dissections were conducted, dividing the gastrointestinal tract into foregut, midgut, and hindgut, and the fat body was also collected. DNA samples were sent to the UNMC Genomic Sequencing Core Facility for 16S sequencing to determine the bacterial communities that comprise the microflora of in the gastrointestinal tract of NV+ and NV- *D. melanogaster*. This data will help us to understand the unique structure of the gut microflora and how it affects the longevity and health of an organism when infected with a non-pathogenic virus, such as Nora virus. The project described was supported by grants from the National Center for Research Resources (5P20RR016469) and the National Institute for General Medical Science (8P20GM103427), a component of the National Institutes of Health.

202 Microbiome transfers adaptive potential in *Drosophila melanogaster*. *L. Henry*[1], J. Ayroles[1,2] 1) Ecology and Evolutionary Biology, Princeton University, Princeton, NJ; 2) Lewis-Sigler Institute for Integrative Genomics, Princeton University.

The microbiome is an integral component of host biology, shaping developmental, physiological, and reproductive phenotypes. From an evolutionary perspective, it remains unclear if and how the microbiome influences host adaptation. During adaptation, both host and microbial genomes evolve in response to stressful environments. If the microbiome is important in host adaptation, then transferring the microbiome into naïve hosts should transfer the adaptive potential in a stressful environment. Here, we test this idea by leveraging >100 generations of adaptation to high sugar diet (HS) in *Drosophila melanogaster*. We first sequenced the microbial communities at multiple timepoints during the course of experimental evolution. Microbial communities respond rapidly (within 5 generations) to HS diet. To test the adaptive potential of the microbiome, we performed a full reciprocal transplant experiment across control (C) and HS adapted populations (i.e. [C and HS fly genotype] x [sterile, C, and HS microbiome] x [C and HS diet]). We profiled the microbial communities (16S amplicon sequencing and qPCR), developmental time, characterized the fatty acid metabolic profile with HPLC, and fly fecundity. We find that microbiome influences developmental time, fatty acids, and fly fecundity. Interestingly, the microbiome influences were stronger on HS diet. On the HS diet, the C microbiome accelerated development, but reduced fecundity in both C and HS fly genotypes. However, the HS microbiome conferred fecundity benefits only in HS diets, especially for the HS genotype. Intriguingly, the HS microbiome in C flies increased variance in fecundity and fatty acid abundance,

suggesting the microbiome leads to decanalization. In addition, we have analyzed the metagenomes of 10 Evolve & Resequence experiments in *D. melanogaster*. We found that the microbiome in evolved lines frequently responds dramatically to different selective pressures. Taken together, our data suggest that the microbiome shapes phenotypic variation to best match the host to local environment. Microbial variation may alter phenotypic variation, enabling hosts to explore the fitness landscapes in novel ways. These results highlight the role microbial evolution may play in driving host adaptation to stressful environments.

203 Microbiota's effect on development in a *Drosophila* Parkinson's disease model. J.A. Parker-Character[1], *G.B. Call*[2] 1) Department of Biomedical Sciences, Midwestern University, Glendale, AZ; 2) Department of Pharmacology, Midwestern University, Glendale, AZ.

Although Parkinson's disease (PD) is generally described as a movement disorder, it is a complex multisystem disorder with patients experiencing a range of symptoms with both motor and non-motor features. One of the most common non-motor ailment that affects the PD population is GI disturbances.There is increasing evidence supporting that changes in the microbiota correlates with the pathogenesis of PD. Recently, researchers have shown in a PD rat model that gut dysbiosis occurs with PD even before dysfunctional motor symptoms were present. The *Drosophila* microbiome is typically comprised of 5-70 different bacterial species deriving from four bacterial families: *Enterobacteriaceae*, *Proteobacteria*, *Acteobacteriaceae*, and *Lactobacillaceae*. It has been demonstrated that the microbiota can influence fly behavior and development. Flies homozygous for the *parkin* loss-of-function allele ($park^{25}$) is an excellent PD model. These flies have reduced dopaminergic neurons, reduced motor ability, loss of olfaction, decreased lifespan and problems with fertility/fecundity. The $park^{25}$ stock is unhealthy and is maintained over a balancer chromosome, though it is not a lethal mutation. Observationally, homozygous $park^{25}$ flies tend to have an increased pupal lethality compared to heterozygous $park^{25}$ flies. To determine if the microbiome can affect development of $park^{25}$ flies, we performed a fecal transfer experiment between w^{1118} (the parent stock from which $park^{25}$ was generated) and $park^{25}$ flies. We had 40 $park^{25}$ males and 40 w^{1118} males deposit fecal matter in separate food vials over a three-day period. We then removed the males, and added $park^{25}$ parents (15 females and 5 males) onto the feces-containing food for 24 hours. Once the pupae emerged, we determined eclosure rate as well as the pupal lethality. Initial results indicate that the microbiome does not affect total pupae numbers or the percentage of homozygous $park^{25}$ pupae. However, there was lower eclosure rate in the $park^{25}$ homozygous flies on $park^{25}$ fecal matter (71%) when compared to the $park^{25}$ homozygous flies raised on w^{1118} fecal matter (87%, $P<0.05$). We are currently determining if there are other developmental effects on $park^{25}$ flies by the microbiome and will be determining the different bacteria present in the two stock populations. This data will be presented at the meeting.

204 Priority effects dictate microbiota composition and influence host lifespan. *W.B. Ludington*[1,5], B. Obadia[2], A. Aranda-Diaz[3], R. Dodge[1], M. Voltolini[4], K.C. Huang[3], M. Sepanski[1], R. Paisner[5] 1) Dept. Embryology, Carnegie Institution for Science, Baltimore, MD; 2) MCB Dept, University of California Berkeley, Berkeley, CA; 3) Bioengineering Dept, Stanford University, Stanford, CA; 4) Lawrence Berkeley National Lab, Berkeley, CA; 5) Dept. Biology, Johns Hopkins University, Baltimore, MD.

The bacteria that occupy an animal gut can have profound effects on their host's fitness. Therefore, it is important to understand how gut bacterial composition is determined through the process of colonization. The fly gut microbiome varies greatly between individuals, leading us to ask what are the natural sources of variation. One prominent factor that causes ecological variation in macroecosystems is the priority effect, whereby an early arriver colonizing species changes the environment for later arrivals. Here we show that priority effects change gut colonization and consequently change the lifespan of flies. We inoculated germ free 5-7 day old mated female flies with two species of commensal gut bacteria each, with one bacterial species inoculated first and the second several days later. We focused on natural fly commensals from the Lactobacillus and Acetobacter genera. We experimentally varied which bacterial strains the flies were exposed to and the order in which each species was introduced. Flies first exposed to L. plantarum were less likely to be colonized by A. indonesiensis and vice versa. However, flies first exposed to L. plantarum were more likely to be colonized by A. pasteurianus. We used high resolution fluorescence microscopy to determine the spatial localization of the strains, suggesting that early arrivals preferentially occupy longitudinal crypts in the fly proventriculus. Electron microscopy confirmed crypt localization and suggested that bacterial cell shape is a key factor in determining the localization to crypts. Overall, we find that both metabolic and physical interactions between bacteria lead to priority effects that cause populations of flies inoculated with the same gut bacteria to have different life expectancy. These results are important to help us understand general principles of human gut colonization.

205 Effect of bacterial genetics on persistence in D. melanogaster. *S. Gottfredson* Brigham Young University, Provo, NV.

This experiment aims to study the correlation between bacterial genotype and persistence in, *Drosophila melanogaster.* The gut microbiome of *D. melanogaster* has been a subject of study for many years. However, it has not been studied in depth why the bacterial species associate and persist with *D. melanogaster.* A common assumption has been that the microbiome is established solely by the diet of *D. melanogaster*. However, recent work suggests that other factors may be at play in the establish of the *D. melanogaster* microbiome. This study will look at the persistence of six different species of known gut microbiota in *D. melanogaster,* 4 lab isolates, and 2 wild isolates. By frequently transferring the flies onto new sterile food three times a day for two days, the microbiome has little time to replenish through the normal means of diet. After the two days of frequent transferring, the flies will be homogenized and the bacterial content will be analyzed. Analysis of the data collected will then be done to check for a correlation between the bacterial genes and the level of persistence in *D. melanogaster.*

206 Establishment and persistence of probiotics in *Drosophila melanogaster*. *A.J. Barron*, N.A. Broderick Molecular and Cell Biology, University of Connecticut, Storrs, CT.

The gut microbiome of *Drosophila melanogaster* has emerged as an excellent model for understanding host-microbiota interactions. The relative simplicity of the *Drosophila* microbiome compared to the more diverse microbial communities associated with vertebrates makes it ideal for elucidating ecological mechanisms that determine how microbes colonize new environments and overcome host defenses, such as the production of reactive oxygen and chlorine species (ROS and RCS, respectively) and the synthesis of antimicrobial peptides (AMPs). We are studying the probiotic bacterium *Escherichia coli* strain Nissle 1917 (EcN) and its ability to colonize the fly gut and provoke epithelial renewal following exposure to oxidants. Our data show that EcN colonizes the fly gut at higher levels than *E. coli* K12 strains and that administering regular doses of the bacterium allows populations in the gut to stabilize. We also show that EcN competes directly with gut commensals both *in vivo* and *ex vivo.* Screening of EcN mutants that are defective in their protective responses to ROS and RCS showed that many of these mutants are diminished in their ability to colonize the *Drosophila* gut. Data collected examining EcN colonization levels in a *Drosophila* line in which ROS/RCS production has been genetically reduced by RNAi-mediated knock-down of Duox will be discussed. We also demonstrate by reporter gene analysis and qPCR that EcN elicits robust AMP production by the immune deficiency pathway. Future experiments will characterize the potential beneficial effects of association with EcN prior to exposure to a pathogen. Overall, this work will broaden our understanding of how an invasive species colonizes a new environment and how the host response affects this process.

207 Characterization of partitiviruses infecting *Drosophila melanogaster*. *S.T. Cross*[1], B.L. Maertens[1], J.R. Fauver[1,2], B.D. Foy[1], G.D. Ebel[1], M.D. Stenglein[1] 1) Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO; 2) Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO.

Metagenomic surveys of wild organisms have generated thousands of new virus sequences. This explosion in described viral diversity is transforming our

understanding of virus evolution and genome structure. But in many cases, basic information about the biology of these viruses is missing. For instance, it is often not even clear what host a particular virus infects. Here we describe our initial characterization of fly-infecting partitiviruses, which appear to be widely distributed in arthropods, and globally common in *Drosophila*. Partitiviruses are segmented, multipartite dsRNA viruses (family *Partitiviridae*) that until recently were only known to infect fungi, plants, and protozoans. Partitiviruses have been found in nearly every wild population of *Drosophila* that has been examined, with prevalence levels comparable to those of *Wolbachia* bacteria. We identified partitiviruses in wild *Drosophila*from Colorado and Haiti, and in multiple species of mosquitoes, including known disease vectors. The Colorado *Drosophila* remained infected over three years, with infection rates ranging from 63-94%, and we established lines of wild-caught, infected flies. We confirmed that these partitiviruses actually replicate in *Drosophila*, and identified sites of virus replication in larval and adult flies. We determined the genome structure of these viruses. We performed phylogenetic analyses and examined concordance between host and virus phylogenies. We quantified transmission routes and efficiencies, and found that these viruses can be transmitted both horizontally and vertically. In both cases, transmission from infected females was more efficient than transmission from infected males. Future studies will be necessary to understand the and impact of partitivirus infection on *Drosophila* biology.

208 Immunity costs associated with meiotic drive. *J. Lea*, R. Unckless University of Kansas , Lawrence, KS.

Meiotic drive systems have been observed across several species ranging from plants to fungi to animals. The non-mendelian inheritance that is responsible for their rapid spread is often associated with chromosomal rearrangement events that surround the responsible driver loci. Several inversions flanking the loci limit or completely prevent recombination. As a result, potentially disadvantageous mutations in proximity to the driver are maintained and less likely to be purged by natural selection. Although a majority of research has focused on mapping the regions responsible for meiotic drive and understanding the mechanisms they produce, minimal work has been done to examine the costs associated with these unfavorable regions – especially costs beyond basic viability and fertility. We studied the costs associated with meiotic drive elements in term of immune defense in *Drosophila melanogaster* segregation distorter and two sex-ratio drive systems in *Drosophila neotestacea* and *Drosophila affinis.* We conducted systematic infections using *Enterococcus faecalis*, *Providencia rettgeri*, *Lysinibacillus fusiformis*, and *Candida albicans* and measured both survival and bacterial load after infection. We followed up these assays with a transcriptomics approach to understand what aspects of the immune response are perturbed in flies carrying the meiotic drive element.

209 Purge of hemolymphatic lipid by Malpighian tubules during infection protects *Drosophila* from ROS damage. *X. Li*[1], S. Kondo[2], B. Lemaitre[1] 1) Global Health Institute, School of Life Science, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland; 2) Invertebrate Genetics Laboratory, Genetic Strains Research Center, National Institute of Genetics, Mishima, Japan.

Infection induces the production of immune effectors to combat invading microbes. However, mounting an immune response is costly and potentially deleterious, requiring host metabolic and physiological adaptation. Thus, immune resistance/host defense during infection encompasses a much broader that goes beyond the mere production of immune effectors. Here, we found that both infection and injury induce in Drosophila the excretion of hemolymphatic lipids into the feces by Malpighian tubules, the insect kidney. This lipid purge is mediated by a novel stress-induced protein Materazzi, that is enriched in Malpighian tubules. We showed that this mechanism protects the hemolymph and the tubules from damage caused by immune-induced reactive oxygen species. Flies lacking materazzi fail rapidly succumb to infection due to their inability to purge hemolymph lipid, that then undergoes peroxidation resulting in high ROS. Thus, excretion of the hemolymphatic lipids by Malpighian tubules is a mechanism used to protect host tissues from excessive ROS during immune and stress responses. Our study uncovers a novel and surprising physiological adaptation to deal with oxidative stress associated with infections.

210 CRISPR Knockout and Functional Analysis of Three Y Chromosome Genes in *D. melanogaster*. *Y. Hafezi*, S. Sruba, S. Tarrash, M. Wolfner, A. Clark Dept Molecular Biology and Genetics, Cornell University, Ithaca, NY.

The functions of gene-poor, repeat-rich regions of the genome are poorly understood and are also understudied due to technical challenges. Yet multiple lines of evidence indicate these areas may be an important source of variation that may drive adaptation and species divergence. Despite its ~40 Mb size, the Y chromosome of *Drosophila melanogaster* contains only 16 known protein-coding genes. These few genes are evolving in an entirely heterochromatic environment where they experience no recombination, male-restricted selection, and one-fourth of the autosomal effective population size. Yet most of the genes originated from autosomal duplications and their reduced nonsynonymous substitution rates suggest they are not degenerating. To directly test their function, we used CRISPR to create deletions that disrupt the transcription start site or coding frame of three genes and test their role in male fertility. One of the genes, CCY, was previously predicted to be required for male fertility and we confirmed that the knockout is male sterile. Surprisingly, however, we found the effect to be dominant, causing sterility even in the presence of an extra wild-type Y chromosome. We also created mutants in two genes with no previously identified functions: PRY and FDY. We found that PRY knockout males are sub-fertile, but preliminary results suggest no detectable effect on fertility for FDY. Because FDY is the newest gene on the Y chromosome, an effect on fertility may be too subtle to detect or may be only apparent during stress. The Y chromosome has been shown to have a significant impact on male fertility, fitness, temperature adaptation, geotaxis, sex-specific aging, position effect variegation and global gene expression. Our CRISPR mutants are a first step in being able to discriminate which roles of the Y chromosome are accomplished by genes versus repeat elements. They are also an important tool for better understanding the evolutionary logic of how and why genes adapt to the bizarre environment of the Y chromosome.

211 Identification of transposable elements contributing to large Y chromosomes in *D. pseudoobscura*. *A.H. Nguyen*, D. Bachtrog Integrative Biology, University of California, Berkeley, Berkeley, CA.

Y chromosomes can vary in morphology and length within populations. *D. pseudoobscura*'s Y differs immensely in shape and size although how such variation arose is unclear. To characterize this variation in DNA content, we first sampled 26 natural Y chromosomes and placed them on isogenic backgrounds through repeated backcrossing. We generated karyotype squashes and estimated nuclear DNA for each Y variant to identify Y chromosomes that differ in size and morphology. Using short- and long-read sequencing, we further characterized two Y chromosomes that contain an extra 24 Mb and 10Mb of DNA compared to the sequenced male Y. We find that most of the DNA gain on the largest Y is due to the accumulation of several transposable elements, with the vast majority (10Mb) being derived from a single over-amplified rolling circle *Helitron* transposon. We will use FISH to confirm the sequencing results using probes targeting high copy number transposons and also address synteny across the Y variants. Overall, we show that transposon over-amplification drives naturally occurring Y polymorphism in *D. pseudoobscura* identified 80 years ago.

212 A Nuclear-Encoded Mitochondrial Duplicated Gene, *CG10396*, Is Essential for Spermatogenesis in *Drosophila melanogaster*. *Mohammadmehdi Eslamieh*, Esther Betrán Department of Biology, University of Texas at Arlington, Arlington, TX.

A previous study of nuclear-encoded mitochondrial genes (N-mt genes) in *Drosophila melanogaster* showed a unique expression pattern for newly duplicated N-mt genes. Many of new genes showed tissue-biased expression (76%; 28/37) while all tissue-biased genes are testes biased. Many of these genes have been found to be essential during spermatogenesis. Here, we are studying the function of a new duplicated N-mt gene of OXPHOS complex IV in *D. melanogaster.* This duplicate is present in all *Drosophila*. OXPHOS complex IV is the last complex in the mitochondrial electron transport chain and has been suggested as one of the major regulators of ATP production. It consists of 13 protein subunits encoded by two different genomes. The three biggest subunits are encoded by the mitochondrial genome while the other 10 subunits and other cytochrome c oxidase-specific regulatory proteins are encoded by the

nuclear genome. *Cytochrome c oxidase 4* (*COX4, CG10664*) is one of the N-mt genes that has been duplicated through DNA duplication and the new copy, *Cytochrome c oxidase 4-like* (*COX4L*, *CG10396*) is assumed to encode a subunit of the same complex and functions in sperm and potentially spermatogenesis. Both genes have different pattern of expression where *COX4* is expressed highly in every tissue while *COX4L* is highly expressed in testes. To understand the function of this new gene, we knocked down this gene in *D. melanogaster* germline using two different RNAi lines driven by *Bam-Gal4*. We also created a knockout strain for this gene using CRISPR-Cas9 technology. The results of both approaches were the same where only complete male sterility was observed. This prominent phenotype along with having energy-related functions, testes biased expression, and also present in *Drosophila* sperm proteome database suggests that males might use different mitochondria in their germline and selection might favor different, higher energy-producing mitochondria in male germline than in female germline and soma. The functional study of this gene adds to our understanding of why so many N-mt duplicates have been retained in the genome for the male germline function.

213 Massive repeats of Wolbachia DNA from lateral gene transfer in the Drosophila ananassae genome. *Julie Dunning Hotopp*, Mark Gasser, Matthew Chung Institute for Genome Science, University of Maryland Sch of Med, Baltimore, MD.

Lateral gene transfers (LGTs) are found in numerous invertebrate genomes. Many LGTs in invertebrate genomes are from *Wolbachia* endosymbiont genomes to the genomes of their insect or nematode hosts. The LGT from the *Wolbachia* endosymbiont *w*Ana to *Drosophila ananassae* is the most massive, constituting >2% of the insect genome (~5 Mbp) and ~20% of the autosomal chromosome 4. We present the complete 1.40 Mbp genome of Wolbachia strain wAna from DNA isolated in a host background without LGT from a single MinION run (max read length=171,039 bp; N50 read length=18,617 bp). MinION sequencing was essential to resolving two near identical ~40 kbp prophage regions that were spanned by multiple >50 kbp reads which revelaed a large deletion from one prohage region, a key difference from the genome of the closely related Wolbachia enodsymbiont wRi. MinION reads (max read length=226,236 bp; N50 read length=19,384 bp) from a *D. ananassae* with the LGT, but not the endosymbiont, were obtained and mapped to this *w*Ana genome, including 12 reads that are >100 kbp. These reads reveal extensive rearrangements of the DNA relative to *w*Ana that should enable assembly and analysis of this fascinating and underappreciated region of the *D. ananassae* genome.

214 The evolution of centromere-associated retrotransposons in *D. melanogaster* populations. *Lucas Hemmer*, Iain Speece, Ching-Ho Chang, Amanda Larracuente Biology, University of Rochester, Rochester, NY.

Centromeres are chromosomal regions necessary for kinetochore attachment and cell division in eukaryotic organisms. Little is known about centromere organization because they reside in rapidly evolving, repeat-rich regions of the genome. These repeats present a challenge for genome assembly and make it difficult to study the role of DNA sequences in centromere function. We recently determined the organization of all *D. melanogaster* centromeres using Pacific Biosciences (PacBio) long-read sequencing technology. We discovered that centromeres are organized as islands of complex DNA rich in retrotransposons and embedded within large blocks of tandem satellite repeats. Interestingly, studies in plants, mammals, and fungi have detected centromere-associated retroelements, suggesting that they are conserved features of centromeres. However, we do not know if retroelements play a role in centromere function or how they shape centromere evolution. The centromere islands of *D. melanogaster*, while unique to each chromosome, share one particular non-LTR retrotransposon (*G2/Jockey-3*), suggesting that it may be important for centromere function, maintenance, or establishment in *Drosophila*. However, *G2/Jockey-3* is not exclusive to centromeres. It is unclear if the enrichment of *G2/Jockey-3* at centromeres reflects an insertion site preference or if selection favors centromeric insertions of *G2/Jockey-3*. We used publicly available DNAseq data from *D. melanogaster*populations to predict *G2/Jockey-3* insertion polymorphisms. We detected polymorphic insertions both inside and outside of centromere islands, suggesting that *G2/Jockey-3* is a recently, or currently, active element in *D. melanogaster*. The pattern, age, and frequency of *G2/Jockey-3* insertions have implications for centromere evolution in *Drosophila* and the possible roles of retroelements in centromere function.

215 Thoracic underreplication predicts minimal Drosophila genome size. Carl Hjelmen[2], V. Renee Holmes[1], *J. Spencer Johnston*[1] 1) Dept of Entomology, Texas A&M University, College Station, TX; 2) Dept. Biology, Texas A&M University, College Station, TX.

Underreplication is the S phase synthesis of only a portion of the genome. The portions of the genome that are not replicated are known to be largely heterochromatic, filled with repeats and noncoding sequences. This phenomenon has been documented throughout many highly polytenic tissues in salivary glands, nurse cells, fat body cells of *Drosophila* and other Diptera. Unique to Drosophila we find that synthesis of DNA stalls between G0 and G1 in the majority of cells of the thorax leaving the genomes underreplicated. We assume here that synthesis in underreplicated thoracic cells proceeds through the euchromatic portion of chromosomes and stalls at heterochromatin as in polytene cells. Further, since genome size has been shown to be linked to the amount of noncoding and repeats sequences, rather than coding sequences in polytene cells, we hypothesize that the amount of replication that occurs in the thorax is directly related to the genome size of a species and that this pattern is consistent. To test this, we determined thoracic underreplication levels for approximately 100 species from the *Drosophila* species stock center. This information allows us to determine the relationship of genome size and underreplication across the Drosophila phylogeny. The amount of underreplication is found to be significantly correlated to genome size across species and sexes. Using this relationship, we can predict how much of the genome is heterochromatic, how much is euchromatic and predict a minimum genome size for *Drosophila* species.

216 Natural variation in sugar tolerance associates with changes in signalling and mitochondrial ribosome biogenesis. *R.G. Melvin*[1,2,3], N. Lamichane[2,3], E. Havula[5,2,3], K. Kokki[2,3], C. Soeder[4], C.D. Jones[4], V. Hietakangas[2,3] 1) Biomedical Sciences, University of Minnesota, Duluth, MN; 2) Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland; 3) Institute of Biotechnology, University of Helsinki, Helsinki, Finland; 4) Biology Department, University of North Carolina at Chapel Hill, NC; 5) Charles Perkins Centre, University of Sydney, Sydney, Australia.

How dietary selection affects genome evolution to define the optimal range of nutrient intake is a poorly understood question. We have addressed this question by analyzing *Drosophila simulans* and *sechellia*, recently diverged species with differential diet choice. *D. sechellia* larvae, specialized to a nutrient scarce diet, did not survive on sugar-rich conditions, while the generalist species *D. simulans* was sugar tolerant. Sugar tolerance in *D. simulans* was a tradeoff for performance on low-energy diet and was associated with global reprogramming of metabolic gene expression. We used a hybridization and phenotype-based introgression method to reveal the genomic regions of *D. simulans* that were sufficient for sugar tolerance. These regions included genes that are involved in mitochondrial ribosome biogenesis and intracellular signaling, such as *PPP1R15/Gadd34* and *SERCA*, which contributed to sugar tolerance. In conclusion, genomic variation affecting genes involved in global metabolic control define the optimal range for nutrient intake.

217 Trans-complementing system uncovers fine workings of CRISPR-based gene drives. *V. Lopez del Amo*[1], A. Bishop[1], E. Bier[1], A. Choudhary[2], VM. Gantz[1] 1) Division of Biological Sciences, University of California San Diego, San Diego, CA; 2) Department of Medicine, Harvard Medical School, Boston, MA.

CRISPR-based gene drives are a powerful technology that can be used to engineer wild populations due to their ability of self-propagating into a population. This technology offers a great promise to tackle vector-borne diseases (e.g., malaria) by suppressing or modifying mosquito population with parasite immunity and therefore decreasing their global burden on public health. CRISPR gene drive approaches lead to the conversion of germline cells from heterozygous to homozygous, biasing inheritance from Mendelian (50%) towards a Super-Mendelian one (~100%). This bias can result in the exponential spread of the gene drive element into a population. This ability to spread has risen risk concerns such as accidental release, demanding strict safety practices

for the development of this technology in the laboratory.

We develop the first trans-complementing gene drive (tGD) system where Cas9 and gRNAs can be kept separate as two different strains, and only when combined have the potential of generating the same biased inheritance of a full gene drive. It allows for the safe optimization of gene drive elements generating a condition comparable to a full gene drive only during the course of experimentation.

First, we demonstrate that a split tGD can trigger simultaneous Super-Mendelian inheritance of two active genetic elements.
Second, this system can be used to analyze different variables in the laboratory phase optimizing the constructs before field applications.
Finally, we show which parameters on the copying process need to be considered when designing a gene drive construct to ensure optimal efficiency.

This research paves the way for the generation of safer gene drive approaches, allowing to speed up the production of improved gene drive systems and moving them faster from the lab to mosquito field applications for public health applications.

218 Characterizing evolutionary strategies in wild *Drosophila* thermal preference via high resolution temporal sampling and broad geographic collections. Jamilla Akhund-Zade[1,2], *Denise Yoon*[1], Alyssa Bangerter[3], Matthew Campbell[3], Nikolaos Polizos[4,5], Eric Wice[6], Julia Saltz[6], Alan Bergland[3], Mason Klein[5], Benjamin de Bivort[1] 1) Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA; 2) Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA; 3) Department of Biology, University of Virginia, Charlottesville, VA; 4) Department of Biology, University of Miami, Miami, FL; 5) Department of Physics, University of Miami, Miami, FL; 6) Department of BioSciences, Rice University, Houston, TX.

Bet-hedging is an evolutionary strategy in which a single genotype encodes a distribution of phenotypes as a means of ensuring that, even when the environment fluctuates unpredictably, some individuals will be fit. This is in contrast with adaptive tracking, or adaptation via natural selection, where phenotypes are genetically determined and the mean phenotype tracks with the environmentally determined optimum. We previously developed a model in which individual *Drosophila* thermal preferences affect life history, and in turn lineage evolution (Kain et al., 2015). This model predicts that bet-hedging is advantageous in regions of high seasonality and short breeding seasons, such as Boston, MA. An adaptive-tracking strategy is favored in locations with long and mild breeding seasons, such as Miami, FL. To test the modeling predictions empirically, we collected *Drosophila* across the U.S. as well as throughout the breeding season. Our hypotheses, deriving from the model, are that flies from northern latitudes will show roughly constant mean preference over the season, higher variability in thermal preference, and low heritability for individual thermal preferences. Our results from weekly seasonal collections in Boston, MA, Charlottesville, VA, and Miami, FL show small fluctuations in mean thermal preference, suggesting that a bet-hedging strategy is common to all locations. Preliminary data from *D. simulans* shows greater variability in thermal preference in isofemale lines collected in Boston, MA compared to Houston, TX. Variability and heritability experiments for *D. melanogaster* are ongoing.

219 Population Genomics of *Drosophila pseudoobscura*. *R.P. Meisel* Biology and Biochemistry, University of Houston, Houston, TX.

Drosophila pseudoobscura is a classic model organism for speciation genetics, chromosome biology, and other areas of evolutionary genomics. Despite its importance in evolutionary biology, there have been no genome-wide studies of genetic variation in *D. pseudoobscura,* only single locus or single chromosome surveys. To address this deficiency, we sequenced the genomes of 66 *D. pseudoobscura* inbred isofemale lines sampled across the species range. We identified single nucleotide and small indel variants across the 66 genomes. We used those data to characterize protein coding and non-coding polymorphism, and we integrated those analyses with divergence from close relatives. We also contrasted patterns of polymorphism and divergence on the autosomes, ancestral *Drosophila* X chromosome, and the *D. pseudoobscura* neo-X chromosome.

220 Tuning gene drive activity with a small molecule. V. Lopez del Amo[1], K. Cox[2], S. Gill[2], B.S. Leger[3], A.L. Bishop[1], E. Bier[1], J. Walker[3], A. Choudhary[2], *V.M. Gantz*[1] 1) Biological Sciences, University of California San Diego, La Jolla, CA; 2) Department of Medicine, Harvard Medical School, Boston, MA; 3) Center for Genomic Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA.

CRISPR-based gene drive constructs can bypass Mendelian inheritance by copying themselves onto the companion chromosome and are this way inherited with Super-Mendelian frequencies. This technology offers tremendous promise for public health as a mosquito or disease control tool, in agriculture for pest suppression and in conservation to remove invasive species that are harming the environment. Here we test a drug-inducible gene drive system targeting the *Drosophila melanogaster ebony*, *white* and *yellow* loci. We show for the first time the use of a small molecule can be used to tune the Super-Mendelian inheritance of a gene drive construct.

221 How gene conversion events shape nucleotide diversity within chromosomal inversions in *Drosophila pseudoobscura*. *S.W. Schaeffer* Dept Biol, Pennsylvania State Univ, University Park, PA.

Drosophila pseudoobscura harbors a wealth of gene arrangements on its third chromosome within natural populations. These arrangements were generated through a series of overlapping paracentric inversion events and have been polymorphic for over one million years. Genomic and transcriptomic analyses have suggested that these inversion mutations were established after they arose because of their ability to suppress genetic exchange in heterozygotes preventing shuffling of alleles among different arrangements. The inverted segments of each gene arrangement contain gene regions whose nucleotide sequence is diverged among inversions separated by undifferentiated regions which unexpected given the expected suppression of recombination in heterozygotes. While single cross overs are suppressed in inversion heterozygotes, genetic exchange can occur via double cross overs or gene conversion events. Gene conversion events can be detected in inverted chromosomes based on observed differentiated segments moving among gene arrangements. We use data from 54 genomes of *D. pseudoobscura*, which carry one of six different gene arrangements, to infer gene conversion tracts across the inverted third chromosome. We used the approach of Bertran *et al*. (1997, The estimation of the number and the length distribution of gene conversion tracts from population DNA sequence data. *Genetics, 146*(1), 89-99.) to infer and map the locations of gene conversion tracts across the third chromosome and ask the following questions: (1) are gene conversion tracts uniformly distributed across the inverted chromosomes; (2) what is the size distribution of conversion tracts; (3) do gene conversion tracts occur in differentiate regions of the inverted segments; and (4) is the frequency of inferred gene conversion tracts equal among all gene arrangements? Schaeffer and Anderson (2005 Mechanisms of genetic exchange within the chromosomal inversions of *Drosophila pseudoobscura*. *Genetics, 171*, 1729-1739.) showed that gene conversion events occur more frequently that mutations based on small number of gene markers on the third chromosome. This study will provide a more comprehensive analysis of genetic exchange among gene arrangements to determine the role that gene conversion plays in homogenizing genetic diversity in inverted chromosomes.

222 Effects of suppressors on *Segregation Distorter* in *Drosophila melanogaster*. *T.J. Mouton*, C.H Chang, A. Larracuente Biology, University of Rochester, Rochester, NY.

Segregation Distorter (*SD*) is a selfish gene complex on the second chromosome of *Drosophila melanogaster* that biases its transmission through the male germline by killing wild type sperm. Males that are heterozygous for *SD* (*SD/SD+*) transmit *SD* to nearly all of their offspring, whereas *SD/SD+* females transmit *SD* fairly, to 50% of their offspring. The main driver locus is on chromosome 2L and the target and modifiers that strengthen drive are located on the

2R chromosome. *SD* chromosomes tend to acquire local inversions that may help keep modifiers that are important for the *SD* complex linked to the main driver. In at least two different populations, *SD* chromosomes with different inversions replaced each other over decades. Suppressors of *SD* (*Su(SD)*) that restore Mendelian segregation are found at high frequencies on chromosomes 2, 3, and X in many natural populations. How these suppressors interact with *SD* chromosomes across populations is unclear. We recently extracted new *SD* chromosomes and an X-linked *Su(SD)* from the Drosophila Genetic Reference Panel (DGRP). Our preliminary results suggest that drivers vary in their sensitivity to *Su(SD)X*, indicating that modifiers of *SD* chromosomes may interact with these suppressors. To test this hypothesis, we measured the effects of *Su(SD)X* on driving ability of *SD* chromosomes from Africa, France, Italy, Ecuador, and four geographic locations in the Eastern US. We found that the effects of *Su(SD)X* vary based on the *SD* inversion type. These results lend insight into the interplay between *SD* and its suppressors, and how these interactions may drive *SD* dynamics in natural populations.

223 Meiotic drive and survival probability of newly inverted chromosomes. *S.A. Koury* School of Biological Sciences, University of Utah, Salt Lake City.

When a new gene arrangement is generated by spontaneous mutation its survival is uncertain and largely unaffected by associated fitness effects. However, if a new chromosomal inversion is introduced into a population already polymorphic for inversions, then its survival probability will be a function of the relative size, position, and linkage phase of the gene rearrangements. This dependence on structural features is due to the complex meiotic behavior of overlapping inversions generating asymmetric dyads, which in turn cause both underdominance and meiotic drive/drag. Therefore, survival probabilities of new inversions can be expressed in terms of the probability of forming an asymmetric dyad via crossing over in meiosis I and the probability of recovery from that asymmetric dyad during disjunction in meiosis II. This model of female meiotic drive was parameterized with data from published experiments on laboratory constructs in *Drosophila melanogaster.* Generalizing this analysis to all possible inversions predicts a bias towards larger, proximally located inversions having a shorter persistence time in populations. These population genetic predictions are consistent with cytological evidence from natural populations of *D. melanogaster*. This research mathematically formalizes a cytogenetic mechanism for female meiotic drive/drag as the major force governing behavior of new gene arrangements entering populations, and therefore determining the genomic distribution of segregating inversion polymorphism.

224 Structural Variation in the Drosophila Nasuta Clade. *D. Mai*, D. Bachtrog Integrative Biology, University of California Berkeley, Berkeley, CA.

The *Drosophila nasuta* clade diverged from the *Drosophila immigrans* group 4 million years ago and comprises 10 to 14 species with varying levels of reproductive isolation (RI). Given how young this clade is, the system provides an opportunity to study the early stages of speciation. It is thought that chromosomal rearrangements, such as inversions, can increase the rate at which RI evolves. Here, we present intra- and interspecific chromosomal rearrangements of 8 species in this group and infer candidate loci that contribute to RI.

Sequence data for 8 species in the *Drosophila nasuta* clade are generated using a combination of single molecule, real time sequencing, nanopore sequencing, and HiC. Genome assemblies are generated from these data using various software: Canu, minimap2, miniasm, Juicer, and 3D-DNA. Paired end short read sequencing of multiple strains per species are used to detect intraspecific inversions. Whole genome alignment software (MUMMER) is used to detect structural rearrangements between species using the corresponding genome assemblies. We find several species specific rearrangements. Further studies on the genes found within these rearrangements may allow us to hone in on important speciation genes in the clade.

225 Chromosomal structural variants in *D.yakuba and D.santomea* and their role in gene formation. *Brandon Turner*, Rebekah Rogers University of North Carolina - Charlotte , Charlotte, NC.

It is the canonical theory that de novo gene formation requires non-coding intermediaries to acquire new mutations prior to loss via pseudogenization. Chromosomal structural variants offer an alternative method of de novo gene formation. This idea is explored by calculating the site frequency of chromosomal rearrangements and SNP's in *D.yakuba* and *D.santomea*. This is used to calculate Tajima's D and produce a list of genes that are compared with mutation locations from previously created gene annotations. The goal is to identify large frequency differences to infer if mutations have helped the flies adapt using this method of gene formation.

226 Molecular mechanisms underlying evolution of testis-specific expression of *de novo* genes. *Shrinivas Dighe*, Helen White-Cooper School of Biosciences, Cardiff University, Cardiff.

There is an ever-increasing understanding of the role of cis-regulatory modules in regulation of gene expression, however in some cases, for example testis-specifically expressed genes, the proximal promoter region alone is absolutely critical for determining tissue specificity. Bioinformatics has failed to identify simple sequence motifs that explain testis-specific expression despite the finding that most testis-specifically expressed promoters are short and rely on the same transcriptional complex, tMAC, for their expression.

de novo genes are derived from ancestral non-coding sequences, and typically show strongly testis-biased expression. Sequence variation in cis-acting regulatory regions presumably generates sites that are recognised by testis-specific transcriptional regulators, such as tMAC. The causative mutations in *de novo* gene evolution can identified by comparing naturally expressing and non-expressing alleles of not-yet-fixed *de novo* genes. Promoters of expressed and non-expressed alleles differ by the presence of SNPs and/or indels.

25/34 segregating *de novo* genes tested required tMAC for expression, while expression of 9/34 was independent of tMAC. We selected one gene from each set (090 and 074 respectively) for functional analysis of the promoters using LacZ expression reporters.

Expression of 090 evolved once; expressing alleles have two deletions and 7 SNPs in their proximal promoter regions compared to non-expressing alleles. Reporter constructs showed that a 7bp deletion just upstream of the TSS is necessary and sufficient to convert a natural non-expressing allele into an expressing allele. Further constructs indicate the deletion does not create a transcription factor binding site per se, rather it alters the distance between two flanking sites.

Expression of 074 has evolved independently at least twice. Two expressing alleles do not share any SNPs or indels not also found in at least one non-expressing allele, yet both are able to drive expression of a reporter gene. The promoter of a non-expressed allele is not able to drive reporter expression. Intriguingly, for both 090 and 074, the reporter mRNA is translationally repressed, and only translated in late spermatids, reminiscent of many more ancient testis-specific transcripts.

Our functional analysis shows that *de novo* genes can acquire expression in testes via several distinct mechanisms, complicating analysis by pure bioinformatics approaches.

227 Do chromatin changes underlie *de novo* gene origin? *L.K. Blair*, Artyom Kopp Evolution and Ecology, UC Davis, Davis, CA.

It is now clear that some genes evolve de novo from non-genic DNA sequence, yet little is known about the mechanisms by which they originate. Eukaryotic gene expression requires functional regulatory sequence with open, accessible chromatin. As such, one hypothesis for *de novo gene* origin is that they preferentially evolve in locations where chromatin is ancestrally permissive (i.e. is somewhat open), since this could require fewer changes to transition from non-expressed sequence to an expressed gene. However, it may also be possible for new “frontier” transcription factor binding sites to evolve near *de novo* gene sequences, and these binding sites both open local chromatin and facilitate transcription. To distinguish between these possibilities, I am using assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq) to compare chromatin states between *Drosophila melanogaster* strains polymorphic for *de novo* gene expression and closely-related species in which the orthologous DNA sequences are not expressed. A signal

of open chromatin exclusive to *D. melanogaster* lineages that express a *de novo* gene would indicate the gain of a pioneer transcription factor binding site. Alternatively, open chromatin across all strains and/or closely-related species is consistent with enhancer changes in regions of ancestrally permissive chromatin, or with the cooption of an ancestral enhancer followed by additional changes. These comparisons will help clarify the underlying regulatory mechanisms of *de novo* gene origin and identify their regulatory sequences.

228 Minimal effects of proto-Y chromosomes on house fly gene expression in spite of evidence that selection maintains stable polygenic sex determination. *J. Son*[1], T. Kohlbrenner[2], S. Heinze[2], L. Beukeboom[3], D. Bopp[2], R. Meisel[1] 1) Department of Biology and Biochemistry, University of Houston, Houston, TX, USA; 2) Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland; 3) Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, Netherland.

Sex determination is the developmental process by which organismal sex is established. Sex determination evolves fast, often due to changes in the master regulators at the top of the pathway. In addition, some species are polymorphic for multiple different master regulators within natural populations. Understanding the forces that maintain this polygenic sex determination can be informative of the factors that drive the evolution of sex determination. The house fly, Musca domestica, is a well-suited model to those ends because natural populations harbor male-determining loci on each of the six chromosomes and a bi-allelic female-determiner. Multiple lines of evidence suggest that natural selection maintains polygenic sex determination in house fly. However, previous work found that there are very few sequence differences between proto-Y chromosomes and their homologous X chromosomes. This suggests that there is not much genetic variation upon which natural selection could act to maintain polygenic sex determination in house fly. To address this paradox, we performed RNA-seq experiments that examine the effects of the two most common proto-Y chromosomes on gene expression. We find that the proto-Y chromosomes do indeed have a minor effect on gene expression, as expected based on the minimal X-Y sequence differences. Our results suggest that, if natural selection maintains polygenic sex determination in house fly, the phenotypic differences under selection are minor and possibly depend on ecological contexts that were not tested in our experimental design.

229 Spermatogenesis expression analysis of *Drosophila*'s Y-linked genes. *C.A. Mendonca*[1], C.C. Avelino[1], G.N.R. Goldstein[1], A.B. Carvalho[2], M.D. Vibranovski[1] 1) University of São Paulo, São Paulo, São Paulo, BR; 2) Federal University of Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, BR.

Despite the sequencing of *Drosophila melanogaster* genome, little is known about Y-linked genes. However, it is well established that some of them are essential for male fertility given that X0 males are viable but infertile. So far, six fertility regions and twelve single-copy genes have been identified on the Y chromosome. For most of them, function remains a mystery as they have been inferred based on domains' similarities. Unlike mammals, *Drosophila*'s Y-linked genes were originated from gene duplications from autosomes. The expression comparison between Y-linked genes from *D. melanogaster* and an autosomal paralog can reveal important consequences of these different genomic contexts. The present work aims at: (1) determining the expression pattern of the Y-linked genes of *D. melanogaster* along spermatogenesis; (2) contribute to the elucidation of their functions based on their expression patterns. In a broader context, it is expected to contribute to the comprehension of the evolution of new genes as they are usually biased expressed in the testes. In *D. melanogaster*, spermatogenesis is spatially and temporally distributed along the testes what enables the separation of mitotic, meiotic and post-meiotic portions, each of them with the predominance of certain cell types. For each phase of spermatogenesis, at least three replicates were made. RNA was extracted and sequenced with Illumina HiSeq 2000 technology. Reads were aligned to a reference genome by Burrows-Wheeler Aligner 0.7.12 and normalized with eXpress 1.5.1. Our results suggest that ten Y-linked genes have the same expression pattern: increase from mitosis to meiosis. One of the exceptions to this pattern is *FDY*, whose expression decreases gradually along spermatogenesis. Since *FDY* is the youngest Y-linked gene originated in the *D. melanogaster* branch, our data reveal the role of the Y-chromosome genomic context on driving the evolution of gene expression along spermatogenesis.

230 Off again, on again: The complex relationships between transposon insertions, piRNA silencing and flanking gene expression. *K. Van Vaerenberghe*[1], D. Miller[2], A. Li[1], A. Erwin[1], C. Cummings[1], E. Grantham[1], K. Hall[2], A. Perera[2], W. Gilliland[3], J. Blumenstiel[1] 1) Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS; 2) Stowers Institute for Medical Research, Kansas City, MO; 3) College of Science and Health, DePaul University, Chicago, IL.

piRNA-mediated silencing functions as a critical mechanism of genome defense against transposable elements. However, as with any immune system, there are costs associated with failure to distinguish host from parasite. In the case of piRNA silencing, this can come in the form of "genomic autoimmunity" since genes that flank transposon insertions can become off-targets of piRNA-mediated silencing. This is presumably because mechanisms of piRNA biogenesis can spread into neighboring genes. However, it is not clear why some transposon insertions have the capacity to mediate silencing of flanking genes, whereas others do not. To investigate this, we have studied three different cases where gene silencing is associated with off-target piRNA biogenesis. In one case, the gene *cdi*, flanking the telomere in *Drosophila virilis*, has been entirely converted into a piRNA cluster. The silencing state for *cdi* also has the capacity to paramutate non-silenced alleles, as long as maternal piRNA provisioning is maintained. In a second case, a *Ulysses* transposon insertion, in the upstream portion of the gene *oysgedart,* triggers silencing and piRNA biogenesis, but only from the sense strand in the germline. Here, there is no signature of piRNA cluster identity and silencing is only maintained in *cis*. In a third case, we identify a *Doc* insertion that triggers piRNA biogenesis and the silencing of multiple flanking genes. In this case, both sense and anti-sense genic piRNAs are observed, but silencing is entirely in *cis* and no maternal effects are evident. Strikingly, in the latter case, we have identified a revertant allele that restores gene expression. The reversion is caused by a *hobo* insertion that nucleates piRNA biogenesis in regions flanking the *hobo* insertion site, but relieves local gene repression by the original *Doc* insertion. These three cases suggest that there is a fine balance that determines whether or not a gene will be silenced, via piRNAs, by flanking insertions. Moreover, the effects of paramutation are limited. Overall, this supports a model for the evolution of piRNA silencing that must carefully balance the needs of genome defense and also limit the effects of genomic autoimmunity.

231 A meta-analysis suggests distinct genetic adaptation mechanisms underlying clinal and seasonal adaptation in *D. melanogaster*. *Y. Yu*, A. Bergland Biology, University of Virginia, Charlottesville, VA.

Populations of short-lived organisms such as *Drosophila melanogaster* locally adapt to spatially and temporally varying selection pressures. Although similar complex fitness-related traits underlie local adaptation across space and time, we still lack an understanding of the genetic architecture of clinal and seasonal adaptation. We hypothesize that the genetic architecture of clinal and seasonal adaptation will be different, due to different patterns of co-variation between distinct selection pressures across latitudinal (spatial) and seasonal (temporal) gradients. To test this hypothesis, we performed a meta-analysis examining allele frequency dynamics through space and time at previously identified eQTL loci. First, we show that eQTLs are enriched genome-wide among clinal but not seasonal SNPs. Second, we tested if eQTLs associated with genes that are highly expressed in certain tissues are enriched for clinal or seasonal SNPs, and found that different tissues are enriched with clinal or seasonal eQTLs. Third, by assessing the phenotypic effects of clinal and seasonal eQTLs and genes, we show that clinal and seasonal adaptation likely follow distinct models. On the one hand, clinal adaptation is in line with an omnigenic model, which emphasizes a hierarchical grouping of the genetic architecture (including core genes with large effects and peripheral genes) in adaptation processes. On the other hand, seasonal adaptation is more likely to follow an infinitesimal model, which suggests that many genes with similar effects contribute to local adaptation. The dissimilar enrichment patterns observed from SNP to tissue level suggest different genetic and physiological basis between clinal and seasonal adaptation. The

distinction of adaptive models highlights the importance of studying clinal and seasonal adaptation by hierarchical grouping of genetic and physiological basis into different tissue or physiology orientated biological contexts. Such distinction can advance our understanding of how fitness related genetic diversity is maintained across space and time.

232 Adaptive evolution and function of uORFs in *Drosophila*. *Jian Lu* School of Life Sciences, Peking University, Beijing, CN.

Upstream open reading frames (uORFs) play important roles in regulating the main coding DNA sequences (CDSs) via translational repression. Despite their prevalence in the genomes, uORFs are overall discriminated against by natural selection. However, it remains unclear why in the genomes there are so many uORFs more conserved than expected under the assumption of neutral evolution. Here, we generated genome-wide maps of translational efficiency (TE) at the codon level throughout the life cycle of *Drosophila melanogaster*. We identified 35,735 uORFs that were expressed, and 32,224 (90.2%) of them showed evidence of ribosome occupancy during *Drosophila* development. The ribosome occupancy of uORFs is determined by genomic features, such as optimized sequence contexts around their start codons, a shorter distance to CDSs, and higher coding potentials. Our population genomic analysis suggests the segregating mutations that create or disrupt uORFs are overall deleterious in *D. melanogaster*. However, we found for the first time that many (68.3% of) newly fixed uORFs that are associated with ribosomes in *D. melanogaster* are driven by positive Darwinian selection. Our findings also suggest that uORFs play a vital role in controlling the translational program in *Drosophila*. Moreover, we found that many uORFs are transcribed or translated in a developmental stage-, sex-, or tissue-specific manner, suggesting that selective transcription or translation of uORFs could potentially modulate the TE of the downstream CDSs during *Drosophila* development.

Reference:

Zhang H#, Dou SQ#, He F, Luo JJ, Wei LP, and Lu J* (2018). Genome-wide maps of ribosomal occupancy provide insights into adaptive evolution and regulatory roles of uORFs during *Drosophila* development. *PLOS Biology.* 16(7):e2003903. doi: 10.1371/journal.pbio.2003903.

233 Adaptation of A-to-I RNA editing in *Drosophila*. Yuange Duan, Shengqian Dou, Shiqi Luo, Hong Zhang, *Jian Lu* School of Life Sciences, Peking University, Bejing, CN.

Adenosine-to-inosine (A-to-I) editing is hypothesized to facilitate adaptive evolution by expanding proteomic diversity through an epigenetic approach. However, it is challenging to provide evidences to support this hypothesis at the whole editome level. In this study, we systematically characterized 2,114 A-to-I RNA editing sites in female and male brains of *D. melanogaster*, and nearly half of these sites had events evolutionarily conserved across *Drosophila* species. We detected strong signatures of positive selection on the nonsynonymous editing sites in *Drosophila* brains, and the beneficial editing sites were significantly enriched in genes related to chemical and electrical neurotransmission. The signal of adaptation was even more pronounced for the editing sites located in X chromosome or for those commonly observed across *Drosophila* species. We identified a set of gene candidates (termed "PSEB" genes) that had nonsynonymous editing events favored by natural selection. We presented evidence that editing preferentially increased mutation sequence space of evolutionarily conserved genes, which supported the adaptive evolution hypothesis of editing. We found prevalent nonsynonymous editing sites that were favored by natural selection in female and male adults from five strains of *D. melanogaster*. We showed that temperature played a more important role than gender effect in shaping the editing levels, although the effect of temperature is relatively weaker compared to that of species effect. We also explored the relevant factors that shape the selective patterns of the global editomes. Altogether we demonstrated that abundant nonsynonymous editing sites in *Drosophila* brains were adaptive and maintained by natural selection during evolution. Our results shed new light on the evolutionary principles and functional consequences of RNA editing.

Reference: Duan Y, Dou S, Luo S, Zhang H, **Lu J*** (2017). Adaptation of A-to-I RNA editing in Drosophila. ***PLoS Genetics***. 13(3):e1006648.

234 Drosophila species as a model system to study the response to high sugar content diet. *Nestor Octavio Nazario-Yepiz*[1], Alejandra Perez-Leaños[1], Pablo Luis Hernandez-Cervantes[1], Eunice Perez-Zaragosa[1], Therese Ann Markow[1,2] 1) Unidad de Genomica Avanzada-LANGEBIO, CINVESTAV, Irapuato, Guanajuato, México; 2) Section of Cell and Developmental Biology, Division of Biological Sciences, University of California-San Diego, La Jolla CA, USA.

We utilized three ecologically diverse *Drosophila* species as a model system to explore the influence of ecological adaptation on fitness and transcriptional response to isocaloric artificial diets differing in their index of protein-sugar. *D. melanogaster* is a cosmopolitan species that breeds in decaying fruit and resembles to individuals exposed to a Western diet in humans (higher in sugar and low in protein). *D. mojavensis* uses a natural diet which is much lower in carbohydrates, and the sister species *Drosophila arizonae* is cactophilic but has been isolated utilizing rotting citrus (higher in sugars than cactus). We measured fitness parameters and transcriptomic response when the three species were shortly exposed as 3rd instar larva to isocaloric diets: diet high in protein relative to sugar (HPLS), diet with equal amounts of protein and sugar (EPS) and diet low in protein but high in sugar (LPHS). Our data showed that the more specialized flies changed the expression of many genes, when comparing the higher-sugar to the lower-sugar diets. Importantly, the genes that changed in these species are principally related to metabolic functions: carbohydrates, lipids and proteins. Furthermore, we will show how the development time and the dry-weight were affected in these species when they reared in the diets during many generations. We suggest that adaptation of these three species together provide a model to examine differences in vulnerability to lifestyle-associated health problems such as metabolic syndrome and diabetes.

235 Genomic dissection of natural variation in resistance to copper poisoning. *E. Everman*, K. Cloud-Richardson, S. Macdonald Molecular Biosciences, University of Kansas, Lawrence, KS.

Metals have complex effects on organism physiology and function. Some metals are required for normal development and homeostasis, and deficiencies can result in disease, while metal poisoning poses risks of neurological and acute organ injury. Natural populations harbor genetic variation that influences resistance to metal poisoning, and toxicity is a genetically complex trait. Genomewide dissection of this natural variation can lead to the identification of genetic factors that contribute to metal resistance, and provide insight into the genes and pathways that regulate metal response. Here, we treat copper as a model metal of interest due to its critical requirement for normal cell function, and similarities between copper metabolism and that of other essential and nonessential metals. Using quantitative trait locus (QTL) mapping in the Drosophila Synthetic Population Resource (DSPR), we identify several candidate genes that are associated with variation in copper resistance. In addition to many novel candidates, several candidate genes have known copper-specific functions, or are involved in the detoxification (Catsup), transport (Zip42C.2), or homeostasis (Ccs) of metal ions. These candidate genes will be validated using RNAi knockdown. Using the multiparental structure of the DSPR, we also present strong evidence that multiple alleles are likely present at the major QTL, and we show that the genomic architecture of copper resistance varies between DSPR mapping populations. Variation in gene expression between high and low copper resistance DSPR strains will be further investigated using midgut- and head-specific RNA sequencing of individuals following exposure to copper. In addition to our work with the DSPR, we examine variation in copper resistance using flies from a natural population and demonstrate that average copper resistance in this population is much higher than in the DSPR. Pollution via metal-containing industrial wastes and leaching of metals into ground water presents a pervasive threat to environmental and human health; our study provides valuable insight into the genomic architecture of metal poisoning susceptibility, and provides candidates for future functional and mechanistic validation.

236 The genetic basis of exploration tendency in a multiparent population of *Drosophila melanogaster*. *Z. Elkins*, L. Storks, A. Rahman, E. King Division of Biological Sciences, University of Missouri, Columbia, MO.

The ability of animals to move throughout their environment to find food, mates, and suitable habitat is critical to their survival and reproduction. However, this behavior can be energetically expensive and potentially costly. As a result, individuals often vary widely in their overall motility, exploration, and dispersal tendency. We used a pool-seq approach using a multiparent population to uncover the genetic basis of exploration tendency in Drosophila melanogaster (the Drosophila Synthetic Population Resource, DSPR).
Our measurement of exploration tendency was the tendency of female fruit flies to move from a starting chamber to a novel fly chamber. We first demonstrated that our measure of exploration tendency has a genetic basis by assaying 20 recombinant inbred lines to estimate the broad-sense heritability of exploration tendency (H2=0.4). To identify the source of this genetic variability, we generated 17 pairs of "high exploration" and "low exploration" pools consisting of 40 - 100 female flies and performed whole genome sequencing. We compared allele frequency and haplotype frequency differences between these pools and identified over 20 regions of the genome suggested in exploration tendency.

237 Genomewide Expression Analysis of the Adult Female Gut in the *Drosophila* Synthetic Population Resource. *S.J. Macdonald*, A.C. Majane Department of Molecular Biosciences, University of Kansas, Lawrence, KS.

Animals are routinely exposed to toxins in their environment; many specialist herbivores exploit dietary resources containing toxic secondary metabolites, agricultural pests commonly encounter insecticides, and humans and other animals deliberately or inadvertently consume an array of xenobiotic compounds throughout their lives, from pharmaceutical drugs to heavy metal-contaminated water. Additionally, there is abundant evidence that populations harbor significant genetic variation for xenobiotic metabolism, leading to among-individual variation in the response to xenobiotics. Since the gut is the primary site of xenobiotic detoxification, in this study we examined gut-specific gene expression variation across a series of distinct strains of *Drosophila melanogaster* to explore the landscape of regulatory variation in this tissue. We dissected adult female gut tissue from the founders of the *Drosophila* Synthetic Population Resource (DSPR), a multi-parental mapping population, using 3-5 biological replicates per genotype. Following RNA extraction we generated standard Illumina paired-end mRNAseq libraries, collected an average of 19 million read pairs per sample, conducted SNP-aware read alignment to the reference genome using HISAT2, and counted reads mapping to genes using featureCounts. DESeq2 revealed 7,721 genes showed significant expression variation across founders. This gene set includes 76 cytochrome P450 genes, the majority of the members of this gene family in the *D. melanogaster* genome. Notably, we saw significant founder-to-founder variation in the gut expression levels of *Cyp12d1* and *Ugt86Dd*, genes we have previously implicated in the control of resistance to caffeine and nicotine, respectively. In addition, we sought to map eQTL (expression Quantitative Trait Loci) contributing to regulatory variation by collecting mRNAseq data for >100 DSPR RILs (Recombinant Inbred Lines), and we will describe our efforts to localize large-effect, cis-regulatory eQTL contributing to gene expression variation in the DSPR. Given our interest in the genetic basis of xenobiotic resistance in flies, and the likelihood that some large fraction of this variation is regulatory in origin, our data will provide a useful resource with which to more precisely resolve the genetic and mechanistic basis of xenobiotic metabolism and resistance.

238 Does *fruitless* affect mate discrimination by *Drosophila sechellia* females against *D. melanogaster* males? *M. Tomaru* Department of Drosophila Genomics and Genetic Resources, Kyoto Institute of Technology, Kyoto, Japan.

Females of *Drosophila sechellia* rarely accept *D. melanogaster* males, whereas *D. melanogaster* females accept *D. sechellia* males fairly well. The discrimination by *D. sechellia* females against *D. melanogaster* males seems to be (partially) recessive, since hybrid females accept *D. melanogaster* males more than *D. sechellia* females do. By using a third chromosome DrosDel deficiency kit, we found that several chromosomal regions in *D. sechellia* are candidates involving female discrimination factors. Hybrid females hemizygous for one of such chromosomal regions copulated with D. melanogaster males at 10% or less, while successful copulation was observed at about 40% in the control crosses. One of the candidate deficiencies is *Df(3R)ED2* (91A5:91F1) that defects *fruitless* which is the master control gene of male courtship behavior. To examine whether *fruitless* affects mate discrimination by *D. sechellia* against *D. melanogaster*, two insertion alleles of *fruitless* (*NP0021* and *5-HA-1629*) were used to obtain hybrid females from the cross between *fruitless* females and *D. sechellia* males. Then, we observed the courtship behavior of a *fruitless/sechellia* hybrid female and a wild-type *D. melanogaster* male. The hybrid females possessing one of the *fruitless* alleles copulated well with *D. melanogaster* males, suggesting that these two alleles do not affect female mate discrimination by *D. sechellia* against *D. melanogaster* males.

239 Tri-hybrid cross identifies new hybrid incompatibility loci between *D. melanogaster* and *D. sechellia*. *Jacob Cooper*, Nitin Phadnis School of Biological Sciences, University of Utah, Salt Lake City, UT.

Hybrid incompatibilities are the result of deleterious interactions between diverged genes in the progeny of two species. Finding hybrid incompatibility genes is a central goal of evolutionary genetics, as their identity reveals critical information about the mechanisms of how species stay isolated from one another. In *Drosophila*, the best characterized hybrid incompatibility is the hybrid male lethality that occurs from crossing *D. melanogaster* females to *D. simulans* males. Hybrid males die due the lethal interaction of at least three genes: *Hybrid male rescue* from *D. melanogaster*, *Lethal hybrid rescue*from *D. simulans* (*Lhr* ^{sim}), and *GST-containing FLYWCH Zinc-Finger Protein* from *D. simulans* (*gfzf* ^{sim}). Loss of the any of these species specific alleles rescues hybrid males. While we were surveying the patterns of hybrid male rescue by depletion of *gfzf* ^{sim} in species closely related to *D. simulans*, we found we were unable to rescue hybrid males in crosses with *D. melanogaster* and *D. sechellia*. *D. sechellia* is a close relative of *D. simulans*, and all previous work indicates that the genetic architecture of its hybrid incompatibility with *D. melanogaster* should be similar to that of *D. simulans* and *D. melanogaster*. Our results indicate that *D. sechellia*'s resistance to hybrid male rescue may contain clues to the evolution of this hybrid incompatibility.

To uncover the genetic basis of *D. sechellia* resistance to hybrid male rescue with *D. melanogaster*, we designed a tri-hybridization cross between the three species. We tested recombinant chromosomes from *D. simulans* and *D. sechellia* for their ability to rescue hybrid males with *D. melanogaster* with depletion of *gfzf* ^{sim}. Using whole genome sequencing, we measured the genetic contribution of each species to the viable hybrid males recovered from the cross. We find that the genetic architecture of *D. sechellia*'s resistance to hybrid male rescue is surprisingly simple, mapping to just two major effect loci. One of these loci is a previously identified hybrid incompatibility gene (*Lhr* ^{sim}), while the other represents a previously unidentified interactor in this hybrid incompatibility. Our results indicate that this new locus may represent a fourth hybrid incompatibility gene required hybrid male lethality between *D. melanogaster* and *D. simulans.* I will discuss the implications of our findings on the continued evolution of hybrid incompatibilities after speciation events, and the potential to identify systems of interacting genes by mapping interspecies variation.

240 Partial behavioral isolation between a DDT-resistant population of *Drosophila melanogaster* and its unselected control population, and their mating potential with other wild type populations. *P.T. Barnes*, P. Winn, M. Argueta, L. Drayson Biology Department, Connecticut College, New London, CT.

Although a DDT-resistant strain of *Drosophila melanogaster* (91R) and its unselected control (91C) were both derived from the same foundation population

obtained from nature in 1952, recent studies have shown significant behavioral isolation between these two populations. Three hypotheses can be proposed for how genetic changes could have occurred to cause the observed behavioral isolation between these two strains: Behavioral and genetic changes occurred (1) in both strains simultaneously, 2) primarily in the 91C strain, or 3) primarily in the 91R strain. The 91C and 91R strains were separately compared to five laboratory wild type "tester" strains by multiple-choice, simultaneous mass-matings. These tester strains have been in the lab, ranging from as old as approximately 1925, up to as recently as 2010. Random mating was consistently observed between 91C and all of the tester strains. However, 91R showed significant behavioral isolation (within-group preferences) with three of the five tester strains. These results tentatively support Hypothesis 3: that behavioral genetic changes have occurred primarily in 91R. Analyses of the mating propensities of females and males, in all the various combinations of stocks, show a variety of significant differences between stocks. However, none of these differences can explain significant within-group preferences. These behavioral and genetic changes may be associated with the intense selection that occurred for resistance to DDT through epistasis, pleiotropy, and/or linkage. In addition, random genetic changes may have occurred during bottlenecks of the 91R stock that happened during the artificial selection regime.

241 Genetic dissection of X-linked hybrid male sterility between *Drosophila simulans* and *D. mauritiana*. *R.A. Villegas*, N. Weldon, G. Mavhezha, C.D. Meiklejohn University of Nebraska-Lincoln, Lincoln, NE.

Intrinsic postzygotic isolation, the inviability or sterility of hybrids due to genetic incompatibilities, is one of the many forms of reproductive barriers leading to speciation. In species with sex chromosomes, hybrid incompatibilities accumulate preferentially on the sex chromosomes and if hybridization results in the sterility of only one sex, it is the heterogametic sex (Haldane's Rule). The underlying evolutionary forces driving these patterns are still debated, but to address this problem it is essential to identify the genes that cause these hybrid incompatibilities. To initiate genetic analysis of hybrid male sterility (HMS), we introgressed a 4-Mb segment of the *Drosophila mauritiana*X chromosome into a *D. simulans*genetic background, resulting in complete male sterility. Through recombination-based genetic mapping, we demonstrate evidence for at least 3 HMS regions within this 4-Mb segment. We identify the proximal-most HMS factor as *Odysseus*(*OdsH*), which was previously discovered as an HMS factor between these two species. In a *D. simulans* genetic background, the *D. mauritiana* allele of *OdsH* (*OdsHmau*) is sufficient to cause complete male sterility. Multiple hypotheses have been proposed regarding the mechanism of *OdsH*-mediated sterility. The *D. simulans* Y chromosome has been implicated in interactions with *OdsHmau*that could cause sterility. We find genetic evidence to support this hypothesis: in an otherwise *D. simulans* genome, the *D. mauritiana*Y chromosome rescues fertility of males carrying *OdsHmau*. A second, non-mutually exclusive, hypothesis proposes that the *D. mauritiana*allele of *OdsH* is misregulated in a *D. simulans* genetic background and this misregulation plays a role in the HMS phenotype. To investigate this hypothesis, we will compare both transcript and protein abundances of OdsH between fertile and sterile introgression genotypes, as well as within pure *D. simulans*and *D. mauritiana*males.

242 Examining the molecular mechanisms of hybrid incompatibilities. *Sarah Gross*, Jacob Cooper, Nitin Phadnis School of Biological Sciences, University of Utah, Salt Lake City, UT.

A fundamental component in our understanding of speciation is a thorough grasp of the genetic and molecular basis of deleterious genetic interactions known as hybrid incompatibilities. While several hybrid incompatibility genes have been identified so far, we still understand very little about the molecular events that underlie incompatible genetic interactions. One of the most well known examples of this phenomena is the inter-species cross between *D. melanogaster* and its closest sister species *D. simulans,* which results in inviable hybrid F1 males. A single hybrid incompatibility between at least three genes, Hmr, Lhr and gfzf is essential for this lethality. The molecular underpinnings of this incompatibility, however, remain unclear. To understand the precise role of gfzf in this hybrid incompatibility, and to uncover the functional effects of species-specific alleles, I am developing a simplified CRISPR-based approach to precisely manipulate the gfzf locus in *D. simulans*. This approach opens the door to precise manipulations and application of cutting-edge tools such as allele swaps, ancestral reconstructions and a variety of epitope tags. Our approach is highly modular, and promises to be easily transferable to other non-model systems where CRISPR-based manipulations are otherwise tedious and inconsistent. Here, I present results from an implementation of our simplified method in Drosophila, and results from recent developments in understanding the molecular basis of hybrid incompatibilities.

243 A dynamic repertoire of male meiotic actin-related proteins arose in the *Obscura* group. C. Schroeder[1], *J. Valenzuela*[1], H. Malik[1,2] 1) Division of Basic Sciences; 2) Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, Seattle, WA.

Actin is an evolutionarily ancient cytoskeletal protein that is critical for many architectural and signaling roles. Before the last common ancestor of eukaryotes, the actin gene duplicated and diversified into the superfamily of actin-related proteins (Arps). Arps, which share the conserved actin fold, specialized early in eukaryotic evolution for varying cellular roles in the cytoplasm and the nucleus. Despite its strict conservation across phyla, the Arp superfamily exhibits rare diversification in *Drosophila* through the expansion of lineage-specific functional paralogs. Using a phylogenomic approach, we discovered four independent Arp gene duplications, which originated in the common ancestor of the *Obscura* group in *Drosophila*. The duplicates exhibit genetic turnover within the *Obscura* group and several are under positive selection. Notable differences in surface residues exist between the duplicates and their parental Arps, suggesting a divergence from canonical protein interactions found among the parental Arps. Intriguingly, these unexpected variants are largely expressed in the male germline, whereas canonical Arps are ubiquitously expressed in a wide array of different tissues. To ascertain the function of these divergent Arps, we generated *D. pseudoobscura* transgenics expressing the duplicates with a GFP tag to localize them in the testis. The duplicates exhibit varying temporal expression throughout sperm development, specifically during meiosis and spermatid elongation. Some localize to distinct structures, including actin cones, which separate bundled spermatids into individual mature sperm. Our findings indicate a surprising dynamic invention of *Drosophila* male-specific Arp paralogs, which may shed light on the evolution and biology of sperm development.

244 Sexual selection rewires reproductive protein networks. *T. Karr*[1], S. Snook[2], H. Southern[3], T. Gossmann[3] 1) School of Life Sciences, Arizona State University, Tempe, AZ, USA; 2) Department of Zoology, Stockholm University, Stockholm, Sweden; 3) Department of Animal and Plant Sciences, University of Sheffield, Sheffield, United Kingdom.

Polyandry drives postcopulatory sexual selection (PCSS), resulting in rapid evolution of male ejaculate traits. Critical to male and female fitness, the ejaculate is known to contain rapidly evolving seminal fluid proteins (SFPs) produced by specialized male secretory accessory glands. The evidence that rapid evolution of some SFPs is driven by PCSS, however, is indirect, based on either plastic responses to changes in the sexual selection environment or correlative macroevolutionary patterns. Moreover, such studies focus on SFPs that represent but a small component of the accessory gland proteome. Neither how SFPs function with other reproductive proteins, nor how PCSS influences the underlying secretory tissue adaptations and content of the accessory gland, has been addressed at the level of the proteome. Here we directly test the hypothesis that PCSS results in rapid evolution of the entire male accessory gland proteome and protein networks by taking a system-level approach, combining divergent experimental evolution of PCSS in *Drosophila pseudoobscura (Dpse)*, high resolution mass spectrometry (MS) and proteomic discovery, bioinformatics and population genetic analyses. We demonstrate that PCSS influences the abundance of over 200 accessory gland proteins, including SFPs. A small but significant number of these proteins display molecular signatures of positive selection. Divergent PCSS also results in fundamental and remarkably compartmentalized evolution of accessory gland protein networks in which males subjected to strong PCSS invest in protein networks that serve to increase protein production whereas males subjected to relaxed PCSS alters protein networks involved in protein

surveillance and quality. These results directly demonstrate that PCSS is a key evolutionary driver that shapes not only individual reproductive proteins, but rewires entire reproductive protein networks.

245 Fitness consequences of long sperm and sperm storage organs of *Drosophila melanogaster*. Susi Zajitschek, Felix Zajitschek, *Halli Weiner*, Mollie Manier Biological Sciences, George Washington University, Washington, DC.

Drosophila males produce some of the longest sperm known, up to 5.8 cm in *D. bifurca*, 20 times the length of the fly. In the female reproductive tract, these sperm are stored within the seminal receptacle (SR), a long, coiled sperm storage organ. Sperm length and SR length are coevolving across the *Drosophila* phylogeny, presumably driven by a Fisherian runaway process mediated by a long-sperm advantage in long SRs and a genetic correlation between these two traits. In long-sperm species, sperm are metabolically costly, producing trade-offs between sperm size and sperm number and between sperm size and development time. However, costs associated with long SRs are not well understood. In this study, we investigate costs and benefits associated with intraspecific variation in sperm length and SR length in *D. melanogaster*. We measured male and female fitness in inbred lines derived from four populations previously selected for long sperm, short sperm, long SRs, or short SRs. Fitness measures included reproductive success (males and females), longevity (males and females), and mating success (males). We found no evidence for fitness tradeoffs for males or females of sperm or SR selection lines, nor did we find evidence for sexual conflict. Both males and females from long-sperm populations had higher fitness, as measured by longevity and reproductive success, respectively. Both males and females had similar fitness between long-SR and short-SR lines, suggesting SR length does not confer a fitness advantage or cost based on the metrics used here. Long-sperm males did not have a mating advantage over short-sperm males, but males from long SR lines were both more attractive (shorter mating latency) and copulated for longer. This result suggests sons of long-SR females may enjoy a fitness benefit under a "choosy daughter" scenario. If female choice is beneficial, females should select for males that could produce choosy daughters. Although previous work found a genetic correlation between sperm length and SR length, we believe a "sexy sperm" hypothesis would not apply here, since sperm length was not significantly different between the long- and short-SR lines.

246 Discerning the historical and genetic relationship between the *Drosophila* germline stem cell gene *bag of marbles* and the bacteria *Wolbachia*. *M. Wenzel*, C.F. Aquadro Molecular Biology and Genetics, Cornell University, Ithaca, NY.

The *Drosophila* protein coding gene *bag of marbles* (*bam*) plays a key role in early male and female gametogenesis by regulating the differentiation of germline stem cells (GSCs). Although the regulation of GSC gene function is essential for reproduction, *bam* shows strong and episodic bursts of protein sequence diversification in several lineages of *Drosophila*. Two hypotheses for the rapid evolution of *bam* include (1) diversifying function of *bam* across the *Drosophila* genus and (2) selective pressure on *bam* from the endosymbiont bacteria *Wolbachia.* Here we are exploring the latter hypothesis based on observations that *Wolbachia* partially rescues the reduced fertility of a *bam* hypomorph mutant in female *D. melanogaster*. One approach we are taking to evaluate the role of *Wolbachia* as an evolutionary driver of *bam* is through investigating how *Wolbachia* functionally interacts with *bam*. The original hypomorph (bam-z) is defined by a nonsynonymous mutation in the region where Bam binds to its key partner Bgcn. We have created a second putative hypomorph in a different functional region of Bam, and are in the process of generating additional hypomorphs through a targeted CRISPR approach and a mutagenesis screen. By testing for a *Wolbachia* interaction in a variety of distinct hypomorphs, along with additional binding assays, we will be able to better understand the nature of the *Wolbachia* rescue of the *bam* hypomorph phenotype. A second approach we are taking to evaluate the hypothesis that *Wolbachia* is an evolutionary driver of *bam* is through investigating the historical interaction between *Wolbachia* and *Drosophila. Wolbachia* can undergo lateral gene transfer (LGT) during host infections, resulting in pieces of its genome becoming integrated into the host's genome. The computational detection of such "genomic fossils" can allow us to identify the tempo and mode of historical *Wolbachia* infections among Drosophila species. Initial analyses of whole-genome sequencing trace files for *D. melanogaster* has revealed several previously-unreported candidate LGTs. We are now extending this approach to additional species to gain a more comprehensive picture of the history of Wolbachia infections across the genus.

247 Determining how *Drosophila* Exprimental Evolution Affects and is Affected by Associated Microbes. Robert Courville [1], *Yasamin Heydary*[1], Samira Sadoughi[1], John Chaston[2], Parvin Shahrestani [1] 1) Biology Department, California State University, Fullerton, Fullerton, CA; 2) Department of Plant & Wildlife Sciences, Brigham Young University, Provo, UT 84602.

The interaction between the microbiome and host, and the effects it has on physiological characteristics of the host, such as longevity, have recently been of high interest. However, the effect of host evolution for longevity differentiation on the microbiome composition has not been investigated, yet. We aim to investigate with two research questions: (1) Do *Drosophila melanogaster* populations that have been experimentally evolved for divergence in longevity differ in their associated microbiome composition and abundance? (2) How does manipulations of the *D. melanogaster* microbiome affect the host rate of evolution? We first analyzed metagenome data from 10 short-lived and 10 long-lived populations of *D. melanogaster* for differences in microbial DNA using Metaphlan. Populations with longer life spans had microbiomes dominated with the phylum *Proteobacteria* whereas the populations with shorter life spans have microbiomes dominated with the phylum *Firmicutes*. This contradicts previous research, in which introduction of *Proteobacteria* to fly microbiomes made them short-lived. We investigated abundance of bacteria from 5 of the short-lived and 5 of the long-lived populations by homogenizing whole flies, plating them on mMRS medium and quantifying the CFU's after incubation. The results supported the metagenomics data, as short-lived populations had higher abundances of *Firmicutes* bacteria than long-lived populations. To further investigate bacterial load differences between the genotypes, we dechorionated a generation of offspring from each of the 10 populations described above and applied two treatments through inoculation: exclusively one *Acetobacter* species and exclusively one *Lactobacillus* species. Between the two methods, we surprisingly found that bacterial load in long-lived flies is 5-6 times lower than short-lived flies. This ratio is even more surprising when accounting for body size, as long-lived flies are almost double in size in comparison to the short-lived flies. A genotype specific restriction of the microbial community is possible, but further research is needed. Second, we will evolve short-lived populations from long-lived populations by selecting for accelerated development and applying four microbial treatments: (1) no microbiome change (2) only *Proteobacteria* present (3) only *Firmicutes* present (4) axenic. Rate of change in host lifespan will be measured, to analyze if microbial conditions have any influence on experimental evolution.

248 The genetic evolution of reproductively isolating male pheromone preference in *Drosophila simulans* and *sechellia*. *M.P. Shahandeh*, T.L. Turner Ecology, Evolution and Marine Biology, University of California, Santa Barbara, Santa Barbara, CA.

Michael P. Shahandeh and Thomas L. Turner

Differences in mating behaviors are a common prezygotic mechanism preventing mating between species. In *Drosophila*, pheromones act as important species-specific signals that prevent costly hybridization. In *D. melanogaster* and *D. sechellia*, females express the primary pheromone 7,11-heptacosadiene (7,11-HD); *D. simulans* females express a different primary pheromone, 7-Tricosene (7-T). *D. melanogaster* and *D. sechellia* males readily court females with 7,11-HD. But for *D. simulans*, 7,11-HD is aversive and suppresses courtship behavior. Where *D. simulans* and *D. sechellia* co-occur, male pheromone preference is the primary mechanism preventing hybridization, accounting for ~71% of the total gene flow restricted between them (Shahandeh et al., 2018). While recent discoveries in neuroscience are advancing our understanding of the molecular basis of this shift in behavior (Clowney et al., 2015; Seeholzer et al., 2018), little is known about the causal genetic changes. To fill this gap in knowledge, we have harnessed the difference in pheromone preference between *D.*

simulans and *D. sechellia*, because they can be coerced to mate and form fertile hybrid offspring in the lab. We created and phenotyped a backcross population of over 400 individuals in an aim to identify the genetic basis of male pheromone response. Using whole genome sequencing in a QTL mapping approach, we attribute a majority of the difference (~66%) in pheromone preference behavior to a single region, suggesting that behavioral isolation may be attributed to a small genomic region with large effect. However, using an engineered set of introgression strains, we show that the effect of this single region is divided among at least three, non-additive loci with different effects on male courtship behavior. We will present our most recent efforts to fine-map these regions to causal genes. Ultimately, we aim to provide an in-depth study of the genetic mechanisms underlying the evolution of a reproductively isolating behavior—a necessary goal of behavioral research, so that we may uncover general patterns in the types of changes underlying evolutionary shifts in behaviors that isolate species.

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249 Accurate, ultra-low coverage genome reconstruction and association studies in Hybrid Swarm mapping populations. *C.A. Weller*, P.A. Erickson, A.O. Bergland Biology, University of Virginia, Charlottesville, VA.

Genetic association mapping studies seeking to uncover the link between genotype and phenotype are often facilitated by panels of sequenced inbred lines, e.g. the *Drosophila* Genetic Reference Panel (DGRP) or *Drosophila* Synthetic Population Resource (DSPR). While inbred reference panels provide a valuable, replicable source of genetic variation, they may not accurately represent naturally-occurring patterns of heterozygosity and nucleotide diversity. To address this problem, we evaluated a mapping population design, which we call the Hybrid Swarm, where dozens to hundreds of inbred lines are randomly crossed for few (e.g. five) generations. The Hybrid Swarm approach has likely remained underutilized due to the difficulty of genome-wide genotyping compared to pre-sequenced inbred lines. To reduce sequencing costs and make the Hybrid Swarm approach feasible, we developed a computational pipeline that can reconstruct whole genomes from ultra-low-coverage (0.05X) sequence data in Hybrid Swarm populations founded by an arbitrarily large number of sequenced inbred founders. Reconstructions of Hybrid Swarm genomes were highly (99.9%) accurate whether genetic variation was drawn from haplotypes in the DGRP or from haplotypes generated by coalescent models across a range of genetic diversity values. We then conducted a power analysis to test the performance of GWAS using the Hybrid Swarm as compared to inbred lines, recombinant inbred lines, and highly outbred populations. To acquire a large sample size of simulated GWAS, we developed a flexible forward-in-time simulator and association test pipeline that stores population genotypes in a highly compressed haplotype block format, reducing computational requirements by two orders of magnitude relative to alternative methods. We report the relative power for different mapping population designs across a range of allele frequencies and effect sizes for tens of thousands of simulated GWAS, with the Hybrid Swarm performing at a rate comparable to highly outbred (F_{50}) populations. Taken together, our work provides a computationally efficient framework for GWAS power analysis and demonstrates the feasibility of the Hybrid Swarm as a cost-effective method of fine-scale genetic mapping.

250 Genetic basis of variation in high sugar induced diabetic-like traits in *Drosophila*. *X. Zhuang*[1,4], F. Morgante[2,4], M. Ludwig[1], S. Park[2], Y. Li[2], M. Stephens[3], G. Bell [2], M. Kreitman [1] 1) Department of Ecology and Evolution, University of Chicago, Chicago, IL; 2) Department of Medicine, University of Chicago, Chicago, IL; 3) Department of Human Genetics, University of Chicago, Chicago, IL; 4) co-first authors.

Drosophila is a well-established model for investigating quantitative traits, and it provides powerful tools for dissecting the contributions of both genes and environment on metabolism. Previous studies revealed that a high sugar diet (HSD) has profound effects on cellular physiology and metabolism in *Drosophila*, including insulin resistance, lipid metabolism, impaired lifespan, and reduced body size. We developed a fly model with diabetic-like traits induced by HSD, and examined HSD induced phenotypes in larvae and in adults across a subset of *Drosophila* Genetic Reference Panel (DGRP). Flies under HSD display an increase in whole body glucose and glycogen level, a decrease in developmental rate, survivorship, body weight, and longevity, compared with flies under low sugar diet (LSD). The examined DGRP lines display a continuous and wide range of these phenotypes and large broad-sense heritability, suggesting great potential for quantitative trait loci (QTL) mapping. To this end, we used one of the HSD induced traits, namely developmental delay, to perform a bulk segregant mapping analysis in advanced intercross populations we developed. This mapping resource consists of 64 DGRP lines, which have been combined into 16 highly recombinant synthetic populations, each of which is founded by 8 of the inbred lines. Fly embryos collected from these populations were reared on HSD and LSD. Flies with extreme phenotypes of developmental rate were then individually barcoded and their genomes were sequenced. Allele frequencies of the extreme phenotypes under each treatment were compared and used to identify single nucleotide polymorphisms (SNPs) associated with the trait. The design of multi-parental advanced intercross populations should greatly increase the power to detect associated variants and provide a greater opportunity for rare variants to be present and for common variants to be represented in the synthetic population, allowing mapping of both rare and common functional variants. We inferred the underlying mosaic founder structure for each sequenced fly using a hidden Markov model (HMM) that takes into account read depth. This allowed us to impute each fly's genotype. Preliminary genome-wide association studies (GWAS) with mixed liner model show SNPs with suggestive association with variation in high sugar induced developmental delay, but also imply a prevalence of small-effect QTLs.

251 *tartan* underlies the evolution of male genital morphology. J.F.D. Hagen[1], C.C. Mendes[1,2], K.M. Tanaka[1,3], P. Gaspar[1], M. Kittelmann[1], *A.P. McGregor*[1], M.D.S. Nunes[1] 1) Oxford Brookes University, Oxford, GB; 2) University of Oxford, Oxford GB; 3) Tokyo Metropolitan University, Tokyo, Japan.

One of the main aims of evolutionary biology is to identify the genetic basis of phenotypic differences between species. The morphology of male external genitalia evolves rapidly between species presumably driven by sexual selection. For example, the anal plates, claspers and posterior lobes exhibit striking differences in size, shape and bristle number and morphology between the males of *Drosophila simulans* clade species, which last shared a common ancestor as recently as 240,000 years ago. However, little is known about the genes and developmental changes underlying this divergence. To address this, we have carried out high-resolution introgression mapping of genes underlying differences in clasper morphology between *D. mauritiana* and *D. simulans*. We identified a region of 177 kb containing only 9 genes that explains approximately 16% of the difference in clasper size between these two species. Expression and functional analyses of these positional candidates revealed that *tartan*, which encodes a transmembrane leucine-rich repeat protein involved in cell affinity, is required for the regulation of clasper development. Moreover, analysis of reciprocal hemizyotes that are genetically identical, except for which species the functional allele of *trn* is from, determined that the *trn* allele of *D. mauritiana* specifies larger claspers than the allele of *D. simulans*. Therefore, we have identified a new gene underlying clasper development and one of the first loci underlying the evolution of genital diversification among species.

252 A mosaic of independent innovations involving eyes shut are critical for the evolutionary transition from fused to open rhabdoms. *A. Zelhof*[1], S. Mahato[1], J. Nie[1], D. Plachetzki[2] 1) Dept Biology, Indiana University, Bloomington, IN; 2) MCBS, University of New Hampshire, Durham, NH.

A fundamental question in evolutionary biology is how developmental processes are modified to produce

morphological innovations while abiding by functional constraints. Here we address this question by investigating the cellular mechanism responsible for the transition between fused and open rhabdoms in ommatidia of apposition compound eyes; a critical step required for the development of visual systems based on neural superposition. Utilizing Drosophila and Tribolium as representatives of fused and open rhabdom morphology in holometabolous insects respectively, we identified three changes required for this innovation to occur. First, the expression pattern of the extracellular matrix protein Eyes Shut (EYS) was co-opted and expanded from mechanosensory neurons to photoreceptor cells in taxa with open rhabdoms. Second, EYS homologs obtained a novel extension of the amino terminus leading to the internalization of a cleaved signal sequence. This amino terminus extension does not interfere with cleavage or function in mechanosensory neurons, but it does permit specific targeting of the EYS protein to the apical photoreceptor membrane. Finally, a specific interaction evolved between EYS and a subset of Prominin homologs that is required for the development of open, but not fused, rhabdoms. Together, our findings portray a case study wherein the evolution of a set of molecular novelties has precipitated the origin of an adaptive photoreceptor cell arrangement.

253 Two recently evolved PDZ domain proteins have diverging functions in stabilizing muscle myofibrils. N. González-Morales, T.W. Marsh, O. Marescal, Y.S. Xiao, *F. Schoeck* Department of Biology, McGill University, Montreal, Quebec, Canada.

Alp/Enigma family members have a unique PDZ domain followed by zero to four LIM domains and are essential for myofibril assembly across all species analyzed so far. *Drosophila melanogaster* has three Alp/Enigma family members, Zasp52, Zasp66, and Zasp67. We show that *Zasp66* and *Zasp67* are gene duplications of *Zasp52* restricted to insects. While Zasp66 has a conserved domain structure across orthologs, Zasp67 domains and lengths are highly variable, indicating it is a recent duplication quickly evolving in different directions in different species. In flies, Zasp67 is expressed exclusively in indirect flight muscles, where it colocalizes with Zasp52 at Z-discs. We generated a CRISPR null mutant of *Zasp67*, which is viable but flightless, without other defects. We can rescue all phenotypes by re-expressing a *Zasp67* transgene at endogenous levels. *Zasp67* mutants show extended and broken Z-discs, indicating that the protein helps stabilize the highly regular myofibrils of indirect flight muscles. In contrast, a *Zasp66* CRISPR null mutant has limited viability, but only mild indirect flight muscle defects illustrating the diverging evolutionary paths these two paralogous genes have taken since they arose by duplication.

254 Rapid evolution of a transcription factor essential for development in *Drosophila*. *B. Kasinathan*[1,2], J. M. Young[2], H. S. Malik[2,3] 1) Medical Scientist Training Program, University of Washington School of Medicine, Seattle WA; 2) Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 3) Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, Seattle, WA.

Evolutionarily young, essential genes violate the dogma that essential genes are preserved over long periods of evolutionary time. We identified an evolutionarily young gene, *Nick Nack (nnk)* in *D. melanogaster* that is essential for larval development. *Nick Nack* is a ZAD-containing transcription factor characterized by a conserved Zinc Finger Associated Domain (ZAD) at the N-terminus and five tandem C_2H_2 zinc finger domains at the C-terminus. ZAD-containing transcription factors are the largest class of transcription factors in arthropods and a majority are lineage-specific factors. We find that since *Nick Nack* first arose 30 million years ago, it underwent multiple independent gains and losses in the *Sophophora* subgenus. In addition, *Nick Nack* shows evidence for recent adaptive evolution between *D. melanogaster* and *D. simulans.* Strikingly, *nnk* null *D. melanogaster* embryos arrest at the second instar (L2) larval stage. In contrast to essential ZAD-containing transcription factors that mediate larval progression by regulating biosynthesis of the molting hormone ecdysone, *nnk* null larvae cannot be rescued with ecdysone. Instead, we find that *nnk* null L2 larvae are unable to initiate the L2 larval transcriptional program. Our findings highlight turnover and rapid evolution of a transcription factor essential for development in *Drosophila.* Our ongoing work seeks to uncover the functional consequences of this rapid evolution on developmental gene regulatory networks and genome architecture.

255 A novel gene specifies species-specific variation in a sexually-selected trait. T.C. Buckman, A.R. Harper, *J.P. Masly* Department of Biology, University of Oklahoma, Norman, OK.

External reproductive structures display one of the more striking morphological differences observed among closely-related species, and this variation in morphology is typically the result of sexual selection and/or sexual conflict. However, little is known about how these two evolutionary forces shape variation at the molecular level to direct such widespread, rapid morphological change. The posterior lobes (PLs) of the genital arch— two recently evolved male genital structures found only among the four species of the *Drosophila melanogaster* species complex— provide a powerful model in which to study the genetic and developmental bases of rapid evolutionary change in a complex structure. The PLs display dramatic differences in size and shape among these species and this morphological variation is a consequence of sexual selection. Using a combination of recombination mapping and gene expression experiments, we identified a strong candidate gene for specifying PL size between *D. sechellia* and *D. mauritiana*. *CG14567* has no known function, although its sequence features and protein domains suggest that it might be a signaling peptide. Our results from expression knock-down experiments in *D. melanogaster* and *D. mauritiana* show that *CG14567* possesses a similar function in both species and appears to be a negative regulator of PL growth. Interestingly, this gene appears to have evolved from a somewhat recent gene duplication event, and its expression level, expression pattern, and expression timing during development have diverged rapidly among the four *D. melanogaster* complex species. This gene also possesses several amino acid substitutions among the species, although population genetic tests show no significant signal of positive natural selection at this locus. We are currently combining genome editing experiments with live-cell imaging approaches to identify how variation at *CG14567* gives rise to species-specific PL morphologies.

256 Natural variation in the maternal and zygotic mRNA complements of the early embryo in Drosophila melanogaster. *A.A. Feitzinger*, S.E. Lott Evolution and Ecology, University of California, Davis, Davis, CA.

Natural variation in the maternal and zygotic mRNA complements of the early embryo in Drosophila melanogaster

Anna A. Feitzinger, Susan E. Lott

Maternal gene products supplied to the egg during oogenesis drive the earliest events of development in all metazoans. After the initial stages of embryogenesis, maternal transcripts are degraded as zygotic transcription is activated, this is known as the maternal to zygotic transition (MZT). Altering the abundances of maternally deposited factors in the laboratory can have a dramatic effect on development, adult phenotypes and ultimately fitness. Zygotic transcription activation is a tightly regulated process, where the zygotic genome takes over control of development from the maternal genome, and is required for the viability of the organism. We asked how the maternal and zygotic pools of mRNA vary within and between natural populations of *D. melanogaster.* In order to maximize sampling of genetic diversity, African lines of *D. melanogaster* originating from Zambia as well as DGRP lines originating from North America were chosen for transcriptomic analysis. Single embryo RNA-seq was performed at a stage before and a stage after zygotic genome activation in order to determine which transcripts are maternally deposited and which are zygotically expressed within and between these populations. Differential gene expression analysis has been used to quantify quantitative changes in RNA levels within populations as well as fixed expression differences between populations at both stages. Furthermore, qualitative analysis reveals a number of dramatic on-off expression transitions within a species during this crucial period of development.

Generally, we find that maternal transcripts are more highly conserved, and zygotic transcripts evolve at a higher rate. We find that there is more within population variation in gene expression than between populations and that expression variation is highest post- MZT between African lines. Additionally, we find fixed differences in expression between African and Non-African populations. Determining the natural variation of gene expression surrounding the MZT in natural populations of *D. melanogaster* will give insight into the extent of how a tightly regulated process may vary within a species, the extent of developmental constraint at both stages and on both the maternal and zygotic genomes, and can reveal expression changes allowing this species to adapt as it spread across the world.

257 Identifying the genetic changes driving network co-option during the evolution of a novel body part. *G.R. Rice*, K. Charles-Obi, M. Rebeiz Biological Sciences, University of Pittsburgh, Pittsburgh, PA.

Understanding how body parts originate is one of the fundamental goals of developmental and evolutionary biology. *What genetic processes are involved in generating new anatomical parts?* One potential mechanism is the co-option of gene regulatory networks. Models of network co-option typically invoke the existence of an upstream factor whose ectopic expression is sufficient to initiate the activation of an entire network in a new tissue. However, despite substantial effort, *identification of upstream factors remains a significant hurdle*. Indeed, most characterized cases of co-option exist between distantly related taxa for which genetic manipulations and detailed analyses of *cis*-regulatory elements are difficult. **Here, we identify and validate an upstream network that was co-opted from a well characterized network to generate a novel morphological feature.**

The small hairs (trichomes) that adorn the body in *Drosophila* are an extensively studied trait for which much developmental and evolutionary information has been obtained. The *shavenbaby* gene is required for the formation of these structures, and mutations in its regulatory region have been implicated in multiple cases of trichome loss. However, instances of trichome gain have not been extensively investigated.

Across the animal kingdom, the most rapidly evolving morphologies occur in reproductive structures. Among *Drosophila* species, a wide variation of genital morphologies exist, including the phallus of *Drosophila eugracilis*, which is covered with over a hundred trichomes that have been implicated in wounding females during copulation. The homologous tissue in *Drosophila melanogaster* lacks trichomes, providing a convenient outgroup for comparisons of development and genetic manipulations. I have found that the presence of trichomes in *Drosophila eugracilis* is correlated with expanded expression of *shavenbaby* and its downstream targets, indicating that regulatory evolution at this locus underlies this dramatic phenotype. In further support of this hypothesis, I have discovered that ectopic expression of *shavenbaby* in the phallus of *Drosophila melanogaster* induces phallic spikes. These results indicate that gene network co-option has led to the gain of a novel trait.

258 Dissecting the shared and divergent genetic architectures of a novel male genital structure and a novel female genital structure. *E. W. McQueen*[1], W. J. Glassford[2], M. Rebeiz[1] 1) University of Pittsburgh, Pittsburgh, PA; 2) Columbia University, New York City, NY.

Investigating the genetic origination and evolution of novel morphologies is fundamental to our understanding of biological complexity. Animal genitalia are complex structures that evolve rapidly, and it is common to see species-level differences in both male and female genitalia. In many systems male and female genitalia are known to share some developmental programming, yet differ in final morphology and function. Comparing the developmental networks of male and female genital traits is therefore an opportunity to investigate how developmental networks deployed in rapidly evolving morphologies operate under the constraints of pleiotropy. However, the degree to which novel male and female genital traits are genetically independent has not been elucidated in any species. We investigated the genetic underpinnings of two genital structures in the *Drosophila melanogaster* subgroup. Males in this subgroup possess a novel genital outgrowth called the posterior lobe, which is a clasping structure. Females of this subgroup have a novel feature on their ovipositors called the oviscapt pouch, which may contact the posterior lobe during copulation. The posterior lobe and oviscapt pouch correlate in size across species, suggesting a genetic or functional association.

Previously, the gene network required for posterior lobe formation was found to have been co-opted from a larval structure. Using gene knockdown, *in-situ* hybridization, antibody staining, and enhancer analysis, we investigated whether this network is shared between the posterior lobe and the oviscapt pouch. We discovered that patterning genes from the posterior lobe network, and even their lobe enhancers, are also involved in the patterning of the oviscapt pouch during this structure's development. We next performed a quantitative trait locus analysis using two closely-related species in this group with divergent genital morphologies to identify shared and unique loci of diversification for the male and female structures. We found evidence that some loci associated with diversification across species may be shared between these two structures. Our discovery of pleiotropy in this system highlights the need to consider the role of genetic linkage in the evolution of novelty and structural complexity.

259 Changes in Phenotypic frequencies and the analysis of stress related traits of *Drosophila takahashii*: a study of seasonal acclimation. *S. RAMNIWAS*, D. Singh UCRD, Chandigarh University, Mohali, IN.

[Aim] Variation in coloration is frequently found in insects, but little is known about its functional value. *Drosophila takahashii* exhibits genetically coded discontinuous variation in abdominal melanisation. To determine whether physiological performance is likely to be affected by melanisation pattern, in this study we investigated the variation in abdominal melanisation and stress related traits among individuals belonging to three different color morphs, and tested the hypothesis that seasonal environmental conditions would enchance the corresponding seasonal phenotype. [Methods] *D. takahashii*flies were collected from altitudnal sites and Mendelian analysis of genetic crosses from true breeding dark and light color strains confirm the occurance of major locus, with dominance of D allele. Ecophysiological traits in populations as well as flies of three body color phenotypes were stretigically analysed. [Results] A significant increase in the frequency of dark allele was observed during the dry season and light flies occur in the wet season, which suggests that climatic selection plays a role. However, intermediate flies were abundant in both seasons. There were significant *F* values for increase in all traits of intermiate phenotypes ($p < 0.001$) due to acclimation but no such acclimation effects were observed in dark and light true breeding strains of *D. takahashii* ($p > 0.42$). [Conclusion] As per our hypothesis, significantly higher physiological tolerance was observed in dark morph under cool-dry stress conditions, and in light morph under hot-wet conditions, respectively, as determine by different traits. Interestingly, intermidate phenotypes showed higher capability to acclimation to both conditions. Further, we found seasonal changes in temperature and humidity to confirm selection pressures on stress related traits.

260 Behavioral and morphological evolution of pest activity in *Drosophila suzukii.* *S. Durkin*, L. Zhao Zhao Lab, The Rockefeller University, New York, NY.

Evolutionarily novel phenotypes, both morphological and behavioral, can give species the opportunity to occupy a new ecological niche. Understanding how and when these phenotypes arise is a fundamental question in evolutionary biology. Here we aim to answer this question by studying the egg-laying behavior of the invasive pest, *Drosophila suzukii.* In contrast to most *Drosophila* species, *D. suzukii* lays its eggs in ripe, as opposed to rotten fruit. This shift is thought to be partly attributable to an enlarged, serrated ovipositor. To understand the relationship between egg-laying behavior and ovipositor morphology, we compared the oviposition preference of four species that represent a range of ovipositor sizes. We found that ovipositor size and ripe fruit preference are generally positively correlated, with *D. melanogaster* having a small ovipositor and preference for rotten fruit, and *D. suzukii* having a large ovipositor and preference for ripe fruit. Unexpectedly, although *D. subpulchrella* has an enlarged ovipositor and is able to oviposit in ripe fruit, they do not prefer to and display an intermediate oviposition preference similar to that of *D. biarmipes.* Our results suggest that the evolution of ripe fruit preference and ovipositor size

is asynchronous along the phylogeny leading to *D. suzukii*. Therefore, the emergence of morphological innovations may have predated behavioral innovations in this species group. With the addition of our ongoing gene regulatory and genomic analyses we will be able to pinpoint not only when the traits associated with *D. suzukii's* pest activity emerged, but how they are reflected at the molecular level.

261 Convergent evolution of dopaminergic gene expression underlying an adaptive trait. *R. Tarnopol*[1], P. Wittkopp[1,2] 1) Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI; 2) Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI.

Recent work in *Drosophila sechellia* has revealed a key role for dopamine biosynthesis genes in mediating adaptive evolution to the toxic host *Morinda citrifolia*. However, the extent to which genes associated with dopamine metabolism are responsible for conferring the octanoic acid (OA) resistance phenotype has not been rigorously studied. Here, the role dopamine biosynthesis pathway genes *ple*, *ddc*, and *catsup* play in conferring the OA-resistance phenotype were identified in a recently characterized OA-resistant population of *D. yakuba*, *D. yakuba mayottensis*. Using qPCR, transcriptional regulation of each of these genes was examined to determine whether OA-resistant populations of *Drosophila* have distinct mRNA profiles for these genes relative to non-OA-resistant populations. The UAS-Gal4 system was used to functionally test the roles of *ple*, *ddc*, *catsup*, and *l(1)sc*, a gene responsible for dopaminergic neuron fate, in conferring the OA-resistance phenotype in non-OA-resistant *D. melanogaster*.

262 Evaluating functional conservation of the rapidly evolving germline stem cell genes, *bam* and *bgcn* . *J. Bubnell*, C. Aquadro Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Although germline stem cell maintenance and differentiation are conserved processes across the *Drosophila* genus, many of the genes involved in these pathways show lineage specific patterns of adaptive evolution. Two of these genes, *bag of marbles* (*bam*) and *benign gonial cell neoplasm* (*bgcn*) partner to initiate gametogenesis by promoting differentiation of the germline stem cell. Despite their role in stem cell regulation and their necessity for female and male fertility, both genes show episodic bursts of amino acid divergence in several *Drosophila* lineages. We're currently testing the hypothesis that *bam* and *bgcn* have acquired their role in germline stem cell differentiation and are now under positive selection in these species. We are using CRISPR-Cas9 to generate *bam* and *bgcn* null alleles in diverse *Drosophila* species and then evaluating germline stem cell function. We have found that *bam*'s core role in gametogenesis is conserved in lineages showing signals of adaptive evolution, *D.melanogaster*, *D. simulans*, and *D. yakuba*, as well as in *D. ananassae* where we have not observed a signal of adaptive evolution at *bam*. These results suggest that changes in *bam*'s function as a stem cell differentiation factor is not driving its adaptive evolution. We are also currently generating *bgcn* null alleles in these species for similar analyses. If the functions of *bam* and *bgcn* are conserved across these species, then the episodic signal of positive selection we observe implies adaptation to other factors present in these lineages. A prime candidate that we are evaluating is the endosymbiotic bacteria *Wolbachia*, that transiently infects *Drosophila*, manipulates reproduction, and genetically interacts with *bam* in *D. melanogaster*.

263 Fine-scale temporal sampling shows evidence of cryptic population structure in a single *Drosophila melanogaster* population. *A.S. Bangerter*, A.O. Bergland Biology, University of Virginia, Charlottesville, VA.

Drosophila melanogaster is an ideal study system for investigating patterns of adaptation and population dynamics in the wild. Temperate populations of *D. melanogaster* show both phenotypic and genetic adaptation to temporally varying selection from season to season. Clinal variation of both phenotype and genotype in temperate populations of *D. melanogaster* are also well characterized. However, coarse-grain temporal and spatial analyses of phenotype and genotype do not always give a full picture of the fine-scale demographic and adaptive dynamics that contribute to the evolution within a population. Fine-scale temporal and spatial structure in *D. melanogaster* populations have not been well addressed with direct genetic evidence. To acquire fine-scale temporal sampling of a wild population, we collected bi-weekly samples of wild *D. melanogaster* from a fruit orchard in central Virginia that start in mid-June and end in early December in order to capture a full seasonal cycle. We performed whole genome sequencing of individual F1 males from 8-17 wild caught females from each sampling time point. This dataset is novel, both in the fine- scale temporal sampling as well as being able to investigate changes in genotypes. Deviations from Hardy-Weinberg equilibrium, kinship patterns within and across time points, and patterns of allele frequency shifts in known seasonally varying SNPs show signals compatible with population structure within time points and through time. Our results suggest our focal orchard population is not entirely panmictic, but rather exhibits both fine-scale spatial and seasonal change in population structure. Understanding this fine-scale structure in a single population will help us answer more broad questions of how populations adapt in response to seasonal change.

264 Comparative transcriptomics provides insights into reticulate and adaptive evolution of *Heliconius* butterflies. *W. Zhang*[1], B. Leon-Ricardo[2], B. Schooten[2], S. Belleghem[2], B. Counterman[3], O. McMillan[4], M. Kronforst[5], R. Papa[2] 1) State Key Laboratory of Protein and Plant Gene Research, Peking-Tsinghua Center for Life Sciences, and School of Life Sciences, Peking University, Beijing, China; 2) Department of Biology, University of Puerto Rico, Rio Piedras, San Juan PR, USA; 3) Department of Biological Sciences, Mississippi State University, MS, USA; 4) Smithsonian Tropical Research Institute, Gamboa, Panama; 5) Department of Ecology and Evolution, University of Chicago, Chicago, IL, USA.

Butterfly eyes are complex organs composed of a diverse group of cell types with interplay between photo pigments and other proteins. They play a central role in visual signaling and are ecologically important in speciation and adaptation. Yet most research on butterfly vision has focused mainly on the photoreceptor cells disregarding the thousands of other genes that characterize the eye compound. We here utilized the whole eye transcriptome to obtain a more holistic view of the evolution of the butterfly eye, as well as a portrait of speciation driven by ancient hybridization.

We investigated signatures of adaptation in the eye transcriptome of an ecologically, behaviorally and morphologically diverse group of Neotropical butterflies. We sequenced and assembled transcriptomes from adult female eyes of eight species representing all major clades of the *Heliconius* genus and an additional outgroup species, *Dryas iulia*. We identified 4042 orthologous genes shared across all transcriptome datasets, and constructed a transcriptome-wide phylogeny, which revealed topological discordance with the mitochondrial phylogenetic tree. The phylogenetic discordance suggests genome-wide introgression among *H. sara*, *H. hortense* and *H. erato.* We then estimated this introgression pattern using additional genome data and proposed an ancient hybridization leading to speciation before the divergence of *H. hortense* and *H. clysonymus*. We also used this new topology to estimate the Ka/Ks ratio for each orthologous cluster and performed further tests for putative candidates using the branch-site model and GA-branch method, separately. Our analysis suggested a greater number of genes subject to positive selection in younger evolutionary lineages. Furthermore, we characterized patterns of expression for a subset of these positively selected orthologs using qRT-PCR. The qRT-PCR results showed changes in expression patterns between species, tissues and sexes, indicating that such faster evolving genes could also be under gene expression divergence and thus regulatory evolution.

We investigated evolutionary changes in the whole eye transcriptome across the morphologically and behaviorally diverse group of *Heliconius* butterflies. We identified candidate eye genes that show signatures of adaptive molecular evolution and provide evidence of their expression divergence between species, tissues and sexes. Our results demonstrate greater evolutionary changes in younger lineages, suggesting a sensory tissue specific selective pressure underlying speciation. Besides, our data suggest an ancient hybridization leading to speciation among focal *Heliconius* species.

265 Effects of genotype by diet interaction on quantitative traits in *Drosophila melanogaster*. *D. Kang*, W. Huang Department of Animal Science, Michigan State University, East Lansing, MI.

Genotype by environment interaction can contribute significantly to phenotypic variation in genetically diverse populations. For example, diet is an important environmental factor that can induce plastic responses for many phenotypes in flies. However, the extent to which the dietary responses depend on the genetic backgrounds of the individuals, a form of genotype by environment interaction, and its genetic basis, remain less understood. Such genotype by diet interaction shares analogy with observations in the human population where the risks of metabolic disorders conferred by different diets apparently change among ethnic groups and among individuals. However, the effects of diets and genetic backgrounds are difficult to separate in humans. The fly model provides a unique design in which genotypes can be replicated and subject to different dietary treatments, such that the effects of genotypes, diets, and genotype by diet interaction can be disentangled. In this study, we used 10 genetically diverse inbred strains from the fully sequenced and deeply phenotyped *Drosophila melanogaster* Genetic Reference Panel (DGRP), to quantify the effects of genotype by diet (standard, high sugar, high protein) interaction on several quantitative traits, including fertility, body weight, resistance to starvation, and developmental time. We found statistically significant effects of genotype by diet interaction that contributed the majority of quantitative trait variation. By further profiling the expression of genes in embryos, brain, and fat body, this study will provide useful insights into the contribution of genotype by diet interaction to quantitative trait variation and the underlying variation in gene expression.

266 Sex-specific influences of the microbiota on *Drosophila melanogaster* life history traits. *R. Hughes*[1], T. Call[1], C. Walker[1], P. Schmidt[2], J. Chaston[1] 1) Plant and Wildlife Sciences, Brigham Young University, Provo, UT; 2) University of Pennsylvania, Philadelphia, PA.

Associated microorganisms ('microbiota') engage in complex interactions with their hosts that influence animal behaviors and adaptive traits. Here we focus on how the microbiota influence life history traits in *Drosophila melanogaster*. *D. melanogaster* is an established model for understanding how organisms adopt different life history strategies in different geographies. For example, *D. melanogaster* in the eastern United States are locally adapted across a latitudinal cline; low latitude populations invest in early reproduction ('fast') whereas high latitude populations favor somatic maintenance ('slow'). We previously showed that the *Drosophila* microbiota, which is dominated by lactic acid (LAB) and acetic acid (AAB) bacteria, also varies with latitude; and that species from the different bacterial orders can dictate the fast-slow strategy of female wild *D. melanogaster.* We hypothesized that manipulating the microbial communities of low latitude and high latitude flies using isolated strains might influence those the flies' phenotypes in a sex-specific manner. To test this hypothesis, we reared *D. melanogaster* either bacteria-free or with different microbial communities that mimic latitude-specific microbiome swaps. We then measured the starvation resistance and lifespan of both male and female flies; and the fecundity of female flies in each treatment. Consistent with our past findings, female *D. melanogaster* starvation resistance was heavily influenced by microbial composition, indicated by microbial influence masking the impact of host genotype on the locally adapted life history strategy. Conversely, host genotype influenced the starvation resistance of male flies more strongly than the microbiota, such that different fly genotypes reared with different microbial communities did not display overlapping phenotypes. Our analysis of female fecundity and of male and female lifespan provide additional context for these findings. Taken together, our data identify a strong sex-dependent effect of the microbiota on life history traits and life history strategy of wild *D. melanogaster.* We discuss these findings within the context of previously demonstrated sex-dependent responses of *Drosophila* (e.g. transcription) to the microbiota.

267 Genotype-by-temperature interactions maintain polygenic sex determination in the housefly. *K. Adhikari*, R. Meisel Department of Biology and Biochemistry, University of Houston, Houston, TX.

Sex determination (SD) establishes sexually dimorphic developmental pathways, either based on genetic differences between males and females or environmental cues. SD systems vary across taxa because they are rapidly evolving. A single master regulatory locus is enough to determine sex in most organisms with genetic SD. However, some organisms have multiple master SD loci in their genome that segregate independently, resulting in polygenic SD. Polygenic SD is predicted to be an unstable intermediate between monogenic SD systems, and the factors responsible for maintaining polygenic SD are poorly understood. Housefly (*Musca domestica*) has a stable polygenic SD system with multiple male and female determiners segregating in natural populations. The male determining factor (*Mdmd*) is commonly found on the Y chromosome (Y^M) and the third chromosome (III^M). Y^M males are found in colder, northern latitudes whereas III^M males are found in southern, warmer latitudes. This suggests that selection operating on a genotype-by-temperature (GxT) interaction maintains this polymorphism. To test this hypothesis, we raised III^M and Y^M males at high and low temperatures, and we studied the resulting GxT effects on two phenotypes: gene expression and extreme temperature tolerance. Using RNA-seq, we identified 247 genes whose expression in testis and 50 genes whose expression in head depends on GxT interactions. We also found that III^M males raised at high temperature are the most tolerant to extreme heat and Y^M males raised at low temperature are the most cold tolerant, suggesting a GxT interaction that includes developmental acclimation. The direction of this GxT interaction is consistent with the III^M chromosome providing a fitness advantage at warmer temperatures and the Y^M chromosome increasing fitness at lower temperatures. Our results therefore support the hypothesis that GxT interactions maintain polygenic SD in housefly through temperature-dependent fitness effects of genetic variation on the Y^M and III^M chromosomes. Our RNA-seq data suggest that the GxT interactions could be mediated through effects on gene expression.

268 Pattern of heredity of carbohydrate, lipid, and protein contents in different nutritional environments. *A.M. Perinchery*, E.G King Biological Sciences, University of Missouri, Columbia, MO.

In order to survive in a changing environment, organisms need to adapt. An organism consumes and stores a finite amount of resources that are used for all daily tasks. These finite sources must be allocated to different life history traits like reproduction or somatic growth. We used a half sibling population derived from the DSPR, the Drosophila Synthetic Population Resource, to understand the pattern of heredity of carbohydrate, lipid, and protein content in different diets and nutrient allocation between reproduction and somatic tissue of Drosophila melanogaster.

We generated a half sibling population by mixing RILs from the DSPR for 5 generations and then set up a half-sibling family design. To generate a family we rotated a single male fly (sire) among three different dams every two days. This process was repeated with 25 males with a total of 75 females. The adult offspring of these crosses were placed onto one of three diets for 10 days: a high sugar, low yeast or control diet. For every family (offspring of one sire plus the three dams), the offspring of one sire-dam pairing were dissected into ovarian and somatic tissue so that energy budget measurements to be measured for reproductive tissue and somatic tissue separately, and could also be added together to get the energy budget components for the total fly body.

269 Epistasis and genotype-by-environment interaction have shared regulatory roles in the transcriptional response to hypoxic and dietary stress among mitonuclear genotypes in Drosophila. *D.M. Rand*, J.A Mossman Ecology & Evolutionary Biology, Brown Univ, Providence, RI.

Mitochondrial function requires the coordinated interaction of genes encoded by the mitochondrial and the nuclear genome. The functions of the mitochondria modulate this intergenomic communication to regulate environmental factors such as dietary input and oxygen tension. The shared role of the mitochondrial-nuclear genome in these functions raises the question of whether gene-by-gene interactions (G x G) that enable mitochondrial function are distinct from the gene-by-environment interactions (G x E) that fuel mitochondrial activity. We examine this question using a Drosophila model of mitonuclear interactions in which experimental combinations of mtDNA and nuclear chromosomes generate pairs of mitonuclear genotypes to test for epistatic interactions (G x G). These mitonuclear genotypes are then exposed to altered dietary or oxygen environments to test for G x E interactions. We use development time to assess dietary effects, and genome wide RNAseq analyses to assess hypoxic effects on transcription, which can be partitioned in to mito,

nuclear, and environmental (G x G x E) contributions to these complex traits. We find that mitonuclear epistasis is universal, and that dietary and hypoxic treatments alter the epistatic interactions. We further show that the transcriptional response to alternative mitonuclear interactions has significant overlap with the transcriptional response to alternative oxygen environments. Gene coexpression analyses suggest that these shared genes are more central in networks of gene interactions, implying some functional overlap between epistasis and genotype by environment interactions. These results are discussed in the context of evolutionary fitness, the genetic basis of complex traits, and the challenge of achieving precision in personalized medicine.

270 Interactions between the sexual identity of the nervous system and the social environment mediate lifespan in *Drosophila melanogaster*. Ewan Flintham[1], Tomoyo Yoshida[1], Sophie Smith[1], Hania Pavlou[2], Stephen Goodwin[2], Pau Carazo[3], *Stuart Wigby*[1] 1) Edward Grey Institute, Department of Zoology, University of Oxford, Oxford, UK; 2) Centre for Neural Circuits and Behaviour, University of Oxford, Oxford, UK; 3) Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Valencia, Spain.

Sex differences in lifespan are ubiquitous, but the underlying causal factors remain poorly understood. Inter- and intrasexual social interactions are well known to influence lifespan in many taxa, but it has proved challenging to separate the role of sex-specific behaviours from wider physiological differences between the sexes. To address this problem, we genetically manipulated the sexual identity of the nervous system—and hence sexual behaviour—in *Drosophila melanogaster*, and measured lifespan under varying social conditions. Consistent with previous studies, masculinization of the nervous system in females induced male-specific courtship behaviour and aggression, while nervous system feminization in males induced male–male courtship and reduced aggression. Control females outlived males, but masculinized female groups displayed male-like lifespans and male-like costs of group living. By varying the mixture of control and masculinized females within social groups, we show that male-specific behaviours are costly to recipients, even when received from females. However, consistent with recent findings, our data suggest the courtship expression to be surprisingly low cost. Overall, our study indicates that nervous system-mediated expression of sex-specific behaviour per se— independent of wider physiological differences between the sexes, or the receipt of aggression or courtship—plays a limited role in mediating sex differences in lifespan.

271 Distinct contributions of sperm and seminal proteins to male reproductive ageing in Drosophila. *I. Sepil*[1], R. Dean[2], J. Steinhauer[3], E. Bath[1], E. Sandham[1], H. Ostridge[1], B. Hopkins[1], N. Buehner[4], M. Wolfner[4], R. Konietzny[5], M-L Thézénas[5], P. D. Charles[5], R. Fischer[5], B. M. Kessler[5], S. Wigby[1] 1) Department of Zoology, University of Oxford, Oxford, GB; 2) Department of Genetics, Evolution and Environment, University College London, London, GB; 3) Yeshiva University, New York, NY; 4) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY; 5) TDI Mass Spectrometry Laboratory, Target Discovery Institute, Nuffield Department of Medicine, University of Oxford, Oxford, GB.

Age-related reproductive declines in males are widespread but generally subtler than for females, and the underlying biology is poorly understood. Ejaculate deterioration in ageing males might arise from changes in the seminal fluid proteins or sperm, yet their relative contributions are not known. Understanding the fundamental processes of male ejaculate ageing is a pressing issue given the strong trends for delayed fatherhood in humans. Here we use label-free quantitative proteomics and fluorescent sperm labelling techniques in the fruit fly, *Drosophila melanogaster*, to reveal the distinct contributions of sperm and seminal proteins to male ejaculate ageing. We reveal that sperm and seminal proteins show strikingly different responses to age and mating history. Sperm production declines with chronological age, reserves deplete under mating but we find no evidence of reduced viability. In contrast the abundance of seminal proteins is maintained, or accumulates in the absence of mating, however we find evidence of qualitative changes in old males, which contribute to reduced reproductive success. We also reveal that age-related reproductive decline can be delayed through a manipulation of the insulin-signalling pathway. Our data show that age-related male subfertility is multi-faceted, but that interventions that delay somatic ageing can potentially provide co-benefits to reproductive health.

272 A rapidly evolving actin-related protein dynamically localizes to critical meiotic structures in the testis. *C. Schroeder*[1], J. Valenzuela[1], H. Malik[1,2] 1) Division of Basic Sciences; 2) Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, Seattle, WA.

Many cytoskeletal proteins are evolutionarily ancient. Notably, the superfamily of actin-related proteins (Arps) originated prior to the last common ancestor of eukaryotes and is highly conserved for many fundamental biological processes, including cell division, motility and chromatin remodeling. Despite its strict conservation across phyla, the Arp superfamily surprisingly exhibits signatures of diversification in *Drosophila*: a unique Arp subfamily known as Arp53D is found in all species of *Drosophila* and is rapidly evolving (positive selection). Rapid evolution among members of otherwise conserved protein families has been shown in other systems to be the result of recurrent adaptation, likely spurred by molecular arms races between genetic elements with opposite evolutionary interests. Canonical Arps are ubiquitously expressed in all tissues, yet intriguingly Arp53D, which has no known function, exhibits testis-specific expression. We hypothesize, based on this rapid evolution and tissue-specific expression, that Arp53D may be engaged in a meiotic conflict in *Drosophila*. Gametic Arps also exist in mammals and like Arp53D, their functions are unknown. What is the function of a divergent gametic Arp? Here we find that Arp53D localizes to two critical germline-specific cytoskeletal structures. During meiosis, Arp53D localizes to the fusome, an organelle that connects all germ cells in a cyst. Following spermatid elongation, Arp53D localizes to actin cones, which separate syncytial spermatids into individual cells and push excess cytoplasm to the end of the sperm tail. Like all Arps, Arp53D contains the canonical actin fold domain but also contains an unusual N-terminal extension that we find is required for Arp53D's unique localization. Our data lend support to the idea that Arp53D is functioning at the interface of a meiotic intragenomic conflict. Using cytological and biochemical approaches, we are continuing to investigate Arp53D's molecular role in sperm development and the impact of Arp53D's positive selection on male fertility. These efforts will elucidate the driving force of Arp genetic innovation.

273 Sex-specific differences in desiccation resistance and the use of energy metabolites as osmolytes in *Drosophila melanogaster* flies acclimated to dehydration stress. *D. SINGH*, S. RAMNIWAS UCRD, Chandigarh University, Mohali, IN.

[Aim] In the Indian subcontinent, there are significant between-population variations in desiccation resistance in *Drosophila melanogaster*, but the physiological basis of adult acclimation responses to ecologically relevant humidity conditions is largely unknown.[Methods] *Drosophila* flies from midland locality—Solan of the western Himalayas were maintained at two different relative humidity conditions by using saturated solutions of phosphoric acid for 5 % RH; and sodium chloride for 50 % RH at 21 °C (Winston and Bates 1960) and all the desiccationrelated traits were measured in non-acclimated and acclimated groups of flies at 5 or 50 % RH. [Results] We tested the hypothesis that increased desiccation resistance in acclimated flies is associated with changes in cuticular permeability and/or content of energy metabolites that act as osmolytes. Under an ecologically relevant humidity regime (~50 % relative humidity), both sexes showed desiccation acclimation which persisted for 2–3 days. However, only females responded to acclimation at ~5 % relative humidity (RH). Acclimated flies exhibited no changes in the rate of water loss, which is consistent with a lack of plastic changes in cuticular traits (body melanization, epicuticular lipid). Therefore, changes in cuticular permeability are unlikely in drought-acclimated adult flies of *D. melanogaster*. In acclimated flies, we found sex differences in changes in the content of osmolytes (trehalose in females versus glycogen in males). These sex specific changes in osmolytes are rapid and reversible and match to corresponding changes in the increased desiccation resistance levels of acclimated flies. Further, the increased content of trehalose in females and glycogen in males support the bound-water hypothesis for water retention in acclimated flies. Thus, drought acclimation in adult flies of *D. melanogaster* involves inducible changes in osmolytes (trehalose and glycogen), while there is little support for changes in cuticular permeability.

[Conclusion] We conclude that sex-specific acclimation differences are associated with differences in the levels of osmolytes, trehalose in females and glycogen in males.

274 Estimating the timing of multiple admixture pulses during local ancestry inference. *Paloma Medina*[1], Bryan Thornlow[1], Rasmus Nielsen[2], Russ Corbett-Detig[1] 1) UC Santa Cruz, SANTA CRUZ, CA; 2) University of Copenhagen, Denmark.

Admixture, the mixing of genetically distinct populations, is increasingly recognized as a fundamental biological process. One major goal of admixture analyses is to estimate the timing of admixture events. Whereas most methods today can only detect the most recent admixture event, here, we present coalescent theory and associated software that can be used to estimate the timing of multiple admixture events in an admixed population. We extensively validate this approach and evaluate the conditions under which it can successfully distinguish one- from two-pulse admixture models. We apply our approach to real and simulated data of *Drosophila melanogaster* We find evidence of a single very recent pulse of cosmopolitan ancestry contributing to African populations, as well as evidence for more ancient admixture among genetically differentiated populations in sub-Saharan Africa. These results suggest our method can quantify complex admixture histories involving genetic material introduced by multiple discrete admixture pulses. The new method facilitates the exploration of admixture and its contribution to adaptation, ecological divergence, and speciation.

275 Environment, but not thermosensation, dictates *Drosophila melanogaster* cold hardening ability. *H. Stone*, P. Erickson, A. Bergland Department of Biology, University of Virginia, Charlottesville, VA.

From a bear's hibernation to a butterfly's journey southward, all organisms living in temperate climates must find a way to survive the lethal cold of the winter. *Drosophila melanogaster*'s strategy includes cold hardening, a process in which prior exposure to cool temperatures results in increased freeze tolerance. While physiological changes associated with cold hardening have been documented, various factors that prompt the process are not yet fully understood. We have tested the impact of thermosensation, seasonal changes, and the nutritional environment on cold hardening. Flies with mutations in genes necessary for thermosensation exhibited comparable cold hardening ability to thermosensory wild-type flies. Additionally, flies with amputated thermosensory organs showed similar cold hardening ability to sham-operated controls. Therefore, thermosensation is not required for cold hardening to occur. To determine the impact of seasonal changes on cold hardening, we tested the cold hardening ability of outbred flies living in outdoor cages. Our assays indicated that the influence of the environment prior to the precooling period persists after two weeks of precooling. These seasonal experiments suggest that warmer temperatures prior to precooling are detrimental to freeze tolerance, while cooler prior temperatures appear to be beneficial. However, we did not observe any transgenerational effects of seasonal environmental exposure, nor did we observe genetic changes in cold hardening ability during the summer and early fall. We hypothesize that late fall and early winter temperatures may prompt selection for increased cold hardening ability. Flies fed cornmeal-molasses-based fly food or rotting fruit exhibited differences in cold hardening, possibly implicating the microbiome or nutritional state as being influential for cold hardening. Our evidence suggests that environmental exposure, especially temperature, is a key factor in a fly's ability to cold harden, and the cold hardening process is not dependent on thermosensation.

276 Examination of meiotic drive on karyotype evolution in *Drosophila virilis* subgroup. *T. Miorin*, R. Rogers, N. Stewart Department of Bioinformatics, University of North Carolina at Charlotte, Charlotte, NC.

Many mechanisms affecting evolutionary forces among species are still unclear, despite the large amount of studies among the diversity that exists. If there are changes in the karyotype of these organisms within the species, alterations to important functions such as meiosis can occur. I have focused on this consequence in two subspecies of Drosophila – *D. americana* and *D. novamexicana*. My work has focused on looking at the polymorphic fusion of the X-4 chromosome in *D. americana*. This fusion is found along the centromere and has shown biased transmissions among the *D. americana* when heterozygous females were collected. Typically this biased segregation is shown in female meiosis, leading to meiotic drive as a force of evolution among the karyotypes. In *D. americana*, there are two centromeric fusions, one between the second and third, and the other along the X and fourth chromosome. *D. novamexicana* has not shown meiotic drive for either the fused or unfused X and fourth chromosomes, as they segregate at a 50/50 ratio.
To study this meiotic drive system further, I performed 10 generations of introgression and backcrosses using virgin females of *D. americana* and the males of *D. novamexicana*. Previous work done had shown that the offspring's embryos showed a bias among the X-4 fusion that leaned towards 57% fused, which supported the notion of meiotic drive. These crosses involved looking at adult offspring. I examined 550 offspring and, using PCR techniques, was able to determine if the offspring had the fused or unfused karyotype. These introgression lines showed the same ratio as before, with 57% containing the fused X-4 and 43% unfused X-4. This leads to the suggestion that meiotic drive is playing a force in the centromere causing the rearrangements of the metacentric chromosomes.

277 Testing the role of genes, within a conspecific sperm precedence locus, on sperm competition in *Drosophila*. *G. Grewal*, B. Morham, A. Civetta The University of Winnipeg, Winnipeg, Manitoba, CA.

Drosophila females can mate with different males setting the stage for postcopulatory sexual selection through sperm competition within the female reproductive tract. If the female mates with conspecific and herterospecific males, the majority of the progeny is sired by the conspecific male. This phenomenon is known as conspecific sperm precedence (CSP) and serves as a postmating prezygotic mechanism of reproductive isolation between species. Whether sexual selection can be a driver of reproductive isolation leading to speciation remains a controversial issue. The identification of genes that influence sperm competition within and between species can provide grounds to link sexual selection and speciation. A previous mapping study identified the 89B cytogenetic position as a likely hotspot for genes affecting CSP. Here, we focus on testing the role of candidate genes within the 89B location on intraspecific sperm competition (ISC). Using tissue expression data, we identified 14 candidate sperm competition genes within 89B. Each candidate gene was knocked down via RNAi using the Gal4-UAS system and sperm competitive ability assayed using transgenic *D. melanogaster* with GFP labelled sperm heads. Our preliminary data suggests that only three gene knockdowns were unable to displace resident GFP sperm in intraspecific competition. It remains to be seen whether these gene knockdowns are also unable to displace resident heterospecific sperm. The inability to displace heterospecific sperm would indicate a genetic association between intra- and interspecific sperm competition, providing a genetic link between sexual selection and CSP.

278 Activating mutations in FGFR btl leads to a competitive advantage in *Drosophila* germline stem cells. *K.H. Le*, L.J. Greenspan, E. Matunis Cell Biology, Johns Hopkins School of Medicine, Baltimore, MD.

The paternal age effect describes how older men are more likely to have children with genetic disorders than younger men. This is attributed to an age-related accumulation of sperm with spontaneous mutations that are thought to originate in germline (sperm-producing) stem cells (GSCs). Stem cells with advantageous mutations affecting intrinsic cellular properties including proliferation rate, survivability, or adhesive properties can out-compete other stem cells. However, competition between GSCs is still poorly understood.

Using the *Drosophila melanogaster* as a genetic model, we find that over-activating the breathless (btl) receptor tyrosine kinase, a homolog of the fibroblast growth factor receptor (FGFR) and a component of the MAP-Kinase (MAPK) signaling pathway, causes mutant GSCs out-compete their wild-type GSC neighbors,

suggesting that this mutation confers a competitive advantage. This suggests a relationship between GSC competition and the paternal age effect because the human homolog of btl is known to cause Apert's Syndrome, a paternal age effect congenital disorder. In contrast, activating mutations in other components of the MAPK pathway—Raf, Ras, and heartless (htl), another FGFR homolog—did not result in more mutant GSCs than non-mutants

To determine the mechanisms by which certain mutations are able to confer an advantage to GSCs, we performed experiments to further characterize btl. Using the LexA/LexAop system, we found that btl was expressed in both the germline and the soma. This is surprising because a lab member had previously shown that heartless (htl), a different FGFR homolog, is expressed in the somatic cells as well, and the expression of these two homologs have been shown to be mutually exclusive in other cases. By observing loss-of-function mitotic recombination clones over time, we found that both btl hypomorphic and null clones were maintained in the niche after 15 days. This suggests that while activated btl appears to confer a competitive advantage to GSCs, endogenous btl is not required for GSC maintenance. Additionally, we assayed for a mitotic advantage by staining for phosphohistone-3 and compared the mitotic index. Interestingly, although btl acts upstream of the MAPK pathway, which typically drives cell growth and proliferation, we found that GSCs with over-activated btl had a mitotic index indistinguishable from neighboring wild-type GSCs. Although further studies are needed to determine the exact type of advantage that over-activating btl confers, our data shows our data shows that the *Drosophila* can be used to find a connection between stem cell competition and human mutations that cause congenital disorders. Further uncovering the mutations and mechanisms that underlie paternal age effect disorders will be a prerequisite for finding ways to prevent mutation accumulation and genetic defects.

279 Role of prostaglandins in *Drosophila* germline stem cell maintenance. *N.M. Green*, T.L. Tootle Anatomy & Cell Biology, University of Iowa, Iowa City, IA.

Stem cells play a crucial role in maintaining adult tissues by generating cellular replacements after tissue injury and aging. Germline stem cells (GSCs) are a unique pluripotent population that reside in both the testes and ovary and give rise to the male and female gametes respectively. *Drosophila* GSCs are one of the most well-studied stem cell models and have aided in uncovering numerous mechanisms of stem cell maintenance. In the *Drosophila* ovary, two GSCs reside in the anterior tip of the germarium and receive signals from their niche environment to divide asymmetrically giving rise to newly differentiating cells while maintaining the GSC pool. Recently, we have found that prostaglandin signaling plays a role in GSC maintenance in the *Drosophila* ovary. Prostaglandins are produced downstream of cyclooxygenase (COX) enzymes. *Drosophila* contains a single COX-like enzyme, Peroxinectin-like (Pxt), which is necessary for prostaglandin synthesis and signaling during oogenesis. Pxt is expressed throughout oogenesis and weakly accumulates in the germarium. Homozygous females for the *pxt* mutant alleles pxt^{f01000} and pxt^{EYO} are sterile and heterozygotes experience a progressive decline in fertility with age. Removal of *pxt* results in alterations to ovarian morphology, including a shift in ovariole composition towards vitellogenic stages and higher percentages of degenerating chambers. Loss of *pxt* disrupts normal germarium morphology causing defects in the organization and formation of fusomes in the dividing cystoblasts. In germaria lacking Pxt, there is an increase in cells with rounded fusomes which resemble GSCs, but are located outside the normal stem cell environment. These data indicate an important role for prostaglandin signaling in stem cell maintenance and provide new insight into the mechanisms required for preserving oogenesis and ovary homeostasis.

280 Adipocyte amino acid sensing in the control of ovarian germline stem cell maintenance. *S. Sahu*, AR Armstrong University of South Carolina, Biological Sciences, Columbia, SC.

Inter-organ communication plays a pivotal role in regulating whole organism physiological responses to changes in environmental factors such as nutrition. Over nutrition, or obesity, can lead to adipocyte dysfunction and is often associated with human diseases, including type 2 diabetes and many cancers. The *Drosophila melanogaster* ovary, a stem cell-supported organ, is highly responsive to dietary changes, showing a severe reduction in egg production when female flies are fed a protein-poor diet. This effect on egg production is mediated by nutrient-sensing pathways acting within the ovary and remote nutrient sensing by other tissues such as the fat body. We have previously shown that amino acid sensing specifically in adipocytes, the major cellular component of the *Drosophila* fat body controls germline stem cell (GSC) maintenance in adult ovaries. Reduced adipocyte amino acid transport leads to loss of GSCs by activation of the amino acid response pathway. We hypothesize that the amino acid response pathway mediates GSC loss by reducing translation of factors necessary for maintenance. The highly conserved AAR pathway further activates two downstream effects that may mediate its control on GSC maintenance: a global reduction in translation and selective up-regulation of ATF4 dependent transcription. We find that RNAi-mediated knock down of translational machinery to reduce global translation specifically in adult adipocytes phenocopies the GSC loss earlier shown by adipocyte specific amino acid transporter knockdown. We also find increased germline cyst cell death leading to a reduction in germarium size. Conversely, crc, *Drosophila* ATF4, over-expression specifically in adipocytes does not influence GSC maintenance. Taken together, these data suggest that AAR pathway activation in adipocytes reduces expression of proteins that may be directly or indirectly involved in maintenance of stem cells. In the future, our goal is to identify adipocyte factors secreted downstream of AAR pathway activity that modulates GSC maintenance. This work will illustrate translational inter-dependency between organs to maintain stem cell-supported tissues.

281 Rbp9 promotes germline stem cell progeny differentiation in the *Drosophila* ovary by directly regulating mRNA translation. *K.L. Ishihara*, X. Zhu, R. Tu, M. Yao, T. Xie Stowers Institute for Medical Research, Kansas City, MO.

The ability of germline stem cells (GSCs) to self-renew and properly differentiate is of paramount importance to genetic continuity. RNA-binding protein 9 (Rbp9), a highly conserved Elav/Hu family protein, was previously shown to regulate GSC differentiation in *Drosophila* ovary, but the underlying molecular mechanisms remain largely unclear. Here, this study shows that Rbp9 regulates early GSC progeny differentiation by directly regulating the translation of the mRNAs encoding some important differentiation factors. Our RNA iCLIP results reveal that Rbp9 directly binds to the 3'UTRs of the mRNAs encoding important differentiation factors, including *bam*, *sxl*, and *mei-P26*. Then, we generated the GFP reporters containing 3'UTR of *bam*, *sxl*, or *mei-P26* with and without the Rbp9 binding sites under the control of the germline-specific *nos* promoter to determine the biological importance of Rbp9 binding. Interestingly, the *bam* 3'UTR without Rbp9 binding sites fails to upregulate the GFP expression in mitotic cysts, while the *sxl* and *mei-P26* 3'UTRs without Rbp9 binding sites fail to downregulate the GFP expression in 16-cell cysts. qPCR results showed no significant changes in *gfp* mRNA levels between the 3'UTRs with and without Rbp9 binding sites, indicating that they are regulated at the translational level via 3'UTRs. Consistently, Rbp9 forms protein complexes with translation regulators, including eIF4A. We are currently investigating the possible mechanisms for the opposing roles of Rbp9 in the regulation of translation between mitotic and 16-cell cysts. Finally, Rbp9 is a well-conserved protein in both structure and function as expression of *elavl2*, a mouse counterpart of Rbp9, can fully rescue the differentiation defects of *rbp9* knockdown ovaries. Therefore, the study of Rbp9 in *Drosophila* should provide important insight into the molecular mechanisms underlying the functions of the Elav family proteins in mammalian development.

282 A novel mutation in *brain tumor* causes both neural over-proliferation and neurodegeneration in adult Drosophila. C. Loewen[2], G. Boekhoff-Falk[3], B. Ganetzky[2], *S. Chtarbanova*[1] 1) Biological Sciences, University of Alabama, Tuscaloosa, AL; 2) Laboratory of Genetics, University of Wisconsin, Madison, WI; 3) Department of Cell and Regenerative Biology, School of Medicine and Public Health, University of Wisconsin, Madison, WI.

An increasing body of evidence indicates that two apparently distinct types of diseases, cancer and neurodegeneration, share common pathophysiological

processes. Mutations in genes involved in cell cycle regulation, inflammation, oxidative stress and protein turnover are characteristic for both types of conditions. Recent epidemiological studies have demonstrated a strong association between Parkinson's disease (a neurodegenerative disorder leading to dopaminergic neuronal loss) and several malignancies such as lung, prostate and brain cancer. Further understanding of the mechanisms that underlie both, cancer and neurodegeneration may open novel avenues for therapeutic strategies and more targeted treatments of these conditions.
In a screen for novel neuroprotective genes we identified a mutation that causes extreme, progressive neurodegeneration in conjunction with massive brain overgrowth. We mapped the mutation to the Drosophila *brain tumor* (*brat*) gene, which encodes a TRIM-NHL RNA-binding protein with established roles in regulating stem cell proliferation in the developing brain and ovary. Neurodegeneration has not previously been associated with *brat* mutations. Our new allele, *brat*^{cheesehead} (*brat*^{chs}), carries a mutation in the coiled-coil domain of the TRIM motif, and is temperature-sensitive. We demonstrate that mRNA and protein levels of neural stem cell genes are increased in heads of adult *brat*^{chs}mutants, and that the over-proliferation phenotype initiates prior to adult eclosion. We also show that in the adult, neurodegeneration coincides with activation of the effector caspase Dcp-1 in cell types such as neural progenitors and glia. Finally, we report that the dual phenotype of *brat*^{chs} flies is enhanced by a mutation in a putative prolyl 4-hydroxylase-coding gene. This represents a previously unknown interaction for Brat that may reveal a new pathway in which Brat functions, and could be relevant to both human cancer and neurodegenerative diseases.

283 Searching for transcription factors associated with Pros and neurodegeneration in the larval brain of Drosophila. *R. Sang* Institute of Genetics Zhejiang University, Hangzhou, Zhejiang Province, CN.

Drosophila neuroblast undergoes cell division in larva stage. Self-renewal and differentiation of the neuroblast is an important question to understand brain development in Drosophila. Prospero(Pros) is a gene which promotes cell differentiation and represses cell proliferation. Here we tried to find transcription factors which could cause Pros entering the nucleus and neurodegeneration. We chose 70 transcription factors which might be important in Drosophila neuroblast. We knocked down these genes in larva brain using RNAi lines, and detected if pros entered the nucleus by immunofluorescence staining. We found 8 of them might influence the pattern of Pros. These genes might play important roles in Pros positioning and neuroblast cell division, and the mechanism still need to be studied.

284 Characterization of the role of *Similar to deadpan* gene in *Drosophila* neural stem cells. *A. Rajan*, H. Komori, C. Lee Life Sciences Institute, University of Michigan - Ann Arbor, Ann Arbor, MI.

Neural stem cells undergo asymmetric cell division to self-renew and produce progenitor cells that generate differentiated neurons. Revealing the mechanisms of neural stem cell self-renewal is an important step in understanding the cause of a variety of neural disorders. The type II neural stem cell (neuroblast) lineage in the fly larval brain provides an excellent *in vivo* model for investigating the mechanisms that regulate self-renewal and differentiation. Previous studies have demonstrated that the *Hes* family genes, *deadpan (dpn)* and *E(spl) genes* have an important role in self-renewal of type II neuroblast. However, the role of another Hes family gene, *Similar to deadpan (Sidpn)* in type II neuroblast self-renewal has not been characterized, yet. To examine if *Sidpn* is required for type II neuroblast self-renewal, we induced GFP-marked clones derived from a single *Sidpn* mutant type II neuroblast in the larval brain. *Sidpn* mutant type II neuroblasts could maintain their identity during the larval stage. To examine if *Sidpn* has ability to promote type II neuroblast self-renewal, we overexpressed *Sidpn* in type II neuroblasts. We found that overexpression of *Sidpn* induced supernumerary type II neuroblast formation in larval brains. These results indicate that *Sidpn* is sufficient to promote neuroblast self-renewal, but not necessary. Since Hes family proteins function by forming a homodimer or a heterodimer with one of Hes family proteins, we tested whether Sidpn protein is able to form a heterodimer with Dpn or E(spl)my in *Drosophila* S2 cells. Sidpn had the ability to form a homodimer and heterodimers with Dpn and E(spl)my. Our findings suggest that *Sidpn* may function cooperatively with other Hes family genes to regulate neuroblast self-renewal in the fly larval brain.

285 Tumor cell fate plasticity in neural stem cell-derived tumors. *H.H.H. Truong*[1,2], F. Froldi[1,2], L.Y. Cheng[1,2] 1) Cancer Research Division, Peter MacCallum Cancer Centre, Melbourne VIC 3000, Australia; 2) Department of Anatomy and Neuroscience, the University of Melbourne, Parkville VIC 3010, Australia.

Neural stem cells (NSCs) have been found to be the tumor cell of origin in various human brain cancers. Similar to human NSCs, *Drosophila* NSCs, called neuroblasts (NBs), can also asymmetrically divide to renew, and generate neurons or glia of the central nervous system. Disrupting either asymmetric cell division or neuronal maintenance allows differentiated cells to dedifferentiate into ectopic NBs, which can give rise to tumor clones. It is known that tumors demonstrate greater plasticity than wildtype cells. Using several *Drosophila* neural stem cell-derived tumor models, we characterise the composition and plasticity of the tumor clones, and explore the function and origin of different cell types in the clones using single-cell RNA-seq.

286 Caliban regulates mitochondria integrity to maintain intestine homeostasis. Dong Li, Zhaoxia Dai, Xiao Du, *Xiaolin Bi* Department of Biological Sciences, Dalian Medical University, Dalian, Liaoning Province, CN.

Precise regulation of stem cell activity is crucial for tissue homeostasis. In Drosophila, intestinal stem cells (ISCs) maintain the midgut epithelium and respond to oxidative challenges by increasing proliferation rates. However, the connection between oxidative stress and mitogenic signals remains obscure. Here we show Caliban as a novel regulator of the cellular redox state, which specifically controls the proliferative activity of ISCs to maintain intestinal homeostasis. We find that Caliban is localized to mitochondria and highly expressed in ECs. Loss of Caliban in ECs causes fragmentation of mitochondria, accumulation of reactive oxygen species and activation of JNK and JAK-STAT signaling pathway, which in turns drives the proliferation of neighboring ISCs non-autonomously. Furthermore, loss of Caliban promotes the progression of tumor growth generated by activating Ras in intestinal progenitor cells. These findings reveal the requirement for Caliban in the control of ISC proliferation and suggest an essential role in the regulation of redox homeostasis, with relevance for development, homeostasis and cancer.

287 Caliban regulates mitochondria integrity to maintain the homeostasis in Drosophila intestine. D. Li, Z. Dai, X. Du, *X. Bi* Department of Biological Sciences, Dalian Medical University, Dalian, Liaoning Province, CN.

Precise regulation of stem cell activity is crucial for tissue homeostasis. In Drosophila, intestinal stem cells (ISCs) maintain the midgut epithelium and respond to oxidative challenges by increasing proliferation rates. However, the connection between oxidative stress and mitogenic signals remains obscure. Here we show Caliban is a novel regulator of the cellular redox state, and specifically controls the proliferative activity of ISCs to maintain intestinal homeostasis. We find that Caliban is localized to mitochondria and highly expressed in enterocytes (ECs). Loss of Caliban in ECs causes fragmentation of mitochondria, accumulation of reactive oxygen species and activation of JNK and JAK-STAT signaling pathway, which in turns drives the proliferation of neighboring ISCs non-autonomously. Furthermore, loss of Caliban promotes the progression of tumor growth generated by activating Ras in intestinal progenitor cells. These findings reveal the requirement for Caliban in the control of ISC proliferation and suggest an essential role in the regulation of redox homeostasis, with relevance for development, homeostasis and cancer.

288 An SH3PX1-dependent endocytosis/autophagy network restrains intestinal stem cell proliferation by counteracting EGFR signaling. *P. Zhang*[1], A. N. Holowatyj[2], T. Roy[1], M. Marchetti[1], S. M. Pronovost[1], H. Liu[1], C. M. Ulrich[2,3], B. A. Edgar[1] 1) Department of Oncological Sciences, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT 84112, USA; 2) Department of Population Health Sciences, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT 84112, USA; 3) Fred Hutchinson Cancer Research Center, Seattle, WA 98109.

How intracellular vesicle trafficking affects stem cell behavior is largely unknown. We screened the *Drosophila*sorting nexins (SNXs) and discovered that one, SH3PX1, is critical for normal gut homeostasis and lifespan. SH3PX1 restrains intestinal stem cell (ISC) division through an endocytosis/autophagy network that includes Shi, Rab5, Rab7, Atg1, 5, 6, 7, 8a, 9, 12, 16, and Syx17. Blockages in this network stabilized ligand-activated EGFRs, recycling them via Rab11-dependent endosomes to the plasma membrane. This activated ERK, Calcium signaling, and ER stress in ISCs, autonomously activating their proliferation. The excess divisions triggered epithelial stress, Hippo pathway inactivation, cytokine (Upd3) and intramembrane protease (Rhomboid) production by enterocytes, catalyzing a feed-forward reaction and ISC hyperplasia. Many autophagy/endocytosis genes are mutated in human cancers, most notably those enriched in microsatellite instable-high and KRAS-wildtype colorectal cancers. Disruptions in endocytosis and autophagy may constitute an alternative route to RAS/ERK activation that could be targeted in EGFR-dependent cancers.

289 Sox100B: a Stress Responsive Transcription Factor that Coordinates ISC Proliferation and Differentiation with a Dosage-Dependent Function in *Drosophila*. *Z. Jin*[1,2], J. Chen[1], J. Wang[1], H. Huang[1], T. Cai[1], R. Xi[1] 1) National Institute of Biological Sciences, Beijing, Beijing, CN; 2) Beijing Normal University, Beijing, Beijing, CN.

The midgut epithelium in adult *Drosophila* normally undergoes constant epithelial renewal or turnover, which relies on the activity of local intestinal stem cells (ISCs). ISCs are also responsive to epithelial damage by accelerated proliferation and differentiation to promote epithelial repair. The fly midgut therefore provides a genetically tractable system for the understanding of the underlying mechanisms of tissue homeostasis and regeneration driven by tissue stem cells. Here, we identified Sox100B, a Sox family transcription factor, which has an important function in coordinating ISC proliferation and differentiation in both normal epithelial homeostasis and stress-induced intestinal repair in *Drosophila*. Sox100B is specifically expressed in progenitor cells and can be upregulated dramatically in response to stress signals especially in pre-mature EBs (Enteroblasts) due to increased EGFR and JAK/STAT signaling pathway activities. Loss of Sox100B compromises both ISC proliferation and differentiation. Interestingly, strong induction of Sox100B by forcibly expressing a Sox100B transgene in ISCs and progenitor cells inhibits ISC proliferation and induces precocious cell differentiation. Only a critical level of Sox100B expression is required to ensure a proper function of ISCs. Moreover, we found that Sox100B acts upstream of another Sox family transcription factor Sox21a and direct targets Sox21a to promote EBs differentiation into ECs after tissue damage. We propose that Sox100B is a dosage-dependent transcription factor, which acts downstream of multiple signaling pathways to regulate ISC proliferation and contributes to rapidly intestinal epithelium turnover through induction of Sox21a as well as other differentiation-priming genes to promote progenitor cell differentiation.

290 Regulation of blood cell transdifferentiation by sensory neuron activity. S. Corcoran[2], S. Tattikota[4], F. Wang[2,6], K. Kukar[2], N. Perrimon[4,5], *K. Brückner*[1,2,3] 1) Broad Center; 2) Cell and Tissue Biology; 3) Cardiovascular Research Institute, University of California, San Francisco, San Francisco, CA; 4) Department of Genetics, Harvard Medical School, Boston, MA ; 5) Howard Hughes Medical Institute; 6) SKLSGB, Southwest University, Chongqing, China.

The resident hematopoietic sites of the *Drosophila* larva are in direct contact with segmentally repeated sensory neuron clusters of the peripheral nervous system (PNS). Blood cells (hemocytes) require the PNS for their survival, proliferation and recruitment to these specialized microenvironments known as hematopoietic pockets (HPs). Signals produced by active sensory neurons such as Activin-β (Actβ) are known to promote proliferation of macrophage-like plasmatocytes, but it remains unclear whether other blood cell types, such as crystal cells, are also under control of these sensory microenvironments. Here, we investigate the molecular and cellular mechanisms that drive formation of crystal cells from oligopotent plasmatocytes. Consistent with previous reports, we confirm by various lineage tracing methods that crystal cells arise through transdifferentiation from active phagocytic, differentiated plasmatocytes. Interestingly, we find that crystal cell production heavily depends on sensory neuron activity and Actβ/dSmad2 signaling in plasmatocytes, exceeding the known effects of these conditions on plasmatocyte proliferation. Moreover, we identified transcriptional target genes that are essential for crystal cell formation, utilizing RNAseq analysis of differentially neuron-stimulated larvae and single cell RNAseq (scRNAseq). Our work focuses on a new chemokine like factor, which is essential for crystal cell formation downstream of neuronal activity. Principles of blood cell transdifferentiation and neuronal regulation of hematopoiesis identified in this model may be conserved across species, and apply to related blood cell populations such as vertebrate resident tissue macrophages and lineage-restricted hematopoietic progenitors.

291 TGFβ/Activin signaling is a switch between homeostasis and stem cell regeneration in the *Drosophila* testis. *Salvador Herrera*[1], Amoyel Marc[2], Lydia Grmai[1], Shally Margolis[1], Rebecca Plessel[1], Michael Burel[1], Michael O'Connor[3], Erika A. Bach[1] 1) NYU School of Medicine, New York City, NY; 2) Department of Cell and Developmental Biology, University College London, London, UK; 3) Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN.

Niches provide a distinct microenvironment for stem cells and secrete short range self-renewal cues that promote 'stemness' in the resident population. However, whether stem cells also secrete signals that maintain the niche remains an intriguing question. The *Drosophila* testis provides an ideal system to address this important issue. In this tissue, twelve quiescent niche cells support two mitotic stem cell pools, germline stem cells (GSCs) that ultimately produce sperm and somatic cyst stem cells (CySCs) that support GSCs and produce somatic support cells. Prior work has shown that genetic ablation of all CySCs in the *Drosophila* testis causes niche cells to exit quiescence and transdifferentiate into CySCs (Hetie, *Cell Reports* 2014). This study demonstrated that CySCs non-autonomously maintain niche cells but the identity of the factors that regulate this process are still not known.

We demonstrate that the secreted Activin antagonist Follistatin (Fs) is expressed in CySCs, and its loss from CySCs causes the progressive and complete loss of niche cells, followed by a loss of GSCs and a failure of spermatogenesis. These data indicate that CySC-produced Fs protects niche cells from local Activin ligands. Consistent with this finding, autonomous stimulation of Activin signaling in niche cells causes all of them to transdifferentiate into fully functional CySCs. We show that in CySCs, expression of *Fs* is positively regulated by the Dp/E2f1 transcription factor complex. Depletion of Dp/E2f1 from CySCs results in the non-autonomous loss of all niche cells. Importantly, ectopic mis-expression of Fs in CySCs depleted for Dp/E2f1 fully rescues the niche loss phenotype.

In sum, we hypothesize that TGFβ/Activin signaling acts as a switch that triggers in the niche a regenerative response aimed to replenish lost CySCs, at the cost of reducing the niche population.

292 Using single cell RNA sequencing to probe the genetic profiles of niche cells in the *Drosophil*a testis. *K.Ann. Conlon*[1], S Mahadevaraju[2], M Akeju[1], J Fear[2], B Oliver[2], E Matunis[1] 1) Cell Biology, Johns Hopkins School of Medicine, Baltimore, MD; 2) National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD.

The *Drosophila melanogaster* testis stem cell niche is a powerful model to study stem cell maintenance and regulation due to its genetic tractability and wealth of prior characterization. The stem cell niche, located at the testis apex, is comprised of post-mitotic somatic hub cells with germline stem cell (GSC)

and the cyst stem cell (CYSC) populations closely adhered. Many signaling pathways have been implicated in the maintenance and regulation of the stem cell populations to the hub. Aberrant expression of genes in these pathways have drastic implications for the maintenance of the stem cell niche and spermatogenesis.
Spermatogenesis involves asymmetric division of stem cells, incomplete mitotic divisions of spermatogonia, and meiotic divisions of spermatocytes into spermatids. The CYSCs also divide asymmetrically to form cyst cells which encapsulate the dividing gonia and spermatocytes. The coordination of these various cell types is required for productive spermatogenesis. In collaboration, our lab has isolated single cells and sequenced a majority of the cell types involved in the different stages of drosophila spermatogenesis. This dataset has been partitioned into somatic and germ cell types by clustering reads into similar expression profiles. These clusters have been verified by comparing their profiles to known cell type markers, targeted DamID expression data, and whole testis RNA-seq. These clusters have been further validated using protein traps lines for genes enriched in clusters. Taken together, these measures suggest that scRNA-Seq is a viable option to access the RNA transcriptome of cells in the testis. However, we believe hub cells, GSCs, or CYSCs are not well represented in our initial dataset. We plan to optimize our protocol for isolating these cell types. This is particularly challenging given hub cells are few in number and are known to express high levels of adhesion molecules including Fasciclin 3, DE-cadherin, DN-cadherin, β-catenin, integrin and are thus more strongly connected than other cells. To overcome this obstacle, I am developing a refined method to isolate these niche cells using enzymatic digestion. We will then perform scRNA-Seq on hub cells, GSCs, and CYSCs from larval testes. By sequencing these cell types, we can use their quality RNA profiles as a scaffold to better understand the niche maintenance and various perturbations of it.

293 Using single cell RNA sequencing to probe the genetic profiles of niche cells in the *Drosophil*a testis. *K.Ann. Conlon*[1], M. Akeju[1], S. Mahadevaraju[2], J. Fear[2], Z. Demere[1], B. Oliver[2], E. Matunis[1] 1) Cell Biology, Johns Hopkins School of Medicine, Baltimore, MD; 2) National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD.

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294 The interplay between N-cadherin and E-cadherin is critical for stem cell niche maintenance. *R. Tu*, S. Wang, X. Song, T. Xie Stowers Institute for Medical Research, Kansas City, MO.

Adult stem cells continuously undergo self-renewal and generate differentiated cells. In the *Drosophila* ovary, two separate niches control germ line stem cell (GSC) self-renewal and differentiation processes. Compared to the self-renewing niche, relatively little is known about the maintenance and function of the differentiation niche, which formed by inner germarial sheath cells (ISCs, also known as escort cells). Here, we show that E-cadherin and N-cadherin have compensatory roles in maintaining ISCs and consequently GSCs. The loss of N-cadherin expression does not affect ISC maintenance and function, while the loss of E-Cadherin causes a slight reduction of the GSC number. Surprisingly, the simultaneous loss of both E-cadherin and N-cadherin expression results in the loss of both ISCs and GSCs. The loss of both E-cadherin and N-cadherin causes the defective ISC proliferation and cell death. Mechanistically, the loss of E-cadherin expression leads to an upregulation of N-cadherin expression in ISCs, suggesting that upregulated N-cadherin can compensate for the loss of E-cadherin in ISCs. Finally, RNA-seq results suggest that E-cadherin-mediated repression of N-cadherin expression might be achieved via the microRNA pathway. We are currently investigating the detailed molecular mechanisms underlying the E-cadherin-mediated N-cadherin repression in ISCs. The loss of E-cadherin in epithelial cells can also cause N-cadherin upregulation in mammals, which is often associated with carcinoma formation, but the underlying mechanisms remain elusive. Our further investigation will not only reveal the role of the interplay between E-cadherin and N-cadherin in the maintenance of the stem cell niche but also help understand the potential mechanisms underlying cancer formation in humans.

295 Identification of Eys as a new regulator of intestinal homeostasis. *C. Penalva*[1], J. Maldera[2,3], H. Cummins[1], R. Sudakaran[2,3], B. Edgar[1,2,3] 1) Huntsman Cancer Institute, Salt Lake City, USA; 2) ZMBH, Heidelberg, Germany; 3) DKFZ, Heidelberg, Germany.

The intestine displays a constant homeostatic self-renewing during which the aged and/or damaged differentiated epithelial cells are replaced by the offspring of the resident adult stem cell. Lack of renewal can lead to atrophy, organ failure, whereas un-necessary stem cell activity can trigger dysplasia, promotes inflammatory disorders, and cancers formation. Thus, the fine-tuned control of intestinal regenerative growth is essential for health. However, many aspects of the mechanisms that regulate intestinal homeostasis remain elusive. The epithelial layer unsure critical functions as it acts as a primary stress sensor, and regulates the proliferative rate of the stem cells by secreting cytokines. Several signaling pathways activation in the epithelium, including MAPK and Hippo, are required for regeneration, however how the epithelial cells sense the damages, how they transduce it to signaling pathways, and how the different actors of this mechanism interact together still need to be address.
To understand better these questions, we performed an RNA sequencing after five different stresses (HS, oxidative stress, JNK activation, induction of apoptosis, P.e. infection). Since the epithelial cells are the most abundant, the sequencing of total RNA from the midgut allowed us to identify putative targets or actors of the intestinal regeneration in the epithelial layer. Among the 44 genes upregulated in every stress tested, we selected *eye shut* for further investigation. Eys is a secreted proteoglycan that is required for the formation of the retina in flies and zebrafish, and for the maintenance of the retina integrity in adult both in human and flies. Eys allows the enlargement of the extracellular matrix between the rhabdomers together with prom, its transmembrane partner, and is positively regulated by rumi. No function has been described for eys, prom or rumi in the gut epithelium yet. We determined that eys is switch ON both at the RNA (qPCR) and protein (Immunofluorescence) level in midgut of flies challenged with P.e. infection, Ecc15 infection, or SDS compared to unchallenged flies. Epithelium-specific Knockdown of Eys or Rumi decrease the stem cell proliferation during P.e., Ecc15, or SDS challenge. Thus,

they are both required for regeneration. The sequencing we did allowed us to identify two new regulators of the intestinal homeostasis that might constitute a regulatory pathway (possibly with prom).

296 EGFR signaling mediates regeneration after injury in the Drosophila testis stem cell niche. L. Greenspan, *M. de Cuevas*, E. Matunis Department of Cell Biology, Johns Hopkins School of Medicine, Baltimore, MD.

Adult stem cells are maintained in niches, which are specialized microenvironments that regulate their self-renewal and differentiation. In the Drosophila testis stem cell niche, somatic hub cells produce signals that maintain and regulate adjacent germline stem cells (GSCs) and somatic cyst stem cells (CySCs). Hub cells normally divide only during embryogenesis and are quiescent in adult flies. However, knockdown of the cell cycle inhibitor Retinoblastoma-family protein (Rbf), or forced expression of cell cycle activators, directly in hub cells causes them to proliferate, leave the hub, and convert into functional CySCs. Over time, these mutant hub cell clusters enlarge and split apart, forming ectopic niches surrounded by active stem cells. Thus, Rbf is an essential intrinsic regulator of niche cell quiescence, niche cell identity, and niche number. We previously showed that complete genetic ablation of CySCs also causes hub cells to proliferate and convert into CySCs. This suggests that CySC ablation alters signaling pathways within the niche, triggering hub cells to re-enter the cell cycle and change fate to replace the missing stem cells. To identify these signaling pathways, we knocked down or overexpressed candidate pathway genes in hub cells and screened for loss of hub cell quiescence. We found that the EGFR-MAPK pathway plays a key role in this process. Normally, in unperturbed testes, EGFR signaling is required in cyst lineage cells for their proper differentiation and function, but it does not play a role in the hub. However, upon forced activation of EGFR signaling in the hub, hub cells proliferate and convert into CySCs. Moreover, after genetic ablation of CySCs, reduction of EGFR causes a reduction in the number of testes that successfully regain CySCs. These preliminary results suggest that EGFR signaling is necessary and sufficient for promoting hub cell proliferation and transdifferentiation to CySCs, demonstrating how an existing signal can be repurposed for recovery after tissue injury. Together, our studies reveal that the precise regulation of niche cells, not just the stem cells they support, is important for tissue maintenance and regeneration.

297 The translational repressor Brat constrains regenerative growth to ensure proper patterning after tissue damage. *R.K. Smith-Bolton*, S.N.F. Abidi Cell and Developmental Biology, University of Illinois Urbana-Champaign, Urbana, IL.

How regenerating tissue undergoes repatterning and ensures replacement of the correct cell types is a key question in regeneration biology. By using genetic tools to damage and induce regeneration in third-instar wing imaginal discs, we have identified mutants that have aberrant patterning in the regenerated structure. Through this screen we have shown that the translational repressor *brain tumor (brat)* is a regulator of both growth and patterning during regeneration. While *brat/+* wing discs regenerated better than controls, the resulting adult wings had disrupted wing margins. The enhanced regeneration in *brat/+* mutants was due to elevated expression of Brat targets Wingless and Myc, as well as elevated expression of Ilp8. However, it was unclear why regenerating tissue would constrain expression of pro-regeneration factors through Brat. We replicated the enhanced regeneration by overexpressing Myc after tissue damage, which was sufficient to reproduce the disrupted margin phenotype. Thus, constraining pro-growth factors appears essential for ensuring proper repatterning of the regenerated tissue.

298 Genetic and epigenetic manipulation of regeneration in *Drosophila* imaginal discs. *R. Harris*[1], M. Stinchfield[1], S. Nystrom[2], D. McKay[2], I. Hariharan[3] 1) Arizona State University, Tempe, AZ 85728; 2) University of North Carolina, Chapel Hill, NC 27599; 3) University of California, Berkeley, CA 94720.

In many examples of regeneration, the capacity of a tissue to regrow following damage declines with age. Understanding the mechanisms that dictate changes to regenerative capacity is vital for developing methods to stimulate or augment regeneration. However, the underlying cellular and genetic events that lead to such changes remain unknown, and tools to unambiguously target regenerating cells for interrogation within a tissue are lacking.

Damaged *Drosophila* imaginal discs regenerate efficiently early in the third larval instar (L3), but progressively lose this ability as they proceed through development. This loss of regenerative capacity correlates with reduced damage-responsive expression of multiple genes, including those required for the growth and re-patterning of tissue, such as Myc, MMP1, and WNT signaling. Our previous work found that the expression of two WNT genes, *wg* and *WNT6*, during regeneration requires a bipartite Damage-Activated Regeneration Enhancer, or DARE, whose activity declines during L3 as a result of epigenetic silencing at the enhancer, leading to a progressive loss of regenerative WNT expression. To expand on these findings, we used whole-genome sequencing approaches to characterize changes in both the transcriptional and epigenetic responses to damage as discs mature, which revealed that DARE sequences occur throughout the *Drosophila* genome and potentially regulate multiple factors crucial to regeneration.

In order to investigate the function of these DARE target genes in disc regrowth, as well as the mechanism by which DAREs become silenced with maturity to limit regenerative gene expression, we have developed a novel ablation system that allows genetic manipulations to be targeted specifically to blastema cells. Using this system, we show that the knockdown of several newly identified DARE targets (including previously uncharacterized genes) in blastema cells reduces the regenerative capacity of discs, thus demonstrating their functional role. Conversely, the targeted knockdown of epigenetic silencing factors, including specific Polycomb group genes, reverses DARE silencing at multiple loci, thus leading to the reactivation of regenerative gene expression and significantly improving regeneration. These results demonstrate the utility of this system in exploring the genetic and epigenetic regulation of regeneration, and manipulations that can augment it.

299 Validating changes in expression of candidate genes due to acute injury in the embryonic *Drosophila* Ventral Cord. *A. Bussetty*, R. Erianne, H. Mistry Biology, Widener University, Chester, PA.

Acute or chronic changes in gene regulation on humans suffering from traumatic brain injury or spinal cord injury are hard to determine and evaluate. The use of genetically tractable invertebrate model systems, such as *Drosophila*, provides avenues by which we might better understand the cellular and molecular mechanisms underlying human spinal cord injury. We are using the embryonic central nervous system of *Drosophila melanogaster* as a model. We have wounded the ventral nerve cord of late-stage embryos using a fine bore capillary needle and harvested total RNA two hours later. As control, we have harvested RNA from embryos of a similar age under similar conditions, but without wounding (mock). Using RNA-seq technology, we determined that of the 11,000 genes expressed in both conditions the expression of 702 genes is changed significantly in response to injury. We are focusing our analysis on selected candidates that show injury-dependent changes in expression. These genes are involved in the regulation of axon guidance, synaptic growth, or the mitotic cell cycle. We have harvested total RNA from uninjured (mock) and injury groups of embryos. We have generated cDNA from these RNA samples, and conducted qPCR analysis to validate the observed changes in gene expression of these candidate genes.

300 Polyploid cell growth restores tissues mechanics post injury. *K.J. Gjelsvik*[1,2], V.P. Losick[1,2] 1) MDI Biological Laboratory, Bar Harbor, ME; 2) University of Maine Graduate School of Biomedical Science and Engineering, Orono, ME.

Polyploidy frequently arises in response to injury, disease, and age-related tissue degeneration. Despite its prevalence, major gaps exist in our understanding of how polyploid cells emerge and alter tissue function. In the adult *Drosophila* epithelium, wound healing is dependent on the generation of multinucleated polyploid cells, which results in a permanent change in the epithelial architecture. Here, we study how wound-induced polyploid cells affect

tissue function by altering tissue mechanics. We have found that the mechanosensor, non-muscle myosin II (Sqh) is activated (phosphorylated) and upregulated during wound healing. Sqh activation is dynamic and required not only early to facilitate wound closure, but also persists in the polyploid cells after healing completes. The upregulation and phosphorylation of Sqh are known to correlate with enhanced tissue tension, suggesting that polyploid cell growth alters tissue mechanics. Using laser microsurgery, we found that the relative tissue tension is significantly enhanced in polyploid epithelial cells and dependent on Sqh activity. Injury to the abdomen also damages the underlying muscle fibers, which are permanently severed. Remarkably, we found that the enhanced polyploid epithelial tension mimics the relative tension of the lateral muscle fibers. Therefore, polyploid cell growth enables the epithelium to adapt and become muscle-like to compensate for lost tissue mechanics.

301 MMPs in Drosophila basement membrane homeostasis and repair. *K. LaFever*, A. Howard, A. Page-McCaw Cell and Developmental Biology, Vanderbilt University, Nashville, TN.

Basement membrane is a sheet-like extracellular matrix that underlies epithelia and surrounds muscles. It is important for the stabilization of epithelia, muscles and other tissues, so repair of this matrix is crucial for tissue function. Feeding DSS (dextran sulfate sodium) to adult Drosophila induces basement membrane damage in the posterior midgut of the fly. In normal feeding conditions, the posterior midgut architecture is uniform but it becomes highly disarrayed after DSS damage because of decreases in matrix stiffness. Damage can be assessed by looking at any of the four core components of the basement membrane (Collagen IV, Laminins, Perlecan and Nidogen).

It is known that MMPs play an important role in tissue repair in other developmental stages, and they have been implicated in basement membrane repair. When knocking down Mmp1 and Mmp2 via RNAi, the gut architecture becomes disorganized, very similar to the guts of DSS-damaged adults. Surprisingly, in both mutants, ColIV and LanB1 levels are significantly reduced only in specific basement membranes, suggesting that Mmp1 and Mmp2 have unexpected specificity in maintaining protein levels during basement membrane homeostasis. We are currently investigating whether the MMPs are required for repair of basement membrane after DSS-induced damage.

302 Combover is required for spermatogenesis independently of the Planar Cell Polarity Pathway. *J. Steinhauer*[1], B. Statman[1], J. Fagan[2], J. Borck[1], D. Edelman[1], A. Jenny[2] 1) Department of Biology, Yeshiva College, New York, NY, USA; 2) Department of Developmental and Molecular Biology and Department of Genetics, Albert Einstein College of Medicine, New York, NY, USA.

Drosophila melanogaster has long been an elegant model to study sperm development because of its similarities to the mammalian system. In flies, spermatogenesis starts with a stem cell daughter, which gives rise to, after mitoses and meiosis, 64 interconnected spermatids that further differentiate and individualize into mature sperm. Spermatid individualization requires intricate control of the actin cytoskeleton, critically depending upon the formation of specialized actin cones adjacent to sperm nuclei and their synchronous movement along the sperm tails. Actin cone movement removes most of the cytoplasm and organelles, as well as interspermatid bridges to ensure that each sperm is separated from its sisters and tightly enclosed by a plasma membrane. Here we show that Combover (Cmb), originally identified as an effector of Planar Cell Polarity (PCP) in the fly wing under control of Rho kinase, has an essential role in sperm individualization. *cmb* mutants are male sterile, with actin cones that contain less actin and fail to synchronously move down the flagella, despite being correctly formed and polarized initially. These defects are germline autonomous and can be rescued by wild-type Cmb, but not by a version of Cmb in which known Rho kinase phosphorylation sites were mutated to alanine. Importantly, the function of *cmb* in spermiogenesis is independent of other PCP genes.

303 *Dm* Ime4 is required for somatic cyst cell permeability barrier function during spermatogenesis. *Antonio Rockwell*, Cintia Hongay Biology, Clarkson University, Potsdam, NY.

Work from several labs in the past six years has revealed that m^{6}A methylation regulates several aspects of mRNA metabolism. *Dm* Ime4 (METTL3), the enzyme that catalyzes this modification in Drosophila, is evolutionarily conserved and essential for development in metazoans and plants. However, the reason why this enzyme is essential in multicellular eukaryotes remains unclear. Our lab has found that *Dm* Ime4 regulates profilin (Chic), another essential and evolutionarily conserved protein necessary for f-actin polymerization, previously shown to be required for maintenance of the somatic cyst-cell permeability barrier in Drosophila spermatogenesis. Chic and *Dm* Ime4 colocalize and are abundant in somatic cyst cells. Using Gal4-UAS RNAi to knock down *Dm ime4* specifically in the somatic cyst cells, we observe aberrant localization of Chic and malfunction of the permeability barrier evidenced by increased permeability to dextran (10K and 40K) compared to sibling controls. The *chic* transcript contains *Dm* Ime4 binding sites in UTRs which could affect its splicing, nuclear transport, or translation. Northern blot analyses revealed that the *chic*-RD spliceoform is present only in the testes of *Dm ime4* somatic cyst-cell knockdown strains. Western blot analyses showed that this spliceoform, contrary to annotated data, seems to be translated in testes of somatic cyst-cell *Dm ime4* knock-downs but not in control siblings. Interestingly, this Chic polypeptide has not been previously shown in published western blots using publicly available anti-Chic antibodies. Furthermore, while Chic is present only in somatic cyst cells in control siblings, it is absent in somatic but present in germline cells in *Dm ime4* somatic cyst-cell knockdown strains. RNA-seq data (public domain) show that the RD isoform is normally abundant in embryogenesis but not in adult gonadal tissue. Therefore, we conclude that ablation of *Dm* Ime4 in somatic cyst cells results in the production of a Chic protein that is developmentally inadequate to perform its function in spermatogenesis. Consequently, this aberrant *chic* expression pattern due to *Dm ime4* ablation compromises the somatic permeability barrier, leading to germline death and reduced fertility in *Dm ime4* somatic cyst-cell knockdown strains. **We hypothesize that *Dm* Ime4 (METTL3) may regulate profilin (*chic*) in other developmental contexts and in other organisms, including mice and humans. Profilin is an essential protein, and establishment and maintenance of cell barriers and domains are important strategies used in development. Our findings could explain the essentiality of *Dm* Ime4 (METTL3) in most multicellular organisms**.

304 Testis-specific sugar transporters of *D. melanogaster*. *Mark Hiller*, Katlyn Heneghan, Emily Fontenoy, Stephanie Hrabar Dept of Biological Science, Goucher College, Baltimore, MD.

Carbohydrates are required to build and maintain cellular structures and provide energy for cells. The *Drosophila melanogaster* genome contains twenty-five genes that have been annotated as encoding SLC2 (solute carrier family 2) proteins that are characterized as sugar transporters. Based on temporal expression during development, we propose that five of the twenty-five genes (*sut4*, *sut3*, *glut3*, *tret1-2*, and *CG14605*) exhibit testis-specific gene expression. Spermatid development is a complex process in which every part of the cell is remodeled. These transporters could provide the energy necessary for undifferentiated germline cells to develop into mature sperm or provide carbohydrates to build cellular structures. Alternatively, the sugar transported could provide energy for motility of mature sperm during mating and fertilization. RT-PCR was used to characterize gene expression and confirmed that each is expressed in adult males but not adult females. All are expressed in testes. Transposible Element insertions for three of these genes were generated by the Gene Disruption Project. However, none of the mutant lines have detected defects in spermatogenesis and the mutants are fertile. We continue to chararaterize the mutant lines to better understant the role of sugar transporters in spermatogenesis.

305 *Tob* is an X-linked gene required for post-meiotic male germ cell maturation. Farnaz Shapouri[1], Franca Casagranda[1], Nicole Siddall[1], Robb de Iongh[1], Mary Familari[2], *Gary Hime*[1] 1) Department of Anatomy and Neuroscience, University of Melbourne, Parkville, Victoria, Australia; 2) School of Biosciences, University of Melbourne, Parkville, Victoria, Australia.

The Tob family of proteins are highly conserved regulators of gene expression. The vertebrate TOB1 and TOB2 family members have been shown to form a complex with CAF1 and the CCR4 deadenylase to regulate mRNA turnover or translation of target mRNAs. We have previously shown that TOB1 is expressed in germ cells during mouse spermatogenesis and utilised the *Drosophila* testis to examine Tob function in this tissue. *Drosophila* has a single, X-linked Tob gene. Generation of a Tob hypomorphic allele and use of RNAi indicate that Tob is required for co-ordinated individualisation of spermatids. Loss of Tob function results in disruption of individualisation complexes and scattered spermatid nuclei. These males also exhibit a decrease in ability to produce progeny. The Tob protein localises to individualisation complexes but it appears to be transcribed in spermatocytes. Thus an X-linked gene product can be made available to all haploid spermatids via transcription in diploid spermatocytes followed by a delay in translation until after meiosis. Since the individualisation complex is formed from the Golgi apparatus we examined the localization of this organelle during sperm differentiation by using an anti-Golgin 84 antibody. Immunofluorescence images indicate that in *Tob RNAi* lines the Golgi density was reduced around the scattered spermatids suggesting that Tob functions in the earliest stages of formation of the acroblast and individualisation complex.

306 Pif1A, the Drosophila homolog of human CCDC157, is essential for spermatogenesis and may underlie idiopathic NOA. *H. Zheng*, X. Yuan, Y. Su, X. Yang, Y. Xi, P. Guo, C. Li, W Ge ZheJiang University, HangZhou, CN.

The dynamic process of spermatogenesis shows little variation between invertebrate models such as *Drosophila* and vertebrate models such as mice and rats. In each case, germ stem cells undergo mitotic division to proliferate and then continue, via meiosis, through various stages of elongation and individualization from spermatogonia to spermatid to finally to form mature sperm. Mature sperm are then stored in the seminal vesicles for fertilization. Errors in any of these stages can lead to male infertility. Here, we identify that *Drosophila* Pif1A acts as a key regulator for sperm individualization. Loss of Pif1A leads to male sterility associated with irregular individualization complex and empty seminal vesicles without mature sperm. Pif1A is highly expressed in the testes of mated male adult flies and the Pif1A protein is expressed at a higher level in male than in female flies. Pif1A is homologous to mammalian coiled-coil domain-containing protein 157 (CCDC157), which is also enriched in the testes of humans and mice. Human CCDC157, with unknown function, was identified to be down-regulated in men with idiopathic non-obstructive azoospermia (NOA). We map the function of *Drosophila*Pif1A during spermatogenesis, showing that Pif1A is essential for spermatide individualization and involved in the regulation of the lipid metabolism genes. Our findings might be applicable for studying the function of CCDC157 in spermatogenesis and other aspects of human male fertility.

307 Role for *nmd* in mitochondria-peroxisome interactions during *Drosophila melanogaster* spermatogenesis. *Willow H. Pagon*, M. Ummer Qureshi, Karen G. Hales Department of Biology, Davidson College, Davidson, NC.

Mitochondria undergo shape changes during *Drosophila melanogaster* spermatogenesis. Hypomorphic mutations in the essential gene *nmd* are associated with unusual mitochondrial phenotypes and recessive male sterility. Msp1, an ortholog of Nmd in *S. cerevisiae*, localizes to mitochondria and peroxisomes and appears to have a role in proper localization of some peroxins to peroxisomes. In the absence of functional Nmd in testes, not only are mitochondria aberrant, but peroxisomes do not form properly. One male sterile *nmd* allele has a P element in the 5' untranslated region of the gene. To explore why this allele is homozygous viable, we hypothesized that the P-element insertion allows transcription from a cryptic promoter in somatic but not germline tissues. Initial quantitative reverse transcriptase polymerase chain reaction data indicate that *nmd* mRNA levels in the testes of these males are significantly lower than mRNA levels in wild-type testes. mRNA levels in the heads of mutant males appear to be unaffected by the P element. These results support our hypothesis regarding the basis of the hypomorphic phenotype.

308 Role for the *SLC25A46* ortholog *CG5755* in *Drosophila* spermatogenesis. *Caroline Phan*, Vivienne Fang, Karen G. Hales Department of Biology, Davidson College, Davidson, NC.

Drosophila spermatogenesis is a context for exploring the molecular basis of mitochondrial morphology. During spermatogenesis mitochondria undergo dramatic shape changes. The delicate balance between fusion and fission is integral to the health of both the mitochondrial network and the cell. In the *Z2-3738* mutant line, in which the testis-specific gene *CG5755* is mutated, the mitochondria become short and fragmented, implicating *CG5755*'s role in mitochondrial shaping. *SLC25A46,* the human ortholog of *CG5755*, localizes to the outer mitochondrial membrane and encodes a protein that interacts with mitofilin, mitofusin, and lipid pathways (Abrams *et al*., 2015, *Nature Genetics* 47: 926). This study was undertaken to characterize *CG5755* by identifying functional connections among *CG5755*, mitofilin, and mitofusin (*fzo),* as well as CG5755 protein localization. We created flies homozygous for *CG5755* mutations with mitofilin simultaneously knocked down by RNAi. Preliminary data did not indicate a genetic interaction. We also generated *CG5755; fzo* double mutants, with a possible synthetic phenotype observed; we detected small or absent nuclei and misshapen nebenkerne (spherical structure formed from the intertwining of two fused mitochondrial aggregates). We developed a *CG5755-GFP* construct to visualize the subcellular localization of *CG5755*. Our data ground *CG5755*'s role in mitochondrial shaping and confirm the conservation of some functional interactions.

309 Small Ubiquitin-like Modifer (SUMO) posttranslational modifications play critical roles in sperm development and transfer to seminal vesicles during Drosophila spermatogenesis. *J.E. Rollins*[1], N Desouza[1], D Quaranto[1], J Pangilinan[1], C Torres[1], S Zenie[1], J Steinhaur[2], P Morris[3] 1) Natural Sci, Col Mt Saint Vincent, Riverdale, NY; 2) Biology Department Yeshiva University, NY, NY; 3) Center for Biomedical Research, Population Council, New York, NY.

Drosophila spermatogenesis is a dramatic, temporally-orchestrated developmental stage-specific process. Sperm production includes marked changes in mitosis and meiosis, chromosomes, transcription, translation, and posttranslational modifications, with striking nuclear remodeling during spermiogenesis. The posttranslational modifier (SUMO) protein has been shown to play diverse roles in many highly conserved cellular processes such as spermatogenesis in various species. The purpose of this study was to define the precise stage-specific timing of Smt3 (Drosophila SUMO)- mediated events during germ cell development, determine whether Smt3-deficiency affects sperm production in heterozygotes and germline knockdown of Smt3 gene. Western blot analysis of SUMOylated proteins in testes from wild type and Smt3 heterozygotes showed evidence of reduced levels of SUMOylation in heterozygotes as well two proteins that fail to get SUMOylated compared to wild type. These candidates will be identified and their role in spermatogenesis will be investigated. For bioimaging, unconjugated Smt3 and Smt3-modified proteins were detected by immunofluorescence using both whole mounts and squash preparations of testis from wild-type and heterozygous Smt3-deficient mutant stocks as well as in flies expressing Smt RNAi. In wild-type flies, Smt3-SUMOylated proteins show strikingly different patterns in most stages of spermatogenesis including spermatogonia undergoing mitosis, resting and meiotically active spermatocytes, and round and elongating spermatids in various stages of nuclear condensation during spermiogenesis as well as the head cyst cells. The testes of heterozygotes showed reduced levels of Smt3 and an altered SUMOylated protein profile compared to wild-type. Interestingly, the reduction of Smt3 signals was readily observed in meiotic spermatocytes; no change for mitotic spermatogonia was apparent. Heterozygote males exhibited a reduced fertility and their testes show a marked defect in sperm transfer to the seminal vesicles. SUMO-modifications were confirmed using human and rodent testis with normal spermatogenesis. When expression was knocked down in the germline no mature sperm were found. Nuclei fail to condense do not condense properly in post-meiotic spermatids and actin cones are formed and scattered throughout the testes but are not bundled around the nuclei. Our data are suggestive that 1) precise timing of SUMOylation events in developing fly germ cells is required for normal spermatogenesis; 2) Smt3-deficiency can result in failure of spermatids to properly undergo spermiogenesis and sperm transfer, findings consistent with marked reduction in fertility. Taken together, our results indicate important roles for Smt3 and SUMOylation during and after meiosis in Drosophila testis.

310 Roles for *CG5050* and *CG5043* during spermatogenesis in *Drosophila melanogaster*. *Caroline A. Miller*, Katherine Copenhaver, Karen G. Hales Biology, Davidson College, Davidson, NC.

Mitochondria undergo shape changes in various cell types, and disruption of genes associated with this morphogenesis has been connected with many human neurodegenerative disorders. *Drosophila melanogaster* spermatogenesis is a context for exploring the basis of mitochondrial dynamics; after meiosis, mitochondria aggregate near the nucleus and fuse into two derivatives, which wrap around each other to form the nebenkern during onion stage. The derivatives elongate beside the growing flagellar axoneme. Males homozygous for the *Z2-2588* mutation have abnormal mitochondrial shaping during onion stage and late elongation. Deficiency complementation mapping and RNA knockdown were used to identify *CG5043* as the mutated gene. A paralog of *CG5043*, *CG5050,* was knocked down using RNA interference to identify a milder mutant phenotype. CRISPR/Cas9 targeted mutagenesis was used to induce mutations in the coding region of *CG5050* in order to better characterize its function. To determine their relative timing of expression, we performed RNA *in situ* hybridization using a DIG-labelled RNA probe to determine the stages of spermatogenesis at which *CG5043* and *CG5050* are transcribed. Preliminary data suggest robust expression of *CG5043* in wild type spermatocytes, with reduced mRNA levels in mutants, consistent with possible nonsense mediated decay of the transcript.

311 Role of *CG4701* during mitochondria and peroxisome shaping in *Drosophila melanogaster* spermatogenesis. *Elizabeth Young*, Victoria Williams, M. Ummer Qureshi, Karen G. Hales Department of Biology, Davidson College, Davidson, NC.

During the development of sperm in *Drosophila melanogaster*, mitochondria undergo dramatic shape changes. Growing evidence suggests that certain gene products link mitochondria and peroxisomes. Males homozygous for mutations in either *nmd* or its paralog *CG4701*, which encode AAA ATPases, exhibit mitochondrial phenotypes in spermatids. *nmd* is expressed ubiquitously in the fly whereas *CG4701* is a testis-specific gene expressed only during later stages of spermatogenesis. Flies lacking functional *nmd* in germline cells also cannot properly form peroxisomes during spermatogenesis. We aimed to define the role of *CG4701* in mitochondria and peroxisome shaping by characterizing multiple alleles. To do so, we used CRISPR/Cas9 to perform targeted mutagenesis in the protein coding region of the gene. The *CG4701* mutant strains commonly exhibit misshapen mitochondrial aggregates with large vacuolar inclusions beginning in onion stage and remaining throughout leaf blade and elongation. We also examined the role of *CG4701* in peroxisomes by driving the peroxisome matrix marker *UAS-SKL-GFP* with the testis driver *Bam-Gal4* to examine the peroxisome matrix phenotype in *CG4701* mutants, as well as in flies with reduced expression of *nmd* plus mutated *CG4701*. To clarify whether *CG4701* is associated with peroxisome biogenesis or simply protein import into the organelle, we also localized the peroxisome membrane marker PMP34-Cerulean to examine peroxisome membranes in *CG4701* mutants. This phenotype appears to vary, generally showing some more mislocalization of PMP34-Cerulean to mitochondria and/or less localization to smaller fluorescent punctae, suggesting an inability of PMP34-Cerulean to localize to peroxisomes.

312 The role of *center divider* on sperm length in males and seminal receptacle length in females of *Drosophila melanogaster*. *M. Stimson*, MK Manier George Washington University , Washington, DC.

Drosophila produce extremely long sperm, measuring up to 5.8 cm in *D. bifurca*. Sperm are stored within the female seminal receptacle (SR), where they compete for fertilization with sperm from other males under a form of postcopulatory sexual selection known as sperm competition. Sperm length and SR length are genetically correlated and coevolve across the Drosophila lineage, perhaps via Fisherian runaway selection. The gene *center divider* (*cdi*) encodes a serine/threonine protein kinase that is homologous to the human *testis associated actin remodeling kinase 1* (*TESK1*), which shows testis-specific expression within round spermatids. *cdi* is involved in cytoskeleton control through phosphorylation of *Cofilin*. Because cytoskeletal dynamics are likely important for spermatid elongation, we sought to examine the role of *cdi* on sperm length using knockdown. We also examined SR length in *cdi* knockdowns, localized *cdi* within both testis and the female reproductive tract, and characterized allelic variation in *cdi* in isolines producing long or short sperm. Within the testis, *cdi* is expressed at the apical end, where germline hub cells actively divide, and in the seminal vesicle, where the final stages of sperm differentiation occur and mature sperm are stored. c*di* knockdowns produced longer SRs, but knockdown had inconsistent effects on sperm length, perhaps because it is expressed very late in spermatogenesis, and our knockdown targeted expression earlier in spermatogenesis. We found strong allelic differences associated with sperm length, but RNAseq data found no significant differences in gene expression, suggesting that *cdi*'s effect on sperm length variation may be explained entirely by sequence level variation. *cdi* represents a potential molecular mechanism for Fisherian runaway selection between sperm and SR length in *Drosophila*.

313 Arms are required for swimming: The role of Wampa in spermatogenesis. *E. Bauerly*, M. Gibson Stowers Institute for Medical Research, Kansas City, MO.

Axonemal dyneins are cytoskeletal motor proteins that are associated with microtubules that form the axoneme of cilia and flagella. This family of proteins utilizes ATP to perform a variety of cellular processes including transportation of cargo, positioning microtubules, and orienting nuclei, and as an axonemal dynein, they provide the beating force for flagellum. The molecular structure of axonemes are highly conserved across eukaryotes and is composed of bundles of microtubules that are arranged in a 9+2 manner. The microtubules in the outer 9 doublets contain an inner and an outer dynein arm that are required for proper movement of the flagellum. Defects in the dynein arms are the leading cause of primary ciliary dyskinesia, which is characterized by chronic respiratory infections, sterility, and situs inversus. Despite the importance of this class of proteins, very little is known about their functions. In fact, in *Drosophila* almost 35% of all genes known or predicted to be dyneins have no known function. We analyzed a previously uncharacterized component of the outer dynein arm, Wampa (wam), by utilizing CRISPR/CAS9 mediated mutagenesis to generate a knockout mutant allele. Surprisingly, homozygous animals were viable and displayed no observable phenotype, however, the mutation resulted in male sterility. Transmission electron microscopy revealed that *wam* homozygous mutants lack outer dynein arms on the axoneme, which leads to loss of flagellum motility in the sperm. Immunofluorescence also revealed a multifaceted role for this gene throughout various stages of spermatogenesis including malformation of the nuclear head and disrupted mitochondrial aggregation. Due to the conserved nature of axonemal dyneins and their essential role in male fertility, our study will aid in the understanding of the functional role of dyneins throughout spermatogenesis and during axonemal formation.

314 Regulation of Somatic Cyst Stem Cell Behavior in Drosophila Testes by Chinmo Interacting Proteins. *M. Claybrook*, L. Rinehart, O. Kerscher, M. Wawersik Biology, William and Mary, Williamsburg, VA.

Gonadal stem cells are essential for homeostasis of reproductive tissue and sexual reproduction. For these stem cells to retain functionality, they must actively self-renew and maintain their sexual identity. In some cases, male gonadal stem cells can adopt a female sexual identity, leading to loss of reproductive capacity and morphological changes that cause the testis to resemble an ovary. Indeed, previous work has shown that somatic sex must be actively maintained in adult male flies. The transcription factor *chronologically inappropriate morphogenesis* (*chinmo*) prevents feminization of somatic cyst stem cells (CySCs) and is necessary for their self-renewal (Ma et al, 2014; Flaherty et al, 2010). When Chinmo functionality is reduced, CySCs fail to self-renew and aggregates of ovarian-like follicle cells appear, causing overproliferation of under-differentiated germ cells. Our current study strives to understand mechanisms of Chinmo action in CySCs. Specifically, it examines the function of Chinmo interacting proteins we have identified using the yeast two-hybrid system. To investigate their function, tissue-specific gene knock down was performed using the Gal4-UAS system and aspects of CySC behavior including self-

renewal, differentiation, and sex maintenance were assayed. Chinmo has a mammalian ortholog, ZFP509, that is also expressed in testes. Gaining better understanding of Chinmo's function in regulating stem cell self-renewal and sex maintenance may, therefore, provide valuable insight into human health issues like gonadal cancers and infertility.

315 Cell non-autonomous regulation of male germ cell proliferation in spermatogenesis. *H. Kose*, K. Kawaguchi, M. Inai, H. Yamada Dept Natural Sciences, International Christian University, Mitaka, Tokyo, JP.

The turnover of differentiated gametes occurs continuously according to the demands from the surroundings. The appropriate kinetics in mitosis is set by the interaction between germ cells and neighboring stromal cells. While much progress has been made for molecular mechanisms of stem cell maintenance, less is elucidated as to the role of cyst cell, one of the somatic cells in male gonad. Previous study showed that in *samuelmoses* mutant spermatogonia over-proliferate due to prolonged activation of Notch signaling pathway. Here we found that the proper termination of Notch activity in this context requires DHR78, an orphan nuclear receptor. Furthermore, DHR78 and Samuel require each other for protein stability as previously reported in salivary gland. A pair of cyst cells need to encapsulate male germ cells for proper sperm differentiation. And they are thought to function as a unit from the observation in the other similar over-proliferation mutant whereby the loss of the signaling in one of the cyst cells alone causes the equivalent phenotype to that of mutant in which both cyst cells lose its function. However, when knock-down of *samuel* in one of the cyst cells lead to the cyst that half of the spermatognia appear to be affected. These results show that in this system there exist at least two independent signalings in operation, which are distinct in terms of the role each cyst cell plays.

316 Elucidating the role of mip120 in *D. melanogaster* oogenesis and beyond. *H. Folse*, J. Lipsick Stanford University, Palo Alto, CA.

The dREAM/MMB complex is a multi-protein complex in *D. melanogaster* that regulates gene expression and cell cycle regulation. Mip120, Mip40, Mip130, LIN-52, and p55-CAF1 make up the MuvB core of the dREAM/MMB complex. Null mutants of *mip120* were reported to exhibit several phenotypes such as reduced longevity, male and female sterility, and a much smaller abdomen in comparison to wild-type. In *D. melanogaster* ovaries, Mip120 is typically expressed in the nurse cells and in the somatic follicle cells. Our lab has previously found that ovaries of *mip120* null mutant flies were much smaller than wild type and egg chamber development arrested between stages 7 and 8. Additionally, the nurse cells of *mip120* null mutants exhibited persistent chromosome condensation and failure of chromosomes to disassemble and disperse. We also found that *mip120* null mutants exhibited upregulation of *benign gonial cell neoplasm* (*bgcn*) via qRT-PCR. *bgcn* functions in stem cell differentiation together with *bag-of-marbles* (*bam*), although *bam* did not exhibit mis-regulation. The goal of this project is to understand the mechanisms by which Mip120 regulates oogenesis and the expression of *bgcn*. Expression of *bgcn* is restricted to the ovarian germarium in wild-type flies. However, using a GFP-tagged *bgcn* reporter, preliminary data has shown mis-expression of *bgcn* in the follicle cells of the ovary in *mip120* null mutants. To determine whether other germline genes are being mis-expressed in the somatic cells of the ovary in *mip120* null mutants, analysis of the ovarian transcriptome will be done using RNA-seq. To observe whether the arrested egg chamber phenotype is due to loss of *mip120* in either the germ cells or the somatic cells, the GAL4=>UAS-RNAi system will be used to specifically knock down *mip120* expression in different cell types and at different stages. Lastly, this system will also be used to knock down expression of the other MuvB complex members to determine whether loss of these other proteins leads to similar defects in oogenesis.

317 Prostaglandins regulate nuclear actin during oogenesis. *Tina Tootle*[1], Dylane Wineland[1,2], Garrett Kimble[1], Daniel Kelpsch[1] 1) Anatomy and Cell Biology, University of Iowa, Iowa City, IA; 2) Genetic Counseling, Arcadia University, Glenside, IA.

Prostaglandins (PGs), lipid signals produced by cyclooxygenase enzymes, have numerous functions, including regulating pain and inflammation, reproduction, heart health and disease, and cancer development and progression. One means by which PGs act is through regulating the actin cytoskeleton. Actin is not solely cytoplasmic, but also localizes and functions within the nucleus. During Drosophila oogenesis there are multiple pools of nuclear actin that exhibit distinct developmental and subnuclear localizations. Specifically, DNase I labels monomeric actin in the nucleolus of every cell. Anti-actin C4 labels monomeric, nucleolar actin in a subset of cells during early oogenesis and recognizes polymeric nuclear actin in both the oocyte and undifferentiated germline cells. Anti-actin AC15 labels polymeric nuclear actin that localizes to the chromatin starting at mid-oogenesis in both germline and somatic cells. Additionally, expression of GFP-Actin results in nuclear actin rod formation during early oogenesis. We find that PGs are required to limit the levels of the different nuclear actin pools. Loss of the Drosophila cyclooxygenase enzyme, Pxt, results in increased GFP-Actin rod formation, increased nuclear DNase I staining, an increased frequency of cells exhibiting C4 nuclear actin, and premature and increased levels of AC15 nuclear actin. Thus, PG signaling is a critical regulator of nuclear actin. Our findings that actin monomers localize to the nucleolus and PGs regulate nuclear actin, along with our previous finding that PGs regulate the structure of the nucleolus, led us to hypothesize that PGs tightly control nuclear actin to regulate the nucleolus. Supporting this hypothesis, loss of the nuclear actin export mediator Exportin 6 results in nucleolar defects. These data suggest that nuclear actin plays a critical role in regulating nucleolar structure. Together our findings lead to the model that PGs tightly control nuclear actin to regulate nucleolar structure and likely function. As the role of PGs in regulating the nucleolus is and both the nuclear localization and functions of actin are likely conserved across organisms, our findings reveal a novel pathway that may play critical roles in both normal and pathological instances. Indeed, high levels of PGs, increased nuclear actin, and nucleolar changes have been independently associated with cancer development and progression.

318 A germline stem cell quality control checkpoint is linked to the structural integrity of the nuclear lamina. *R. Cupp*, T. Duan, P. Geyer Biochemistry, University of Iowa, Iowa City, IA.

Germ cells represent the only cellular lineage that bridges generations. To safeguard offspring fitness, germ cells use quality control pathways to ensure transmission of strong gametes. Drosophila germline stem cells (GSCs) possess a newly described quality control checkpoint pathway that monitors the function of the nuclear lamina (NL). This extensive protein network lies beneath the nuclear envelope and provides structural support for nuclear functions such as transcription, replication and genome integrity. We have found that loss of the NL protein Otefin (Ote) activates a novel checkpoint pathway, involving the kinases ATM- and Rad3-related (ATR) and Checkpoint kinase 2 (Chk2). In the absence of Ote, germ cell differentiation is blocked and GSCs die. We have found these effects are linked to the thickening and lobulation of the NL, suggesting that NL deformation is responsible for checkpoint activation. To test this model, we are defining effects of loss of two Ote interacting partners, the chromatin binding protein Barrier-to-autointegration factor and the multi-AT hook protein, D1. Both of these chromatin proteins are involved in nuclear envelope reformation following mitosis. We show that knockdown of either BAF or D1 causes GSC loss that is associated with NL thickening and lobulation. Notably, this GSC loss is suppressed in *chk2* mutants, indicating that the NL checkpoint pathway is activated. Further, we find that over-expression of D1 improves NL phenotypes and provides partial rescues of $ote^{-/-}$ GSC loss. Taken together, these data provide support for the postulate that structural deformities of the NL are responsible for activation of the GSC quality control pathway.

319 The *Drosophila* ribosomal protein paralogue RpS5b functions specifically in germ cells. *S. Jang*, M. Buszczak Molecular Biology, UT Southwestern Medical Center, Dallas, TX.

Ribosomes translate mRNA to protein and contain many ribosomal proteins and ribosomal RNAs (rRNA). These ribosome components are thought to be ubiquitously expressed and homogenous across tissues. However, recent studies have identified 'specialized ribosomes' that translate specific pools of mRNAs in different tissues. These specialized ribosomes can contain distinct ribosomal proteins, unique post-translationally modified ribosomal proteins, or specific types of rRNAs. In addition, some ribosomal proteins can function outside of the ribosome. *Drosophila* encodes for a number of ribosomal protein paralogues. Individual paralogues often exhibit tissue-specific expression. Here, we characterize *ribosomal protein S5b* (*RpS5b*), which shows enriched expression in germ cells. A CRSIPR/Cas9 generated *RpS5b* mutant does not show a classical *Minute* phenotype, unlike many other ribosomal protein mutants.
However, *RpS5b* mutant females exhibit sterility, marked by developmental arrest and degeneration of egg chambers. Mutant egg chambers have abnormal nucleolar morphology and bi-layered follicle cell phenotype. We found Notch signaling is impaired in mutant egg chambers, resulting in abnormal follicle cell divisions after stage 6. RpS5b is expressed and functions exclusively in germ cells, suggesting the follicle cell phenotype arises in cell non-autonomous manner. RpS5b exclusively sediments with monosomes and polysomes, suggesting this ribosomal protein has a role in mRNA translation. We propose there are specialized ribosomes in *Drosophila* and will continue to test whether specialized ribosomes contribute to germ cell development.

320 Neuronal Octopamine - Matrix metalloproteinase signaling regulates germline stem cell proliferation in female *Drosophila melanogaster*. *Yuto Yoshinari*[1], Tomotsune Ameku[1], Shu Kondo[2], Yuko Shimada-Niwa[3], Hiromu Tanimoto[4], Takayuki Kuraishi[5], Ryusuke Niwa[6,7] 1) Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan; 2) Genetic Strains Research Center, National Institute of Genetics, Japan; 3) Life Science Center of Tsukuba Advanced Research Alliance, University of Tsukuba, Japan; 4) Graduate School of Life Sciences, Tohoku university, Japan; 5) Faculty of Pharmacy, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Japan; 6) Faculty of Life and Environmental Sciences, University of Tsukuba, Japan; 7) AMED-CRES, AMED, Japan.

Germline stem cells (GSCs) are key to produce gametes for the propagation of the species. Recently, our group has reported that mating stimulus induces GSC proliferation in *Drosophila* ovary. The proliferation is under control of the male seminal fluid protein Sex Peptide and its specific receptor SPR [Ameku and Niwa, *PLOS Genetics* 2016; Ameku, Yoshinari et al., *Fly* 2017; Ameku et al, *PLOS Biology* 2018]. However, it remains unclear which neuroendocrine pathways transmit the SP-SPR-mediated mating input to the ovary.
To solve this problem, we conducted transgenic RNAi screen. We knocked down a number of transmembrane receptor genes with pan-neuronal and ovarian somatic cell specific drivers. Then, we examined the number of GSCs in virgin and mated females of each RNAi condition. We found that 109 transmembrane receptors as possible candidate regulators for GSC proliferation.
Among these candidates, we focused on Oamb, a G protein-coupled receptor for the insect monoamine neurotransmitter octopamine. We found that Oamb in the ovary positively regulates post-mating GSC proliferation via downstream Ca^{2+} signaling. We also found that a small subset of octopaminergic neurons projecting to the reproductive system, and neuronal activity of these neurons was required for post-mating GSC proliferation. Moreover, we identified Matrix metalloproteinase-2 (Mmp2) as a downstream component of octopamine-Ca^{2+} signaling to induce GSC proliferation. These results suggest that neurotransmitter-dependent reorganization of extracellular environment of GSC niche is required for mating-induced GSC proliferation.

321 Novel structures in the germ line of Drosophila ovaries. *A.P. Mahowald* Whitehead Institute for Biomedical Sciences, Cambridge, IL.

During an ultrastructural study of the Drosophila germarium, focused on the structure of the escort cells in relation to the development of 16-cell ovarian cysts, I discovered a series of novel structures in both the germ line stem cells and in the developing cystocytes in region 2a of the germarium. These structures have been found in a specific strain of Drosophila melanogaster, the Oregon R wild type flies that had been growing in population cages. Initially, they seemed to be related to the fibrous structures characteristic of the Drosophila germ line (also called vasa-positive bodies), but further study has identified a number of unique features that I believe are related to each other.
A small cylindrical structure. 0.3mm x 1mm in size, composed of two juxtaposed rough endoplasmic reticula membranes, with a 40nm amorphous, electron dense area between the two ER membranes. Within the amorphous layer 44 equally spaced "microrods" are found, forming a cylinder. Each microrod is 10 nm in cross-section with a central hole, approximately 5nm in size.
A second structure appears related to the cylinder. It is as though the cylindrical electron dense structure has become stretched out as a flat leaf. Tangential views of this structure show electron densities with the same periodicity as seen in the microrods in the cylinder. These flat structures can also appear as single leaves, or in multiples of two or three.
My current efforts are to understand how the structures form, how they relate to each other, and what function they might have.
(This work has been done in the laboratories of Terry Orr-Weaver and David Page at the Whitehead Institute.)

322 Cytoplasmic polyadenylation as a mechanism for translational regulation of *gurken* (*grk*) mRNA during oogenesis. *A. Norvell*, A. Tino, Q. Oivacce, S. Ro, S. Khan Dept Biol, Col of New Jersey, Ewing, NJ.

Spatial and temporal control of Grk activity during oogenesis is essential for proper patterning of the egg and future embryo, and the complex pattern of Grk protein expression is tightly controlled through a combination of *grk* mRNA localization and translational repression. During the mid to late stages of oogenesis, *grk* mRNA is localized to the dorsoventral corner of the oocyte, where its restricted expression directs the D-V pattern of the egg and future embryo. A number of proteins required for Grk protein translation (Encore [Enc], Vasa, oo18 RNA binding protein [Orb], PolyA Binding Protein 55B [PABP55B] and for translational repression (Squid [Sqd], Bruno [Bru] and Cup) have been identified via genetic screens and biochemical studies. We have found that *grk* mRNA is subjected to cytoplasmic polyadenylation during mid-oogenesis and that this process appears to be important in achieving high levels of localized Grk protein required for D-V patterning. We are currently investigating which of the known translational regulators participate in *grk* polyadenylation at this stage and whether this molecular mechanism is utilized for other aspects of *grk* translational regulation, such as the Grk translational repression initiated during the meiotic checkpoint.

323 Defining the dynamic changes in mitochondrial metabolism that drive cellular quiescence. *S. Yue*, M. Sieber Department of Physiology, UT Southwestern Medical Center, Dallas, TX.

The ability to enter, maintain, and exit cellular quiescence are key features of oocytes and adult stem cells in many organisms. Both adult stem cells and oocytes are stored for long periods of time and quiescence is thought to prevent oxidative damage and the depletion of stored nutrients to ensure the function of these cells when they are activated during wound repair and after fertilization. Interestingly, recent work has shown that changes in mitochondrial metabolism play a major role in maintaining adult stem cells and mature oocytes during periods of quiescence. However, due to the difficulty of isolating quiescent cells *in vivo* the aspects of mitochondrial metabolism that drive quiescence remain largely unknown. The *Drosophil*a oogenesis system allows us to combine advanced model system genetics with the ability to isolate large quantities of quiescent oocytes that we can use for biochemical studies of the mitochondria. We utilize this system to examine the metabolic mechanisms that underlie cellular quiescence.

In our initial studies we discovered that, as oocytes become quiescent in late oogenesis, mitochondria enter a low activity state of mitochondrial respiratory quiescence (MRQ). MRQ is regulated by glycogen synthase kinase 3 (GSK3), which suppresses mitochondrial activity by inducing Electron transport chain (ETC) remodeling. Metabolomic analysis of GSK3-RNAi oocytes suggests GSK3 promotes MRQ by inhibiting fatty acid oxidation and purine biosynthesis, critical aspects of the early embryonic metabolic program. Consistent with these observations we have found that GSK3 regulates the protein levels of mitochondrial trifunctional protein (MTPα) and electron transfer flavoprotein subunit alpha (ETFα) which are key components of the fatty acid beta-oxidation (FAO) pathway. RNAi-mediated silencing of these enzymes triggers premature depolarization of mitochondrial membrane potential in early follicles similar to phenotypes we observed with insulin inhibition. Currently, we are studying how GSK3 regulates FAO via MTPα and ETFα in oocytes. We are also examining how these downstream factors contribute to ETC remodeling and oocyte developmental competence. Overall, these studies provide novel insights into the mitochondrial mechanisms that govern cellular quiescence and provide a foundation to understand how mitochondria are remodeled during reproductive development.

324 Different programs of oogenesis for *Drosophila melanogaster* and the jewel wasp *Nasonia vitripennis*. *P.M. Ferree*, K.J. Eastin W. M. Keck Science Center, Pitzer College and Scripps College, Claremont, CA.

D. melanogaster has long served as a model for understanding insect oogenesis. Indeed, few studies have been performed in other insects, and as a result, little is currently known about the diversity of egg formation across the insects. To begin to address this point, we performed cytological analyses of developing oocytes in the jewel wasp, *Nasonia vitripennis*. In particular, we examined the distribution of the germ cell nuclei and ring canals during early, middle, and late stages. Like in *D. melanogaster*, egg chambers in *N. vitripennis* consist of fifteen nurse cells and one oocyte. However, we observed several striking departures between these two insects. First, unlike in *D. melanogaster*, in which ring canals adjacent to the oocyte increase in diameter throughout oogenesis, ring canals do not change in size in wasps. Additionally, in wasps, the ring canals connected to the oocyte disappear between mid and late stages, and a 'tunnel' forms to connect the oocyte to adjacent nurse cells. Finally, and most interestingly, whereas in *D. melanogaster* four ring canals connect the oocyte to adjacent nurse cells, in *N. vitripennis*, the oocyte only connects to nurse cells through three ring canals. Closer inspection with 3D-reconstructions from confocal imaging revealed a unique connection of nurse cells and ring canals in *N. vitripennis* that can only be explained by asymmetric mitotic divisions during egg chamber formation. We propose that this distinct mitotic division pattern may occur through directed control of the spindle apparatus in the dividing germ cells. Broadly, our work underscores remarkable differences in gametogenesis among the insects.

325 Molecular chaperone *Tetratricopeptide repeat protein 2* (*Tpr2*) is essential for germline stem cell self-renewal and timely cyst divisions in *Drosophila* oogenesis. *Morgan Phillips*, Elizabeth Ables East Carolina University, Greenville, NC.

Steroid hormones influence cell proliferation and cell fate in developing and injured tissues. Although steroid hormone signaling has been well-studied, the precise mechanisms by which cells specifically receive steroid hormones remains largely uncharacterized. In *Drosophila* and many other insects, the primary steroid hormone is ecdysone, which is necessary for reproduction. Ecdysone effects have been well studied in the ovary; for example, ecdysone signaling through the Ecdysone Receptor promotes germ cell proliferation, differentiation, and survival. It is unclear, however, how Ecdysone Receptor expression or signaling is regulated in germ cells. We previously identified the molecular chaperone encoded by *Tetratricopeptide repeat protein 2* (*Tpr2*) in a reverse genetic screen as a possible connection between ecdysone signaling and germline stem cell self-renewal. The human homolog of Tpr2, DNAJC7, can form complexes with Hsp90 and Hsp70 in vitro. Tpr2 is thought to function as a recycling cochaperone, aiding protein folding and dimerization of the glucocorticoid and progesterone receptors. Ecdysone signaling is necessary for *Drosophila* germline stem cell function and cyst divisions. We therefore hypothesized that Tpr2 may promote ecdysone signaling in early germ cells. As an initial test of this hypothesis, we used CRISPR mutagenesis, genetic mosaics, and germline-enhanced RNAi techniques to investigate whether *Tpr2* is necessary for germ cell mitotic divisions. In the absence of *Tpr2*, germline stem cell self-renewal is abrogated, suggesting that *Tpr2* is autonomously necessary for germline stem cell activity. Further, germ cell mitotic divisions are delayed in *Tpr2* mutants, leading to fewer cysts per germarium. Our preliminary data suggest that *Tpr2* mutant germ cells are slow to complete S phase, indicative of an overall slower cell cycle. Taken together, our data suggests that, like *Ecdysone Receptor*, *Tpr2* is essential for cell cycle control in germ cells. Our future directions will test whether Tpr2 promotes ecdysone signaling in germ cells. Our studies help elucidate the molecular mechanisms by which steroid hormones promote cell division.

326 Cell atlas of the *Drosophila* ovary by single cell sequencing. *K. Rust*[1], L. Byrnes[2], K. Lee[3], K.S.Y. Yu[3], J. Sneddon[2], AD. Tward[3], TG. Nystul[1] 1) Departments of Anatomy and OB/GYN-RS, UCSF, CA; 2) Diabetes Center, UCSF, CA; 3) Department of Otolaryngology-Head and Neck Surgery, UCSF, CA.

The *Drosophila* ovary is a complex organ in which multiple cell types contribute to the production of mature oocytes. In the germarium, germ cell differentiation is first supported by inner germarial sheath cells and then assisted by different types of follicle cells, which are produced by follicle stem cells. Relatively little is known about the early steps of follicle cell differentiation. In addition, number and position of follicle stem cells has recently been called into question. To characterize the different cell types of the *Drosophila* ovary more carefully we performed single cell RNA-sequencing and obtained transcriptomes of 10,694 cells of the early, previtellogenic stages. Using the bioinformatic tool CellFindR, we identified 21 clusters with distinct expression profiles, corresponding to all known cell types in the ovary and identifying previously undescribed subgroups among them. Using Monocle, which orders cells in pseudotime to predict lineage differentiation, we identified known as well as novel markers of distinct cell types and subtypes. We screened reporter lines for approximately 80 candidate genes and identified over 20 genes with cell type specific expression. Among these are novel markers for inner germarial sheath cells and several types of follicle cells. One particularly interesting gene was expressed in a subset of prefollicle cells, potentially representing the earliest known marker of differentiation.

We chose Gal4 lines expressing in cells at and around the 2a/2b border, the putative position of follicle stem cells, to drive the G-TRACE system to investigate which cells contribute to the follicle cell population. Interestingly, while gene expression profiles of cells anterior and at the 2a/2b border strongly overlapped, we found that only cells at the 2a/2b border contribute to the follicle cell population, revealing distinct locations for follicle stem cells versus inner germarial sheath cells. Together, our analysis reveals a previously unappreciated heterogeneity among cells of various types in the ovary and presents a valuable source to identify cell type specific expression.

327 Nuclear hormone receptor *ftz-f1* is necessary in both the germline and soma to promote oocyte development. *S. McDonald*, A. Beachum, H. Berghout, E. Ables Dept. of Biology, East Carolina University, Greenville, NC.

Gamete production is intimately tied to the nutrition status of the organism. Integration of nutritionally-dependent physiological signals to molecular mechanisms controlling oogenesis, however, remains largely uncharacterized. Nuclear receptors (NRs) link physiological status to a cellular transcriptional response and are important mediators of reproduction, physiology, and tissue homeostasis. For example, mammalian NR5 family members *SF-1* and *LRH-1* have roles in gonadogenesis and sex steroid production. Two NR5 family members are encoded in the *Drosophila* genome: *Hr39*, which is necessary for female reproductive tract development, and *ftz-f1*, whose role in oogenesis has not been explored. Given that *Hr39* is not intrinsically required for oogenesis, we hypothesized that *ftz-f1* may fill a conserved NR role in *Drosophila*. *Ftz-f1* is expressed throughout the ovary, including germline stem cells (GSCs), germline cysts, and several populations of somatic cells. Using *Flippase/Flippase Recognition Target* (*Flp/FRT*) mediated clonal analysis and tissue specific RNAi, we

analyzed the effects of loss of *ftz-f1* function in oogenesis. Germline-specific knockdown of *ftz-f1* resulted in fewer GSCs as female flies aged. GSCs harboring dual copies of the null *ftz-f1*^{ex7} mutation were more frequently displaced from the niche, as compared to controls. Moreover, *ftz-f1*^{ex7} mutant GSCs displayed increased EdU incorporation, suggesting that *ftz-f1* regulates the S/G2 transition in GSCs. Loss of *ftz-f1*is also associated with the accumulation of GSC progeny (cystoblasts and two cell cysts) and delayed oocyte specification. Taken together, our results indicate that *ftz-f1* directly promotes the timing of germ cell division and specification. Further, reduced *ftz-f1* function in ovarian somatic cells leads to enlarged germaria and increased numbers of 16-cell cysts, suggesting impaired follicular encapsulation, and premature follicle death. Our future experiments seek to elucidate how *ftz-f1* regulates germ cell divisions, cyst encapsulation, and follicle survival. Our data add to a growing body of literature underscoring the importance of nuclear receptors in the control of reproduction.

328 Spermatogenic stage-specific expression in Drosophila species by next generation sequencing. *C. Avelino*, C. Mendonça, G. Goldstein, M. Vibranovski Department of Genetics and Evolutionary Biology, Biosciences Institute, São Paulo, São Paulo, BR.

Spermatogenic stage-specific expression in *Drosophila* species by next generation sequencing

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The *Drosophila* spermatogenesis presents an interesting developmental morphology as cell types are temporally distributed along the testis in a gradient along the proximal-distal axis (Cenci *et al*.,1994). This feature makes *Drosophila* testis an excellent model for studying spermatogenesis dynamics. Vibranovski *et al.* (2009) developed a technique to isolate the major stages of spermatogenesis, and using microarray techniques, generated a transcriptome database that has been explored in diverse studies in the last decade (https://mnlab.uchicago.edu/sppress/). The development of similar databases for other species is crucial for comparative genomics studies related to spermatogenesis. Next generation transcriptomic sequencing also present a significant advance by providing an extensive and deep expression landscape of the spermatogenesis. In order to obtain a comparative spermatogenic stage-specific transcriptome using NGS, we performed the isolation of three major phases of spermatogenesis: mitosis, meiosis, and post-meiosis cells, which are enriched in apical, proximal and distal regions of the testis, respectively. For the isolation of the cells, we used the method developed by Vibranovski *et al*. (2009). The isolation was performed in three species of *Drosophila genus: D. melanogaster, D. willistoni, D. mojavensis*. As cell samples from isolated regions have low RNA concentration, the best strategy to obtain an appropriated RNA quantity and quality for sequencing is the low-input protocol for library construction, that has proven to work on picograms of total RNA (Combs *et al.,* 2015).We found differences of morphology e quantity of RNA for each species, becoming necessary an adaptation of the method developed by Vibranovski *et al.* (2009) for each specie. RNA-seq experiments in single cell isolates from larvae testis have been proven to be more efficient on the separation of different male germline stages than our method. However, our database, made from adult testis, additionally provides the post-meiotic expression component valuable to comparative studies of sperm development.

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329 Regulation of recombination by the synaptonemal complex. *Katie Billmyre*[1], Cori Cahoon[1], Emily Wesley[1,2], Matt Heenan[1], R. Scott Hawley[1,3] 1) Stowers Institute for Medical Research, Kansas City, Missouri 64110; 2) University of Missouri-Kansas City, Kansas City, Missouri 64110; 3) Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, Kansas 66160.

In meiosis, large numbers of double strand breaks (DSBs) are precisely directed into crossover or gene conversion (noncrossover) fates, and repair from the sister chromatid is largely prevented. This precise control of DSB fate occurs in the context of and is dependent on the synaptonemal complex (SC). There has been extensive work regarding the structure and function of the SC, but how it regulates some meiotic events is unknown. Removal of the transverse filament SC protein, C(3)G results in a complete loss of SC structure and repair of meiotic DSBs by a pathway that does not lead to crossing over or gene conversion. To investigate the function of the SC in meiosis we constructed three in-frame deletions within the coiled-coil region of C(3)G using CRISPR/Cas9. These deletions result in fragmentation of the SC at three different times: early pachytene, early-mid pachytene, and mid pachytene. All three of these deletions result in slightly different meiotic defects including defective centromere clustering, pairing, and recombination. We have found that the X chromosome displays different recombination phenotypes than the autosomes in response disruptions in SC structure suggesting crossover distribution is potentially regulated differently on the sex chromosomes versus the autosomes. Additionally, our studies showed that the SC is necessary to maintain meiotic pairing in early prophase. These unique mutations in c(3)G allows us to investigate for the first time the temporal requirement of the SC during meiosis.

330 Investigating the impact of genetic background in synaptonemal complex maintenance. *Emily Wesley*[1,2], Katie Billmyre[1], R. Scott Hawley[1,3] 1) Stowers Institute for Medical Research, Kansas City, Missouri 64110; 2) University of Missouri-Kansas City, Kansas City, Missouri 64110; 3) Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, Kansas 66160.

The formation of haploid gametes during meiosis is essential for successful sexual reproduction. The synaptonemal complex (SC) is a multiprotein complex constructed between homologous chromosomes during meiosis and is required for the maturation of double-stranded breaks (DSBs) into crossover events. Crossovers bind chromosomes together and ensure that meiosis is completed properly. We are interested in the role of a transverse filament protein component of the SC that spans the width between two homologous chromosomes in meiosis. The loss of the transverse filament results in complete loss of the SC and crossover events. We observed that flies which were heterozygous for a null allele of the transverse filament protein displayed differences in the timing of SC disassembly between stocks. We tested three stocks of flies heterozygous for the null allele with differing wild type backgrounds and found that one wild type stock caused early disassembly of the SC in the heterozygous background. This suggests possible mutations in modifier or enhancer regions of the stock. We plan to utilize sequencing and mapping techniques to identify these mutations. Our goal is to uncover a genetic basis for these defects in SC maintenance, so that we may develop a better understanding of how the SC is maintained.

331 *Sina* interactions during female meiosis. *Alyssa Jones*[1,2], Stacie Hughes[1], R. Scott Hawley[1,3] 1) Stowers Institute for Medical Research, Kansas City, Missouri 64110Hawley Lab, Kansas City, MO; 2) University of Missouri-Kansas City, Kansas City, Missouri 64110; 3) Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, Kansas 66160.

Synapsis of homologous chromosomes is crucial for the proper exchange of genetic information during meiosis. The synaptonemal complex (SC) is a multiprotein structure that forms between two homologous chromosomes and facilitates chromosome pairing, synapsis and recombination. Mutations in the E3 ubiquitin ligase *seven in absentia* (*sina*) cause SC components to form rod-like polycomplexes rather than normal ribbon-like tracts along chromosome

arms. The aberrant SC formation in *sina* mutants suggests that Sina degrades a crucial regulator of normal SC formation. Subsequently, these polycomplexes fail to promote proper levels of genetic exchange and chromosome segregation. Studying *sina* mutants allows us to understand how structural components of the SC interact in both polycomplexes and normal SC. We are using a yeast two-hybrid system to test physical interactions between Sina and SC proteins, as well as potential members of the Sina protein complex. Discovering Sina interactors will inform us on what this protein may be targeting in the Drosophila germline and what cofactors are required for its meiotic functions.

332 Determining requirements for pairing and conjunction between the X and Dp(1;3) chromosomes in male meiosis. *C.A. Hylton*, J.E. Tomkiel Dean Biology Department, The University of North Carolina at Greensboro, Greensboro, NC.

Meiosis in Drosophila males lacks crossing over and utilizes two separate mechanisms to regulate disjunction of sex chromosomes and autosomes. Sex chromosomes pair through intergenic spacer (IGS) repeat sequences within the heterochromatic rDNA on the X and Y, whereas autosomes pair between euchromatic homologies distributed along the arms. Pairing appears to be regulated at the chromosome level as well, but the rules for pairing are not completely understood. For example, an rDNA transgene restores pairing and segregation of the Y chromosome from an rDNA-deficient X chromosome, but the same rDNA transgene inserted on an autosome does not segregate from the Y (McKee and Karpen, 1990). Y chromosomes bearing chromosome 2 euchromatin, however, do pair and segregate from an intact chromosome 2 (McKee et al. 1993). Together these observations suggest that chromosome level mechanisms exist to regulate pairing and/or conjunction at different sequences. We developed a FISH pairing assay and monitored pairing and its relationship to disjunction to determine what governs these events. We previously established that Y chromosomes bearing as few as 120 Kb of X euchromatin pair, disjoin, and direct amphitelic segregation from an rDNA-deficient X chromosome. Here, we report on the ability of Dp(1;3) chromosomes to pair with and segregate from the X. We tested a collection of BAC-inserted X euchromatin at position 65C ranging in size from 21 to 177 Kb to determine size and sequence requirements for pairing and conjunction. In addition, we tested several X euchromatic transpositions larger than 200 Kb. Our data show that, in contrast to duplications of X material on the Y, duplications of X material on chromosome 3 are not as effective in directing segregation. In early prophase, however, pairing can occur between homologies on the X and chromosome 3. We suggest this approach will be useful to define the mechanisms regulating the establishment of conjunction and segregation between paired sequences.

333 Identifying new synaptonemal complex components. *Camila Aponte*[1,2], Stacie Hughes[1], R. Scott Hawley[1,3] 1) Stowers Institute for Medical Research, Kansas City, Missouri 64110; 2) University of Missouri-Kansas City, Kansas City, Missouri 64110; 3) Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, Kansas 66160.

The synaptonemal complex (SC) serves an important role in proper chromosome segregation during meiosis. The SC consists of two lateral elements attached to the homologous chromosomes that are joined by transverse filaments and central element proteins. During drosophila female meiosis, absence or improper formation of the SC can lead to chromosome nondisjunction. To identify new SC components or regulators contributing to SC formation, I am conducting a RNAi knockdown screen. Within the female germline, available RNAi stocks will be driven using Nanos Gal4 and screened for their effect on SC formation. Stocks that generate abnormal SC or fail to form SC will be kept for further analysis. The objective is to reveal new proteins involved in the formation or regulation of the SC that may have been missed through previous forward genetic screens.

334 The Role of Rough deal (ROD) in Meiosis of Male *Drosophila*. *Q. He*, B. McKee BCMB Department, University of Tennessee, Knoxville, TN.

Meiosis is a specialized cell division that involves one round of DNA replication following by two rounds of chromosome segregation. Meiosis I is considered as a reductional cell division where homologs separate to opposite spindle poles while meiosis II is an equational cell division in which sister chromatids segregate. Fidelity transmission of genetics information between generations relies on faithful chromosome segregations during meiosis, which prevents the production of aneuploid gametes. ROD is an essential component in spindle assembly checkpoint (SAC) and monitors the connection between kinetochores and microtubules during cell division, but its function in meiosis has not been fully characterized. In present study, the results show that mutations in *rod* give rise to a high rate of chromosome non-disjunction (NDJ) during meiosis of male *Drosophila*, particularly in meiosis I. The rate of sex chromosome NDJ is much higher in spermatocytes of *rod* mutants (27%) compared to that in control flies (1%) and 90% NDJ events are observed in first division. Meanwhile, NDJ events also take place on autosomes and the NDJ rate of 4th chromosome is 16.2% in *rod* mutants and 0.8% in control flies respectively. Remarkably, NDJ analysis on 2nd chromosome reveals that the number of progenies produced by flies with *rod* mutations is 100-fold higher that control flies. Cytological data is consistent with the results of NDJ analysis and uncovers that chromosome NDJ occurs on both sex chromosome (12%) and autosome (34%) in meiosis I. Intriguingly, instead of reductional segregation, sex chromosomes undergo equational segregation in 4% of anaphase I spermatocytes in *rod* mutants, but this phenomenon is not observed in control flies. Moreover, extensive chromosome bridges appear in anaphase I of spermatocytes with *rod* mutations. Taken together, the high rate of chromosome NDJ indicates that ROD plays a critical role in meiotic chromosome segregation and abnormal separation pattern of sex chromosomes implicate that mutations in *rod* might lead to the perturbation on chromosome configuration or orientation during meiosis I. However, Immunostaining by using anti-SNM and anti-MEI-S332 antibodies reveal that the two major mechanisms that stabilize homologous chromosomes and sister chromatids - homolog conjunction and centromeric cohesion are largely unaffected in *rod* mutant flies during meiosis I. In the following studies, we will combine genetic and cytological approaches to examine whether ROD is required for chromosome orientation *Drosophila* meiosis I.

335 dTopors RING domain is required for nuclear structure and chromosome segregation in male meiosis. *A. Binder*, J. Tomkiel Dean Biology, University of North Carolina at Greensboro, Greensboro, NC.

The E3 dual Ubiquitin/SUMO-1 ligase dTopors is required for spermatocyte nuclear morphology and male meiotic chromosome transmission. Null mutations in *dtopors* both perturb nuclear structure, resulting in nuclear blebs, and display prophase I chromatin condensation defects and anaphase I bridges leading to chromosome nondisjunction. dTopors localizes to the nuclear lamina and directly interacts with lamin Dm0 (Capelson and Corces 2005) suggesting a structural role for dTopors in the lamina. dTopors also contains an evolutionarily conserved RING domain presumed to be required for its ligase activity. To investigate whether the RING domain is needed for structural maintenance of the nuclear lamina and/or for proper chromosome segregation during meiosis we created a putative separation-of-function mutant. Using CRISPR-Cas9 we altered *dtopors* resulting in a protein with a five amino acid deletion within the RING domain. This includes the deletion of a conserved cysteine shown to be necessary for ligase activity of related RING domain proteins. Using this mutation we performed genetic and cytological assays of chromosome segregation as well as indirect immunofluorescence examination of components of the nuclear lamina. Our results indicate that the RING domain is required for both the structural maintenance of the nuclear lamina and for proper chromosome segregation in meiosis. While we cannot rule out separate functions of dTopors in nuclear structure and chromosome segregation, the most parsimonious model is that a defect in lamina assembly leads to errors in chromosome segregation. Thus, we suggest that dTopors enzymatic activity is directly required for proper lamina assembly in spermatocytes, and the observed chromosome condensation and transmission defects are secondary consequences of perturbed nuclear organization.

336 The beta-karyopherin protein Ipo9 is required for meiosis in *Drosophila*. *V. Palacios*, M. Buszczak Department of Molecular Biology, University of Texas Southwestern Medical Center at Dallas.

Germ cells are responsible for the propagation of sexually reproducing organisms. From a genome-wide CRISPR/Cas9 loss-of-function screen for genes required for *Drosophila* fertility, we found that loss of the highly-conserved protein Importin-9 (Ipo9) impairs fertility in female and male flies. Ipo9 belongs to the Importin β/karyopherin family, which is known to transport cargoes from the cytoplasm into the nucleus through nuclear pores. Studies using human cell lines show that Ipo9 helps to traffic ribosomal proteins, histone core proteins and transcription factors into the nucleus. However, little is known about the role of Ipo9 in an in vivo context. My project focuses on understanding how Ipo9 influences *Drosophila* germline differentiation, function and embryogenesis. Tagging the endogenous gene shows that Ipo9 exhibits enriched expression in meiotic cells, suggesting a function in this process. FISH reveals that $ipo9^{KO}$ displays chromosome segregation defects in meiosis I during oogenesis. Eggs produced by $ipo9^{KO}$ female flies appear grossly normal. However, embryos derived from these eggs fail to complete embryogenesis. Similar to females, $ipo9^{KO}$ male are also sterile, and form abnormally structured sperm that lack motility. $ipo9^{KO}$ testes show mitochondrial number and size defects at the onion-stage following the second meiotic division, suggesting that these cells fail to undergo proper meiosis as well. In addition, deletion of the N-terminal domain of Ipo9, which is necessary for nuclear trafficking, fails to rescue the sterility associated with $ipo9^{KO}$, consistent with a nuclear trafficking defect. Overall, this study will advance our understanding of how germ cells differentiate to form functional gametes.

337 *Sex lethal* activation in the female germline is dependent on the transcription factor Sisterless A. *R. Goyal*, K. Baxter, M. Van Doren Department of Biology, Johns Hopkins University, Baltimore, MD.

In *Drosophila*, sex determination in both the soma and germline is controlled by the genetic switch ***Sex lethal*** (***Sxl***). XY (male) germ cells expressing *Sxl* are able to produce eggs within an XX (female) somatic gonad, demonstrating that *Sxl* is sufficient for female germline identity (Kobayashi Lab, PMID: 21737698). In both the soma and germline, the presence of two X chromosomes leads to *Sxl* expression. However, the mechanism of X chromosome counting in the germline differs at both *cis*- and *trans*-levels, and we are investigating this autonomous mechanism.

RNA-FISH against nascent *Sxl* transcripts reveals that *Sxl* expression in germ cells beginsat stage 5 of embryogenesis. Interestingly, we do not see evidence that the *Sxl* sex-specific "establishment" promoter ($SxlP_E$) is activated before the "maintenance" promoter ($SxlP_M$), as is the case in the soma. We also find that the DNA elements regulating $SxlP_E$ in the germline are different than in the soma; the genomic region upstream of $SxlP_E$ is insufficient for activation in the germline, and intronic sequences downstream of $SxlP_E$ are also required for germline expression. We are now identifying the specific minimal enhancers that are necessary and sufficient for germline $SxlP_E$ activation.

We are also studying the *trans*-acting factors that regulate *Sxl* in the germline. It has been reported that the X chromosome "counting" genes important for activation of $SxlP_E$ in the soma and germline are different. However, we found that one of these genes, the X-chromosome-encoded transcription factor **Sisterless A** (**SisA**) is essential for germline *Sxl* activation. Germline depletion of *sisA* induces an ovarian tumor phenotype similar to loss of *Sxl* in the germline. Further, this tumor phenotype is accompanied by loss of Sxl expression and can be rescued by germline Sxl expression. Using both RNA-FISH and an endogenously tagged *sisA* allele, we have observed *sisA* expression in early germ cells prior to the onset of *Sxl* expression. We are currently addressing whether SisA is a direct transcriptional activator of $SxlP_E$ in the female germline and whether it acts alone or in combination with other X chromosome counting elements.

Through this work we aim to understand how genetic sex determination is regulated in the germline, and how the sexual identity of the germline interacts with sex-specific somatic development to control proper gametogenesis.

338 Atypical Chitinases regulates cell-cell signaling in the ovary. *Pradeep Kumar Bhaskar*, Mark Van Doren Department of Biology, Johns Hopkins University, Baltimore, MD.

The establishment of sexual identity in the germline is critical for the sex-specific development of germline stem cells and the production of sperm vs eggs.Germ cells depend on signals from the somatic gonad and their own sex chromosome genotype for successful sexual development and gametogenesis. When the "sex" of the germline fails to match the "sex" of the soma, germline development is severely disrupted. How somatic signals and germ cell intrinsic cues act together to regulate germline sex determination is a key question about which little is known in any organism.

In an RNAi screen for genes affecting germline sex determination, we identified Chitinase 10 (Cht10) as a gene that causes an ovarian tumor phenotype when depleted in the germline, similar to germline sex determination genes. Cht10 is a member of a class of atypical chitinases that lack chitinase activity due to mutations in their chitinase domains, and are instead proposed to act as extracellular signaling molecules. These were first discovered as Imaginal Disc Growth Factors (IDGF's) due to their ability to promote proliferation and growth of Drosophila Imaginal Disc cell lines (Bryant lab). Interestingly, animals that lack endogenous chitin, such as mice and humans, have retained these atypical chitinases. Studies in humans have identified atypical chitinases such as Cht3L1 (YKL-40) as disease markers associated with a variety of pathological conditions such as inflammatory diseases and cancer.

We are exploring the role of Cht10 in the ovary. CRISPR-derived mutants for Cht10 exhibit a strong ovarian defect, but this phenotype has interesting differences from germline-specific RNAi. In addition, we have observed changes in MAP-Kinase signaling in Cht10 loss of function, supporting a role for these atypical chitinases in regulating cell-cell signaling. We are also studying the relationship between Cht10 and other genes that generate an ovarian tumor phenotype. This work will establish a new role of Chitinases in gonadal development and will have implications related to ovarian fertility, which remains unexplored.

339 An anciently conserved protein is required for sperm motility in *Drosophila melanogaster*. *F. Busser*[1], R. Snow[1], H. Florman[2], G. Findlay[1] 1) Department of Biology, College of the Holy Cross, Worcester, MA; 2) Department of Cell and Developmental Biology, University of Massachusetts Medical School, Worcester, MA.

In many animals, successful reproduction requires sperm cells to swim to and then fertilize an egg. Defects in sperm motility can therefore lead to infertility. Previous work in mice identified a gene that is required for correct sperm swimming; males in which this gene was disrupted were severely sub-fertile. We have identified an ortholog of this gene in *Drosophila* and are using the fly system to characterize its role in male fertility. RNAi knockdown of *Drosophila sperm gene 1* (*dsg1*) replicated the sub-fertile phenotype observed in mice. Specifically, sperm from knockdown males were unable to localize to the sperm storage organs of the female reproductive tract, suggesting a motility defect. We have used CRISPR/Cas9 to develop a putative null allele of *dsg1*. Homozygous knockout males are severely sub-fertile and produce sperm that fail to localize properly within the female. We are currently using these males, in conjunction with fluorescent markers of sperm heads and tails, to develop microscopy methods to image and quantify sperm motility. We are also producing an antibody to study the localization on sperm of Dsg1 protein and its potential interactions with other regulators of sperm motility. Our results suggest that *dsg1* may have a conserved function in sperm motility across diverse animals and enable the use of *Drosophila* as a model system to study this conserved gene.

340 Investigating the role of octopamine and *Tdc2*$^{+}$ neurons in female sperm discrimination. *D.S. Chen*, S.L. White, A.G. Clark, M.F. Wolfner Molecular Biology and Genetics, Cornell University, Ithaca, NY.

In many species, females mate with multiple males sufficiently to result in the concurrent presence of sperm from those males in the female's reproductive tract. When this happens, the sperm from rival males compete for opportunities to fertilize the female's eggs. Sperm competition involves complex ejaculate-ejaculate and ejaculate-female interactions, so sexual selection and sexual conflict can influence the evolution of both its male and female contributors.
In *Drosophila melanogaster*, sperm characteristics and seminal fluid proteins have been shown to influence a male's competitive ability, but the mechanisms by which females sense and respond to sperm attributes are less well characterized. We previously showed, using tissue-specific gene knockdown, that the proper functioning of octopaminergic *Tdc2*$^{+}$ neurons in doubly mated females can influence sperm competition outcomes (in prep). Our preliminary results, collected from octopamine (OA) synthesis mutant females, also suggest that OA-less females have different sperm competition outcomes than wild-type control females. To further investigate the role of OA in the female's mediation of sperm competition, we are thermogenetically manipulating *Tdc2*$^{+}$ neurons to ask if and when these neurons are required for wild-type sperm competition outcomes. Finally, we ask if the effects of OA on sperm competition is attributable to a population of *Tdc2*$^{+}$/*doublesex (dsx)*$^{+}$ neurons, which have extensive innervation in the female reproductive tract and are known to mediate postmating responses. This project will expand our knowledge of the female nervous system's influence on her reproductive physiology, and inform future investigations on the evolution of male-female communication of sperm quality.

341 Sex-specific ecdysone signaling regulates gonad stem cell niche development. E. Jimenez, *L. Grmai*, M. Van Doren Department of Biology, Johns Hopkins University, Baltimore, MD.

Sexual dimorphism underpins development of nearly all metazoans; in *Drosophila*, a critical effector of sexual dimorphism is the gene *doublesex* (*dsx*), which undergoes alternative splicing to generate male or female isoforms Dsx^{M}/Dsx^{F}. While Dsx orthologs are present throughout evolution, much is yet to be determined about how they effect sexual dimorphism at the molecular level. Dsx is expressed in most dimorphic tissues including the somatic gonad, and proper gametogenesis relies on sex-specific cues from somatic niche cells. In the testis, male GSCs are supported by a tight cluster of post-mitotic cells termed the hub, while the female GSC niche includes both cap cells, a cluster of post-mitotic cells, and the terminal filament (TF), a single stack of cells at the apex of each ovariole. Our previous work has shown that *dsx* is required to ensure the correct niche forms in each sex: *dsx* mutant gonads initially specify a hub regardless of chromosomal sex, but during L3 approximately half of both XX and XY gonads "switch" to form TFs. Analysis of *dsx* genomics data uncovered the *Ecdysone receptor (EcR)* as a putative Dsx target, prompting us to examine its role in niche formation. While mammalian steroid hormone signaling is known to be dimorphic, many known roles for the insect steroid hormone ecdysone are monomorphic. Interestingly, at the time when *dsx* mutant niches "switch", both EcR protein and downstream EcR-dependent transcriptional activity are present in the female, but not male, somatic gonad. Because of this, we hypothesized that modulating ecdysone activity would skew the ratio of *dsx* mutant gonads that form a hub vs. TF. We found that depleting *EcR* in *dsx* mutants skewed gonads toward hub formation, while *EcR* over-expression led to a significant decrease in the percentage of *dsx* mutant gonads with a hub. From this, we conclude that female-biased ecdysone signaling in the developing gonad is ensured by the activity of Dsx, and that this dimorphism in EcR activity is required to secure the hub vs. TF decision. Further work will be required to identify direct Dsx targets and dimorphic elements in the ecdysone pathway. Importantly, our finding that ecdysone signaling is dimorphic in *Drosophila* gonads raises the possibility that sexual dimorphism governs ecdysone activity in other tissues.

342 Male-specific small peptides are encoded by a large "ncRNA" within the *Drosophila* Bithorax complex. *C. Immarigeon*, Y. Frei, R. Maeda, F. Karch Genetics and Evolution, Geneva University, Genève, Genève, CH.

Polypeptides shorter than 100 amino acids have long been excluded from genomics and proteomics studies. However, recent analyses reveal that these small peptides are actually abundant products of eukaryotic genomes. They are encoded by small open reading frames (sORFs) present in virtually every class of PolII transcripts, in all organisms. The contribution of these peptides to cellular functions remains largely unknown. However, the few examples of well-studied small peptides (PGC, Pri/Tal, SLN/PLN/SCL, hemotin, sex peptide…) demonstrate that size is not what matters for biological significance.
A conserved sORF lies in the Bithorax complex (BX-C) of Drosophila, a well-known locus containing three homeotic genes and their regulatory sequences. This sORF is part of a large "non-coding" transcript called *MSA* spanning the intergenic region between *abd-A* and *Abd-B*. We knocked-in a GFP in frame with this sORF within the BX-C and show that this sORF is translated specifically in "secondary cells" of male accessory glands, the prostate-like organs of insects. We confirmed that *MSA* is translated by performing secondary cell-specific TRAP showing a strong association of *MSA* with ribosomes.
By using a combination of genetics, proteomics, immunohistochemistry and behavior analysis we hope to be able to understand the role of this small peptide.

343 Fighting flies with context. *J.T. Alkema*[1], H. Doornbosch[2], A. Alesho[1], F. Lucassen[1], S. de Jong[1], B. Wertheim[2], M.D. Dicke[1] 1) Laboratory of Entomology, Wageningen University, Wageningen, NL; 2) GELIFES, Groningen University, Groningen, NL.

The invasive pest species *D. suzukii* causes extensive agro-economical damage in West-Europe and North-America. Traditional management techniques fail to prevent this, and thus research is being conducted globally into integrated pest management strategies (IPM). One of the elements of an IPM of specific interest is the Push-pull strategy, in which repellent stimuli on crops (push) in cohort with attractive lures (pull) aim to sequester and kill the pest. Unfortunately, even with an extensive library of *D. melanogaster* knowledge, it remains difficult to find effective and efficient stimuli. We investigate whether this can be ascribed to context- and state-dependent response behaviour.
We tested whether flies respond differently to know attractants when they are looking for a site to either forage, mate, or oviposit. We further devised the foraging states into females searching nutrients for their own needs and those searching nutrients for oogenesis.
Finally, we investigated whether there is an aggregative or social structure to the searching behaviour of the pest. We observed how females and males respond to crop stimuli when they are inhabited by varying groups of conspecifics. There definitely appears to be a difference in the way males and females respond to such environments, yet we seem to only be scratching the surface of the underlying social and/or mating mechanisms that regulate this.

344 Satellite Repeats Are Associated with Host Tolerance of an Active Transposable Elements. *J. Lama*[1], E. Kelleher[1], S. Srivastav[1,4], S. Tasnim[1,3], D. Hubbard[1,2] 1) University of Houston, Houston, TX; 2) Texas A&M, TX; 3) University of Texas Medical branch,Galveston, TX; 4) Boston University School of Medicine,Boston, MA.

Transposable elements (TE) are mobile genetic parasites, whose unrestricted activity in germline cells disrupts gametogenesis and causes sterility. Host genomes can respond to these fitness costs of TEs by evolving resistance, in which TE proliferation is regulated directly, or by evolving tolerance, where the fitness consequences of TE proliferation are minimized without impacting their activity. Although resistance to TEs via small-RNA mediated silencing pathways, such as the piRNA pathway, is the focus of extensive research, less is known about host factors that could confer cellular tolerance to TE activity. Furthermore, because small-RNA mediated regulation of TEs is ubiquitous, tolerance phenotypes are often masked, making them challenging to study.
Hybrid dysgenesis systems, in which resistance is short-circuited due to an absence of maternally deposited piRNAs, provide a unique opportunity to study tolerance. We, therefore, used *P*-element hybrid dysgenesis to reveal natural genetic variation in germline tolerance across a panel of >600 recombinant inbred lines using quantitative trait locus (QTL) mapping. We discovered a complex peak in the centromeric and pericentromeric region of chromosome 2,

which is associated with female germline tolerance to *P*-elements. Females harboring tolerant alleles of the QTL are more likely to produce gametes in the presence of *P*-element activity than those containing sensitive alleles. Genomic analysis revealed that dosage of *Responder* (*Rsp*), a satellite repeat found in pericentric heterochromatin of chromosome 2, was positively correlated with tolerance. We further discovered that strains carrying a tolerant allele produced significantly more piRNAs and siRNAs targeting *Rsp* for heterochromatin formation and that the expression of multiple histone proteins differs between tolerant and sensitive QTL alleles. We propose that incomplete packaging of *Rsp* satellite repeats in dysgenic germline may enhance genomic instabilities triggered by *P*-activity, and thereby reducing tolerance.

345 Structure-function analysis of germ granule nanoparticles in Drosophila. Hieu D. L. Vo[1], Samuel J. Tindell[1], Margaret Hagen[1], Claire Umstead[1], Jimiao Zheng[1], Ming Gao[2], *Alexey L. Arkov*[1] 1) Department of Biological Sciences, Murray State University, Murray, KY; 2) Biology Department, Indiana University Northwest, Gary, IN.

Germ cells in different organisms contain large dynamic ribonucleoprotein granules whose components are required for germline development. The detailed structure and function of these germ granules are not understood well. The landmark protein components of the granules include Tudor-domain containing polypeptides, Piwi proteins, which associate with small regulatory piRNAs, and RNA helicases involved in the regulation of germ granule RNA. Here, we present a systematic analysis of the functional assembly of specific molecular complexes (germ granule nanoparticles) within a larger granule, centered around the scaffold Tudor protein and its direct interacting partner Piwi protein Aubergine. Using in vitro biochemical assays and purified granule components as well as proteomics approaches, we are studying the detailed mechanisms of the germ nanoparticles' assembly and our data point to the inherent properties of different individual proteins to drive the assembly of the germ granules, and to the importance of post-translational modifications in this process.

346 Downregulation of homeodomain transcription factor Cut is essential for follicle maturation and ovulation. *E. Knapp*[1], J. Sun[1,2] 1) Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT; 2) Institute for Systems Genomics, University of Connecticut, Storrs.

Proper development and maturation of a follicle is essential for successful ovulation and reproduction; however, signals for follicle maturation and ovulation are poorly understood. In *Drosophila,* a new follicle/egg chamber buds off from the germarium and develops through 14 morphologically distinct stages to become a mature follicle competent to ovulate. Our recent work characterized the stage-14 follicles and demonstrated that upregulation of the zinc-finger transcription factor Hindsight (Hnt) in somatic follicle cells promotes Oamb expression in all stage-14 follicle cells and Mmp2 expression in posterior follicle cells. Octopamine (OA) released from neuronal terminal activates Oamb to induce Mmp2 activity and follicle rupture. In addition, we also found that a NADPH oxidase (Nox) is also upregulated in stage-14 follicle cells and activated by OA/Oamb signaling to produce reactive oxygen species (ROS) for follicle rupture. It is unclear how follicles transition into stage-14 with such molecular signature changes. Here, we found that the homeodomain protein Cut and zinc-finger transcription factor Ttk69 are expressed in somatic follicle cells prior to stage 14 and downregulated in stage 14, opposite to Hnt expression. Our study reveals that extended Cut expression into stage-14 follicle cells leads to inhibition of Hnt, Oamb, Mmp2 expression and follicle rupture. In addition, Cut promotes Ttk69 expression, which further inhibit Nox expression and ROS production in stage-14 follicle cells. In conclusion, we identified a novel follicle cell transition in late oogenesis, in which downregulation of Cut is essential for somatic follicle cells to shift into a state competent to elicit follicle rupture and ovulation.

347 Directing testis specific gene expression: Nucleosome dynamics and transcriptional regulators. *K. Jindrich*, G. Beattie, R. Mitchell, N. Kent, H. White-Cooper School of Biological Sciences, Cardiff University, Cardiff, UK.

Spermatogenesis relies on the coordinated transcription of a large number of genes, many of which are exclusively expressed in testes. In *Drosophila*, the testis-specific transcriptional program is principally activated in spermatocytes, by the testis meiotic arrest complex TMAC. Loss of any TMAC component causes meiotic arrest, where testes fill with developmentally arrested primary spermatocytes. Interestingly, defects in chromatin remodelers NURF and NuRD also result in meiotic arrest but their role in regulating testis genes remain largely unexplored. Here we investigate (1) whether NuRD and NURF are working with TMAC to direct testis specific transcription and (2) the role of chromatin structure in the regulation of testis specific genes. Using RNA-seq, we compared the transcriptome of manually purified spermatocytes from wild-type and NURF, NuRD and TMAC mutant testes. Transcriptional changes in NuRD and NURF mutants were distinct from one another and milder than those caused by loss of TMAC. In concert with TMAC, NURF and NuRD promote transcription of testis-specific genes. Independently, they also repress transcription of non-testis specific genes in spermatocytes. To uncover the impact of chromatin architecture on spermatocyte gene expression, we generated genome-wide maps of nucleosome positions in wild-type and mutant spermatocytes by MNase-seq. Our data suggest that NuRD globally decreases nucleosome density at promoters, irrespective of whether associated genes are responsive to NuRD activity. Integrating this with ongoing chromatin analysis in NURF mutants, we suggest that these chromatin remodelers fine-tune gene expression in spermatocytes by facilitating access to transcriptional repressors and activators such as TMAC. Strikingly, we also find a surprising lack of coherent nucleosome positioning at highly expressed testis genes. We conclude that NuRD and NURF are involved in several strategies that independently specify precise gene expression in testis. This work highlights the interplay between different regulatory mechanisms to ensure cell specific expression, but also suggests that nucleosome positioning might be less critical in terminally differentiated cells than expected based on studies of cultured cells.

348 RNAseq reveals a role for accessory gland proteins and long non-coding RNAs in sperm length variation in *D. melanogaster*. *M. Manier*[1], M. DeNieu[1], K. Borziak[2] 1) Biological Sciences, George Washington University, Washington, DC; 2) Dept. of Biology, Syracuse University, Syracuse, NY.

Drosophila produce some of the longest sperm known, up to 5.8 cm in *D. bifurca*. These sperm are stored within the female reproductive tract in the seminal receptacle (SR), a long, coiled specialized sperm storage organ. Studies in *D. melanogaster* have shown that longer SRs select for longer sperm during sperm competition, driving a Fisherian runaway process fueled by a genetic correlation between sperm length and SR length. In order to identify candidate genes that may be involved in sperm length variation, we compared testis transcriptomes between inbred lines derived from populations previously selected for long sperm or short sperm. RNA was extracted from testes of 100 males from two biological replicate short sperm and long sperm isolines with three technical replicates per line (total of 12 samples), all sequenced across two replicate channels on an Illumina HiSeq 2100. We identified approximately 250 differentially expressed genes that included accessory gland proteins (Acps) known to be transferred to females during mating and not previously known to be expressed in testis, unnamed protein-coding genes, and long non-coding RNAs (lncRNAs). Of the 77 genes upregulated in long sperm testes at least 3-fold, 45% were unknown protein-coding genes, 36% were Acps, and 9% were lncRNAs. Of the most differentially expressed genes with RPKM of at least 100, half were Acps. Our results point to a role for lncRNAs and Acps previously not known to be expressed in testis in sperm length variation in *D. melanogaster*.

349 *Drosophila* CTP synthase regulates collective cell migration through an endosomal-recycle pathway. *L. Pai*[1,2,3], P. Wang[1] 1) Dept Biochem & Molec Biol, ; 2) Molecular Medicine Research Center, Chang Gung Univ. Taoyuan, TW; 3) Liver Research Center, Chang Gung Memorial Hospital, Lin-Kuo, TW.

Cytidine triphosphate synthase (CTPsyn) is a nucleotide metabolic enzyme for CTP production which is involved in DNA, RNA, phospholipid biosynthesis. Phosphoinositides (PIs) are short-lived membrane phospholipids that are involved in many biological functions, such as regulation of endocytic and exocytic processes. *Drosophila* border cell migration, a collective cell migration, is regulated by asymmetrical signaling established by endocytosis and exocytosis. Here, we found that the function of CTPsyn in PIs biosynthesis is required for border cell migration. Depletion of CTPsyn in border cell cluster resulted in delayed migration which was further enhanced by mutations of Pis and PI4KIIIa, enzymes in PIs synthesis. Knockdown of CTPsyn in border cell cluster resulted in disturbing the polarized-distribution of activated RTKs, but not DE-Cad, and aPKC. Consistently, the migratory defect in CTPsyn mutant was also enhanced by

knockdown of Cbl, an E3 ubiqutin ligase required for the internalization of activated RTK to establish asymmetric distribution. CTPsyn genetically interacted with molecules in vesicle trafficking, including Rab5, Sec3, and Sec5, in border cell migration. In addition, the asymmetric distribution of Rab11-mediated exocytosis and the distribution of $PI(4,5)P_2$ at the leading edge of border cell cluster were altered by reduction of CTPsyn. In sum, these results suggest that the CTPsyn regulates polarized sorting of RTK during collective cell migration through endo/exocytosis.

350 Investigating the function of mucin-type O-glycosylation in the *Drosophila*reproductive system. *L. Zhang*, Kelly Ten Hagen Developmental Glycobiology section, NIDCR/NIH, Bethesda, MD.

There are abundant glycans expressed in the mammalian reproductive system and alteration of glycosylation has been found in human reproductive disorders, suggesting that glycans may play important roles in reproductive function. In this study, we use the *Drosophil*a reproductive system as a model to study the function of mucin-type O-glycans. Microarray data show that some O-glycosyltransferases (PGANTs), which initiate the mucin-type O-glycosylation modification, are highly expressed in both adult male and female reproductive systems. Staining of HPA lectin in both reproductive systems revealed that there are abundant O-glycosylated proteins contained in the large granules in secretory cells. RNAi to *pgants*in the male accessory glands caused alteration of O-glcosylation. Additionally, we found one heavily O-glycosylated protein, CG11098 is highly expressed in reproductive system. RNAi to *cg11098*in male accessory glands or female spermathecae leads to the disruption of secretory granule formation. Our results suggest O-glycans play important roles in both adult male and female reproductive systems.

351 Drosophila accessory gland secondary cells and post-mating sperm dynamics. *B. Hopkins*[1], I. Sepil[1], C. Wilson[2], S. Wigby[1] 1) Department of Zoology, University of Oxford, Oxford, UK; 2) Department of Physiology, Anatomy, and Genetics, University of Oxford, Oxford, UK.

In the male fruit fly *Drosophila melanogaster*, the seminal fluid protein (Sfp) sex peptide promotes male fertilisation success through the dual functions of reducing female receptivity to remating, thus lowering sperm competition risk, and stimulating fecundity. The production of many Sfps takes place in the accessory glands, a pair of lobed, secretory organs that branch off from the ejaculatory duct. Although 96% of the accessory gland is composed of so-called 'main cells', which are tasked with the production of sex peptide, the distal tips of the glands also house a small population of functionally mysterious secretory cells, known as secondary cells. These grow in response to age and mating frequency and secrete exosomes of unknown cargo that have been shown to fuse with sperm and associate with the female reproductive tract. Previous work has shown that blocking the development of secondary cells compromises the long-term retention of sex peptide in the female sperm storage organs leading to diminished fecundity and faster return to sexual receptivity. However, more specific targeting of the secretory activities of secondary cells, through the localised suppression of adult BMP-signalling, generates a curious decoupling of sex peptide phenotypes, where fecundity remains normally stimulated but females show a heightened receptivity to remating. Using the same approach, I confirm the decoupling of female post-mating phenotypes and find that these disrupted secondary cell males sire a greater proportion of offspring relative to controls when mating first under competitive conditions. However, I find no such advantage when matings are with mated females. I also present the results of quantitative proteomics that identifies how the composition of seminal fluid is influenced by loss of secondary cell secretion, and fluorescent tagged sperm count data that more directly probes how secondary cells influence sperm in the female reproductive tract.

352 A robust transposon-domesticating response from germline stem cells. *S. Moon*, M. Cassani, Y. Lin, L. Wang, K. Dou, Z. Zhang Embryology, Carnegie Institution for Science, Baltimore, MD.

The heavy occupancy of transposons in the genome reflects existing organisms have survived from myriad rounds of transposon invasions. However, which host cell types are responsible for transposon domestication and how they achieve it remain unclear. We show that the germline stem cells can initiate a robust adaptive response that rapidly domesticates invading transposons by activating DNA-damage checkpoint and piRNA production. We find that temperature modulates the *P-element* transposon activity in germline stem cells, establishing a powerful tool to trigger transposon hyper-activation. Facing vigorous invasion, *Drosophila* first shut down entire oogenesis and induce selective apoptosis. Interestingly, a novel adaptive response occurs in ovarian stem cells through the activation of DNA-damage checkpoint. Within 4 days, the hosts amplify *P-element*-silencing piRNAs, repair DNA damage, subdue transposon, and reinitiate oogenesis. We propose that this robust adaptive response bestows organisms the ability to survive from periodic transposon invasions throughout evolution.

353 A modERN project update: transgenic GFP lines and Transcription Factor ChIP-seq. *Alec Victorsen*[1], Rachael Niemiec[1], Martha Wall[1], Michelle Kudron[3], Louis Gevirtzman[4], Bill Fisher[5], Esther Chan[2], Weiwei Zhong[2], Jinrui Xu[3], Mike Cherry[2], Valerie Reinke[3], Sue Celniker[5], Mark Gerstein[3], Kevin White[1], Bob Waterston[4] 1) University of Chicago, Chicago, IL; 2) Stanford University, Stanford, CA; 3) Yale University, New Haven, CT; 4) University of Washington, Seattle, WA; 5) University of California at Berkeley, Berkeley, CA.

As members of the modERN and ENCODE projects, we are attempting to characterize the binding sites of the ~800 transcription factors (TFs) in the model organisms D. melanogaster and C. elegans, and the ~1600 TFs in Humans. TFs regulate gene expression in part, by binding to regulatory sequences in the genome. These regulatory elements can be found using ChIP-seq (Chromatin-Immunoprecipitation followed by sequencing) in which the TF-DNA (chromatin) complexes can be purified and the DNA sequenced. To assay more than 3000 TFs, we have chosen to use transgenic TF proteins tagged with green fluorescent protein (GFP). This allows us to use a well characterized GFP antibody to isolate the TF chromatin complexes rather than focusing on validating TF specific antibodies. While we initially created the TF-GFP fusions in Bacterial Artificial Chromosomes (BACs) and fosmids, we have begun to introduce the GFP tag by CRISPR. To date, as part of modERN and ENCODE we have generated ChIP-seq data for more than 800 TFs.

354 Analysis of the sequential activation of downstream targets of Notch signaling during *Drosophila melanogaster* egg chamber development. *M. Rowe*, D. Jia Department of Biology, Georgia Southern University, Statesboro, GA.

Living organisms require complex interactions between cells and proper regulation of these interactions to influence biological processes. Of these complex networks, one of the most distinguished is the Notch pathway, which is highly conserved and is linked to several aspects of development. Dysregulation or improper functioning of this signaling pathway often results in defects during development, and can be a causative mechanism for initiation and development of certain cancers. Despite previous research entailing the importance of this signaling pathway and the organismal processes that it may be involved in, less is known concerning the sequential activation of major Notch downstream targets during normal development. As timing of activation may be linked to many developmental processes, our research investigates the sequential activation of major Notch downstream targets including Broad, Cut, and Hindsight using *Drosophila melanogaster* egg chamber development as a model. Upon standard dissection and immunostaining techniques, analysis of egg chambers through confocal microscopy and further characterization was performed using MATLAB and a toolbox designed to systematically identify egg chamber development stage based on area, ratio, and additional morphological criteria. Preliminary results suggest that each downstream target is expressed at slightly different times during development, with a high percentage of egg chambers showing initial Broad upregulation in stage 4/5, followed by Hindsight upregulation in early stage 5, and Cut downregulation in later stage 5 to early stage 6. As activation of downstream targets is often accompanied by cell cycle switches, these results may prove significant as a comparative model for future developmental research using *Drosophila*. Furthermore, our experimental design and methodology demonstrates a novel technique for characterization of egg chamber developmental stages that improves accuracy of stage identification, reduces reliance on visual determination, and can be useful for many areas of research.

355 Downregulated Broad is required for proper border cell migration. *S. Defreitas*, C. Driskell, D. Jia Department of Biology, Georgia Southern University, Statesboro, GA.

Development in *Drosophila* is highly regulated by the timing and spatial cues of hormones and signaling pathways. Broad (Br), is a group of zinc finger transcription factors with four isoforms (Br-Z1-Z4) that result from alternative splicing. Br is an early response gene of the steroid hormone ecdysone which is essential for the initiation of metamorphosis (Karim et al.,1993; Fletcher and Thummel, 1995) and a target of Notch signaling during *Drosophila* oogenesis (Jia et al., 2014). Here, we report that regulation of Br expression is important for proper border cell migration. Br expression is induced by Notch signaling at stage 5 of development however, our studies have shown that Br expression can decline in border cells in the presence of Notch in the middle stages of egg chamber development. The JAK/ STAT pathway along with ecdysone are present in high amounts during the onset of border cell migration to downregulate Br in the presence of Notch signaling for proper border cell migration.

356 Pointed is necessary and sufficient for establishing the posterior end of the follicular epithelium. *C. Stevens*, R. Caur, N. Yakoby Rutgers University, Camden, NJ.

The anterior-posterior axis during Drosophila oogenesis is regulated by a small number of cell signaling pathways. The Janus-kinase/Signal Transducers and Activators of Transcription (JAK/STAT) is activated in both posterior and anterior ends of the follicular epithelium. Previously shown, JAK/STAT activation is required for the expression of decapentaplegic (dpp), the bone morphogenetic protein (BMP) signaling ligand, which consequently activates this pathway in the anterior follicular epithelium. In the posterior, JAK/STAT works in concert with the epidermal growth factor receptor (EGFR) to express the ETS-transcription factor pointed (pnt). Pnt was shown to control the dorsal midline width, which sets the distance between the two dorsolateral domains of the respiratory dorsal appendages primordia. Here we show that Pnt is necessary for determining the posterior fate of the follicular epithelium. Ectopic Pnt expression in the anterior follicular epithelium renders cells incapable of exhibiting native differentiation, including stretch and centripetal follicle cell movement. Furthermore, ectopic Pnt expression in the anterior pole cells ceases border cell migration entirely. Our results indicate that this inhibition is due to Pnt sufficiency to repress anterior fate formation through loss of BMP signaling. This complex signaling and transcriptional network provide insight into the establishment of the anterior-posterior axis of the fly.

357 MEF2 and Tinman collaborate to generate the lumen of the heart in Drosophila by activating the collagen gene Multiplexin. *T.L. Lovato*, R.M. Cripps Biol, Univ New Mexico, Albuquerque, NM.

The embryonic Drosophila heart is a linear tube composed of a heart proper and aorta. The heart possesses a lumen and inflow tracts, through which hemolymph enters and is pumped anteriorly through the narrow aorta. In Mef2and Tinman mutants, the heart lumen is reduced. The expression of the collagen protein Multiplexin(Mp) is also reduced in Mef2 mutants and absent in Tinmanmutants. We have identified the enhancer of the Multiplexingene and demonstrate the ability of both MEF2 and Tinman to activate it in cell culture. We have mapped the physical interactions of MEF2 and Tinman through pull-down experiments and have identified structural abnormalities in the adults of Mef2/tindouble-heterozygote null mutants. These flies also demonstrate functional abnormalities as seen by increased arrhythmias and long diastoles. The high conservation of the transcriptional networks that direct the development of Drosophila and vertebrate hearts makes Drosophilaan ideal model for studying the genetic complexities of cardiac development.

358 Stop codon readthrough of a POU/Oct transcription factor regulates *Drosophila* development. *Y. Zhao*, B. Lindberg, S. Esfahani, X. Tang, S. Piazza, Y. Engström Stockholm University, Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm, SE.

Stop codon readthrough is a mechanism commonly utilized by viruses to add plasticity to the proteome without expanding the genome. Recent studies indicate that readthrough is more pervasive in eukaryotes than previously predicted. Approximately 700 genes have been proposed as candidates for stop codon readthrough in *Drosophila*. The underlying mechanisms and functional importance of this phenomenon is, however, still unknown at large and remains to be deciphered.

Here we studied the impact of readthrough in the POU/Oct transcription factor *drifter(dfr)/ventral veins lacking* (*vvl*), a key factor in the expression of ecdysone biosynthesis genes in *Drosophila*development. We show that stop codon readthrough of *dfr/vvl*mRNA occurs at a high relative rate in the prothoracic gland, resulting in a novel, extended isoform of the protein Dfr-Large (Dfr-L). We applied CRISPR/Cas9 to generate Dfr-L frameshift mutant lines in the ORF immediately downstream of the annotated stop codon. This resulted in prolonged larval development and delayed metamorphosis. RNA-seq revealed changes in metabolism, immunity, and ecdysone-associated genes. We observed compromised expression of the steroidogenic enzymes Nvd, Dib, and Sad at mRNA level and protein level in the prothoracic gland in the mutant larvae, which was dependent on the Dfr-L but not Dfr-S. The expression of Phm, however, was specifically dependent on the short isoform, suggesting complementary functions of the isoforms in the ecdysone biosynthesis pathway. Overexpression of the small Dfr-S and large Dfr-L isoforms as well as RNA interference (downregulating both isoforms) in the prothoracic gland all led to developmental arrest, but at different stages of development. This could be partly rescued by ecdysone feeding. By inducing flp-out clones in the prothoracic glands, we demonstrated that overexpression of either Dfr-L or Dfr-S diminshed the other isoform, respectively. Our findings reveal a novel regulatory mechanism, in which the relative level of stop codon readthrough produces alternative transcription factor isoforms with different regulatory potential of downstream targets and processes.

359 Effects of cooperative HP1 binding at transcription start sites. *J.M. Schoelz*, N.C. Riddle Biology, University of Alabama-Birmingham, Birmingham, AL.

The Heterochromatin Protein 1 (HP1) family is a family of non-histone chromosomal proteins with key functions in chromatin structure, DNA repair, DNA replication, and gene regulation. The HP1 family is conserved from yeast to humans, and many genomes have multiple copies of HP1 genes. In the *Drosophila* genome there are three somatically expressed HP1 genes: *Su(var)205*/HP1a, *HP1b* and *HP1c*. HP1a is essential for the nucleation and propagation of heterochromatic domains and as such is associated with gene silencing. In contrast, HP1B and HP1C localize throughout heterochromatin and euchromatin and share a large percentage of their binding sites, but their effect on transcriptional status is not clear. For instance, tethering of HP1C to transcription start sites (TSSs) is associated with transcriptional activation, while tethering of HP1B produces transcriptional silencing. Additionally, a substantial percentage of HP1 binding sites are shared by all three HP1 paralogs. The co-localization of HP1 paralogs highlights the need to consider how individual HP1 proteins interact in order to fully understand the role of the HP1 family in gene regulation.

Here, we integrate transcriptomic and epigenomic next generation sequencing data to characterize TSSs bound by different combinations of HP1 proteins. Using RNA Polymerase II ChIP-seq and GRO-seq datasets, we find HP1 binding is associated with increased promoter proximal pausing. Classifying TSSs by different combinations of HP1 proteins shows that increased pausing is specifically associated with co-binding of HP1B and HP1C. Additionally, HP1-bound TSSs are enriched for histone modifications correlated with RNA polymerase II pausing, such as H2B-ubiqutination, H3K79 monomethylation, and H4K20 monomethylation as well as the pausing factors M1BP and NELF. These findings illuminate potential functional roles of HP1 proteins in gene regulation and underscore the need to consider the combined action of HP1 proteins when examining functional consequences of HP1 binding.

360 Transcriptional hubs in genome activation. *C.A. Rushlow*, S-K. Huang, R.S. Rajappachetty Dept Biol, 1009 Silver Center, New York Univ, New York, NY.

Zelda is a pioneer transcription factor that activates the early genome (Liang et al., 2008). Understanding its ability to open chromatin and boost transcription

by RNA Polymerase II (Pol II) will shed light upon basic transcriptional mechanisms in development. Here we focus on the earliest Zelda target genes, which like Zelda, are ubiquitously expressed. To obtain a genome-wide view of these genes, we examined RNA expression, Pol II ChIP, and Zelda ChIP profiles from staged embryos (de Renzis et al., 2007; Liang et al., 2008; Chen et al., 2013; Blythe and Wieschaus, 2015; Sun et al. 2015). We compiled a list of 55 genes that are bound by Zelda and Pol II in nuclear cycle 12, and lose expression in *zelda* mutants. We also examined the distribution of Pol II by antibody staining. Preliminary results indicate that in the wild type nucleus, Pol-II forms on 43.6+/-5/53 speckles as well as two large foci (819±97nm). The two large foci are histone locus bodies (HLBs) as FISH signal with antisense H3 and H4 probes perfectly co-localizes with them. Staining with antibodies against Mxc, a major component of HLBs (White et al., 2011), also confirms they are HLBs. In *zelda* mutants, number of Pol-II speckles drops to ~5-6 while the two larger foci become even larger (1023±89nm), indicating that: 1) Pol II redistributes in the absence of early transcription, and 2) the Pol II speckles are hubs of Pol II concentrated at the early gene loci. To test this hypothesis we performed Pol II antibody staining alongside FISH with several early gene RNA probes. Data will be presented that visualizes localization of Pol II at the early gene loci, as well as a correlation between the map distance between sets of loci and their relative positions in nuclei of early embryos.

361 A DNA/RNA dual-activity topoisomerase stimulates transcription of RNA polymerase II. *S. Lee*[1], W. Shen[1], Y. Joo[1], Y. Xue[1], Y. Ding[2], Y. Zhang[1], K. Zhao[2], A. Sharov[1], W. Wang[1] 1) Lab of Genetics, National Institute on Aging, NIH, Baltimore, MD; 2) System Biology Center, National Heart, Lung and Blood Institute, NIH, Bethesda, MD.

Topoisomerase 3 beta (Top3β), is a unique topoisomerase, because it can change topology for not only DNA, but also RNA. Increasing evidence shows that Top3β can act in cellular processes on both DNA and RNA. For example, Top3β can stimulate transcription on DNA by reducing R-loops. In addition, Top3β forms a complex with two RNA binding proteins; Tudor domain containing 3 (TDRD3), and Fragile X Mental Retardation Protein (FMRP); and this complex may facilitate translation of mRNAs important for neurodevelopment. Moreover, Top3β has been shown to interact with siRNA machinery to promote heterochromatin formation and transcriptional silencing. Interestingly, *Top3β* mutation has been linked to autism and schizophrenia, and its inactivation mice leads to shortened life-span, indicating its importance in mental health and aging. However, the mechanism of how Top3β regulates gene expression at DNA or mRNA levels remains unclear. Here we used ChIP-seq and RNA-seq to identify genes that are directly bound and regulated by Top3β-TDRD3 complex, and investigated the underlying mechanisms.

Our RNA-seq using fly heads shows that *Top3β* mutant exhibits down-regulation of about 500 genes, many of which are related to immune response, phototransduction, and visual function. ChIP-seq shows that Top3β and its two partners, TDRD3 and FMRP, co-binds a subset of these genes, suggesting that the entire Top3β complex may regulate these target genes. The ChIP-seq also shows that Pol II signals are decreased in many Top3β-regulated genes in *Top3β* mutant, suggesting that Top3β promotes recruitment of Pol II. In addition, both initiation and elongation forms of Pol II are decreased in *Top3β* mutant, suggesting that Top3β may enhance both initiation and elongation during transcription. Transgenic expression of wildtype *Top3β* can largely rescues the reduced pol II recruitment, whereas the catalytic mutant of *Top3β* has decreased activity in this assay, indicating that Top3β depends on its topoisomerase activity to stimulate transcription. Furthermore, our data from analyzing a mouse model are consistent with the findings from the *Drosophila*. Together, these data consistent with a model that Top3β utilizes its topoisomerase activity to solve DNA topological problems, leading to increased amount Pol II and enhanced transcription of specific genes.

362 CrebA directly activates regulators of secretion. *D.M. Johnson*[1], M.B. Wells[1], R. Fox[1], J.S. Lee[2], A. Bastien[1], M. Slattery[2], D.J. Andrew[1] 1) Department of Cell Biology, Johns Hopkins School Medicine, Baltimore, MD; 2) Department of Biomedical Sciences, University of Minnesota, Duluth, MN.

CrebA/Creb3L-like proteins coordinately regulate expression of the protein machinery of secretory organelles and boost expression of secretory cargo. To gain insight into the mechanism by which these bZip transcription factors regulate target gene expression in a tissue-specific manner, we developed an assay to identify CrebA DNA binding sites in the developing embryonic salivary gland (SG) of Drosophila. We have discovered that CrebA binding is linked to open chromatin and the presence of consensus binding sites previously identified through *in vitro* assays. We show that CrebA functions as a transcriptional activator and that it is necessary and/or sufficient for the expression of ~80% of the tested SG expressed genes to which CrebA binds *in vivo*. We demonstrate the importance of the consensus sites in CrebA transcriptional activation by mutating the consensus sites in the context of the endogenous enhancers of two of the newly discovered CrebA transcriptional targets, Xbp1 and Tudor-SN (TSN). Finally, we describe secretory roles for both of these direct CrebA target genes, Xbp1 and TSN. The mammalian orthologues of both CrebA and Xbp1 have been implicated in the unfolded protein response. Our findings suggest that the ER stress induced by drugs and excess unfolded proteins activates endogenous pathways that normally function to accommodate the increased secretory load of professional secretory cells. Altogether, these findings emphasize the role of the CrebA/Creb3L-like proteins in the direct regulation of secretory capacity and suggest that many/most of the additional direct targets of CrebA identified in this study function in secretion.

363 Uncovering isoform-specific roles of GAGA Factor and its function during early embryo development. *M. Gaskill*, T. J. Gibson, A. Iyengar, M. Harrison Dept. of Biomolecular Chemistry, University of Wisconsin School of Medicine and Public Health, Madison, WI.

Following fertilization, two specified germ cells must be reprogrammed to form a totipotent zygote. Transcription factors are essential for this transformation in cellular identity by driving widespread changes in gene expression. In *Drosophila,* the transcription factor Zelda reprograms the early embryo by facilitating chromatin accessibility and activating transcription from the zygotic genome. Data from our lab and others suggest the essential transcription factor GAGA Factor (GAF) functions with Zelda to shape chromatin accessibility and activate the zygotic genome. While GAF has been extensively studied *in vitro*, technical challenges have limited our ability to determine the role of this important factor in embryonic development. While there are at least two GAF isoforms generated through alternative splicing, a single isoform is expressed during the first six hours of development. Apart from the developmental regulation of isoform expression, little is known about the specific developmental roles of these isoforms. Using Cas9-mediated genome engineering we established tools to investigate the role of GAF in the embryo. To identify functional distinctions between the two isoforms, we mutated each isoform individually. Unexpectedly, given that isoform expression is developmentally regulated, these individual mutants are viable and fertile. This suggests functional redundancy between the isoforms or that a minimal portion of the short isoform is sufficient for activity. We also created isoform-specific, GFP-tagged proteins and used them to image GAF protein dynamics during early development. We demonstrated that GAF is already localized to subnuclear foci as early as an hour after fertilization (nuclear cycle 10). We then used these endogenously tagged proteins to identify GAF-bound loci in the embryo at time points spanning the first few hours of development. Together these data will determine if GAF chromatin occupancy is dynamic or stable as the zygotic genome is being reprogrammed and provide insights into the role of GAF and isoform-specific expression in this process. Future experiments will leverage these tools to further define the role of GAF in reprogramming the embryonic genome.

364 Diversification of retinoblastoma protein function associated with cis and trans adaptations. R. Mouawad[1], P. Himadewi[1], J. Prasad[1], D. Thorley[1], D. Kadiyala[1], N. Wilson[1], P. Kapranov[2], *D.N. Arnosti*[1] 1) Department of Biochemistry & Molecular Biology, Michigan State University, East Lansing, MI ; 2) Institute of Genomics, School of Biomedical Sciences, Huaqiao University, Xiamen, China.

Retinoblastoma proteins are eukaryotic transcriptional co-repressors that play central roles in cell cycle control, among other functions. Although most metazoan genomes encode a single retinoblastoma protein, gene duplications have occurred on occasion: in the vertebrate lineage, leading to three genes

encoding RB, p107, and p130, while separately in the Drosophila lineage an ancestral *rbf1* gene and a derived *rbf2* gene. Structurally, Rbf1 resembles p107 and p130 most closely, and mutation of the gene is lethal, while Rbf2 is more divergent, and is not essential for development. Rbf1 has been demonstrated to be a potent repressor of canonical cell-cycle promoters, unlike Rbf2. The retention of *rbf2* over 60 million years in the entire Drosophila lineage points to essential functions, however. We show here that Rbf2 regulates a broad set of cell growth control related genes, and can antagonize Rbf1 on specific sets of promoters. *rbf2* null mutants exhibit abnormal development of the female reproductive tract, with reduced egg laying, while heterozygous null mutants exhibit an increased rate of egg deposition, suggesting that the normal function of this protein is critical for optimal control of fertility. The structural alterations found in conserved regions of the *rbf2* gene suggest that this gene was sub- or neofunctionalized to develop specific regulatory specificity and activity. We define cis regulatory features of Rbf2 target genes that allow preferential repression by this protein, indicating that it is not merely a weaker version of the ancestral protein. The specialization of retinoblastoma function in Drosophila may reflect a parallel evolution found in vertebrates, and raises the possibility that cell growth control is equally important to cell cycle function for this conserved family of transcriptional corepressors.

365 A novel role for Blimp-1 in the transcriptional repression of the Hippo pathway in postmitotic photoreceptors. *J.R. Bunker*[1], P. Boodram[1], G. Call[2], J. Rister[1] 1) Biology, UMass Boston, Boston, MA; 2) Midwestern University, Glendale, AZ.

For the visual system to discriminate colors, subsets of photoreceptors must express specific color-sensing rhodopsins in a mutually exclusive fashion. In *Drosophila*, R8 photoreceptors either express blue-sensitive Rhodopsin 5 (Rh5) or green-sensitive Rhodopsin 6 (Rh6). This cell-fate decision is regulated by the Hippo pathway. In its canonical role, the Hippo pathway acts as a tumor suppressor pathway in which the kinase Warts (Wts) phosphorylates the oncogene and transcriptional coactivator Yorkie (Yki). This sequesters Yki in the cytosol and inhibits the activation of downstream proliferative and anti-apoptotic genes. In the absence of phosphorylation by Wts, Yki localizes to the nucleus and also promotes Hippo pathway activity, creating a negative feedback loop. However, this negative feedback is absent in postmitotic R8 photoreceptors. Instead, components of the Hippo pathway act as a bi-stable switch: either *wts* is expressed (Hippo ON), leading to Rh6 expression, or *wts* is transcriptionally repressed (Hippo OFF), leading to Rh5 expression. The mechanisms that control this transcriptional regulation of *wts* in postmitotic R8 photoreceptors are not well understood.

Blimp-1/PRDM1 is a transcriptional repressor with many known functions in mammals, for instance it promotes the maturation of B lymphocytes into plasma cells as well as photoreceptor cell fate in the developing murine retina. Its roles in *Drosophila* however, are less well defined. Here we identify a novel role for Blimp-1 in transcriptionally regulating *wts* in postmitotic R8 photoreceptors. Photoreceptor-specific RNAi-mediated knockdown of *Blimp-1* as well as *Blimp-1* null mutant clones caused a loss of Rh5 and a gain of Rh6, suggesting that Blimp-1 promotes Rh5 fate and represses Rh6 fate. Concomitant RNAi-mediated knockdown of *wts* and *Blimp-1* rescued this mutant phenotype, indicating that Blimp-1 acts upstream of Wts to induce Rh5 fate. To determine if *wts* transcription was affected by the loss of *Blimp-1*, we used a *wts* enhancer that was sufficient to drive reporter expression exclusively in the Rh6-expressing photoreceptors. As expected, *Blimp-1* null mutant photoreceptors expressed the *wts* reporter, confirming that Blimp-1 is required for the transcriptional repression of *wts*. Moreover, we identified two conserved motifs in the same *wts* enhancer that closely resembled previously reported Blimp-1 binding sites. As expected, mutating these motifs caused a de-repression of the reporter in the Rh5-expressing photoreceptors. Taken together, our data support the model that the transcriptional repression of *wts* in postmitotic R8 photoreceptors is mediated by the repressor Blimp-1. We are currently testing whether this regulatory relationship also exists in the growth context.

366 Using Spineless gene expression to understand the Mechanisms of Transvection. *A. Chen*, J. Han Johns Hopkins University, Baltimore, MD.

Stochastic gene expression is required for the development of sensory systems including photoreceptors and olfactory neurons. Breakdowns in stochastic fate specification can lead to disorders such as color blindness, autism, lymphoma, and immunodeficiencies. The Drosophila retina provides an excellent model for the study of stochastic gene expression, as R7 photoreceptors randomly express the light-detecting proteins Rhodopsin 3 (Rh3) or Rhodopsin 4 (Rh4) to define R7 photoreceptor subtypes. The decision to express Rh3 or Rh4 is mediated by the transcription factor Spineless (Ss), which is stochastically expressed in 65% of R7s.

The ss gene locus contains a complex cis-regulatory logic. Different DNA elements regulate ss between chromosomes in a process called transvection. Through transvection, alleles of ss with different expression frequencies act between chromosomes to activate and repress expression, yielding an intermediate ratio of Ss^{ON} to Ss^{OFF} cells.

We examined the role of ss regulatory elements in mediating transvection by characterizing two mutant alleles of ss, one with a silencer inversion and another with an uncharacterized mutation in its upstream regulatory region. We crossed these alleles with lines that contained CRISPR deletions of specific ss regulatory elements to determine the DNA elements required for ss transvection.

We found that the silencer element is able to perform transvection and repress gene expression from the alternative chromosome, even when it is located megabases away. Additionally, we found that an intact promoter and enhancer on the same chromosome are necessary to act between chromosomes and repress ss expression, consistent with previous data that activating ss transvection requires both elements on the same chromosome. In the future, we will sequence the uncharacterized ss allele, examine additional CRISPR deletion lines, and use DNA fluorescence in situ hybridization (FISH) to examine the effects of these mutant ss alleles on homologous chromosome pairing. Our work will characterize the DNA regulatory mechanisms required for gene activation and repression between chromosomes.

367 *REDfly:* The *r*egulatory *e*lement *d*atabase for *Drosophila*. *M.S. Halfon*[1,2,3], S.V.E. Keränen[4], S.M. Gallo[2,5] 1) Departments of Biochemistry, Biological Sciences, and Biomedical Informatics, University at Buffalo-SUNY, Buffalo, NY; 2) NY State Center of Excellence in Bioinformatics and Life Sciences, Buffalo, NY; 3) Molecular and Cellular Biology Department and Program in Cancer Genetics, Roswell Park Comprehensive Cancer Center, Buffalo, NY; 4) Independent consultant; 5) Center for Computational Research, University at Buffalo-SUNY, Buffalo, NY.

The REDfly database provides a comprehensive curation of experimentally-validated *Drosophila cis*-regulatory modules (CRMs, "enhancers") and transcription factor binding sites (TFBSs). The database seeks to include all functionally tested sequences, both with and without observable regulatory activity and regardless of whether they have activity redundant with other, shorter regulatory sequences, so that all experimental data are available for exploration. Graphical views show the position of each CRM within its genomic locus, and the location of each CRM with respect to its associated gene is provided. A key REDfly feature is extensive expression pattern annotation for each CRM's activity using the *Drosophila* anatomy ontology, which allows for detailed searching of the data at varying levels of granularity. REDfly currently covers over 800 publications and contains more than 23,000 records of reporter constructs regulating over 830 genes, including over 6400 "minimal" CRMs from transgenic in vivo reporter assays and over 10,000 from cell-culture assays, as well as over 2200 TFBSs. Recent developments include the start of curation of predicted *cis*-regulatory modules in addition to experimentally-verified ones (>8000 current records), improved search and filtering, and increased interaction with the authors of curated papers. Within the next year, we will introduce an expanded data

model that will capture information on temporal aspects of gene regulation, regulation in response to environmental and other non-developmental cues, sexually dimorphic gene regulation, and non-endogenous (ectopic) aspects of reporter gene expression. REDfly is freely accessible at http://redfly.ccr.buffalo.edu and can be followed on Twitter at @REDfly_database.

368 A Vestigial myoblast enhancer is positively regulated by Twist and Notch signaling whereas cut signaling suppresses enhancer activity during the development of *Drosophila* thoracic muscles. *P. Paudel*[1], T. Lovato[1], R. Cripps[2] 1) Biology, University of New Mexico, Albuquerque, NM; 2) Biology, San Diego State University, San Diego, CA.

One of the challenges in muscle development research is to delineate how muscles with unique fiber types are formed. In *Drosophila*, thoracic muscles are of two types: direct flight muscles (DFMs) and indirect flight muscles (IFMs). The correct patterning of each of these muscle categories requires the coordination of specific executive regulatory programs. Previous findings have showed that the regulatory gene *cut*(ct) is required for DFM development, whereas *vestigial*(vg) is required for IFM development. During the larval stage, both of the DFM and IFM adult myoblasts are localized in the outer epithelial layer of wing discs where Notch and Twist are highly expressed. We have identified the vg enhancer that integrates these signals. Preliminary data have showed that Twist can bind to three sites within the enhancer, and Notch, which functions through Suppressor of Hairless Su(H), binds to a single site within the vg enhancer. Our Beta-gal stain has showed no expression in the wing disc myoblasts in Su(H) mutant suggesting that vg is positively regulated by Notch Signaling. However, it is not clear why vg is not expressed in DFM since the positive regulators, Twist and Notch, are expressed in both myoblast types. To delineate this reciprocal effect of vg and ct in larval wing discs, we hypothesize that either wingless (wg) positively regulates vg in IFMs, or alternatively, wg represses the ct and ct represses vg in DFM myoblasts. Since it is not clear how vg shows loss of activity in DLM, our proposed experiments can provide significant new insight into how vg enhancer is regulated in wing disc myoblasts, and more broadly can inform how intrinsic and extrinsic signals are integrated at the genomic level to influence cell fate.

369 Using natural variation in *Drosophila* to uncover how genetic architecture impacts heat-shock recovery and hormesis. *K.G. Owings*, C. Y. Chow Department of Human Genetics, University of Utah, Salt Lake City, UT.

Cells and organisms must adapt to adverse conditions and changing environments. An ongoing challenge is to understand how an individual's genome can respond to numerous, often simultaneous insults over the individual's lifetime. A long-observed example of an organism's ability to cope with several environmental insults is the phenomenon of hormesis, whereby conditioning organisms with low levels of stress improves their ability to withstand subsequent stress and results in beneficial health outcomes. This phenomenon was first observed in flies in 1958 by Maynard Smith, who noted that a transient exposure to heat stress increases the lifespan of female *Drosophila melanogaster.* In the years following this first report, researchers have expanded Smith's work and found that along with extending lifespan, mild heat stress can also provide flies with increased resistance to secondary stress events. The mechanism behind this preconditioning phenomenon and the impact of genetic variation on this response is still not understood. Characterizing the impact of genetic diversity will allow us to identify modifiers of heat shock hormesis that were overlooked when evaluating the phenomenon in a single strain. My research utilizes the natural genetic variation of the *Drosophila* Genetic Reference Panel (DGRP), which is a collection of 200 fully-sequenced, inbred lines derived from a natural population. My research examines how heat shock hormesis is altered by genetic background and aims to identify modifier genes of this response. I will investigate the impact of transient heat stress on lifespan, fecundity, and subsequent stress resistance in the DGRP. Strains with strong and weak hormesis will then be subjected to secondary stresses to determine how this phenomenon might protect certain genotypes from multiple stresses. This work has important implications for both health and evolution.

370 Investigating the evolutionary conservation of insulator sequences in *Drosophila*. *L. Manoj*[1], M. Fujioka[2], J. Jaynes[2], H. Mistry[1] 1) Biology, Widener University, Chester, PA; 2) Dept. Biochemistry & Mol. Biology, Thomas Jefferson University, Philadelphia, PA.

Temporal and spatial control of gene expression regulates normal development in multicellular organisms. Chromatin is subdivided and organized into discrete domains via the action of specialized DNA sequences known as boundary or insulator elements. The *even-skipped* (*eve*) locus of *D. melanogaster* has been studied to identify regulatory sequences that dictate *eve* expression in specific tissues and at precise developmental time points. At the 3′ end of the *eve* locus is a Polycomb response element (PRE) flanked by an insulator (*homie*), which functionally isolates *eve* regulatory DNA from the essential housekeeping gene *TER94*. To test whether the PRE and insulator activities have been conserved during evolution, we looked at other *Drosophila* species. In *D. erecta*, which is closely related to *D. melanogaster*, *eve* is linked to *TER94*. In the more distantly related species, *D. pseudoobscura* and *D. virilis*, *eve* is adjacent to *CG30421*. Using comparative sequence analysis, we have identified and isolated DNA fragments homologous to the *D. melanogaster* PRE-insulator region from these 3 species.

The *D. melanogaster* PRE-insulator fragment is capable of facilitating enhancer-promoter interactions in *trans* (transvection) by homologous pairing. Using this assay, we tested whether pairing function is conserved. We cloned each fragment into both a reporter transgene and an enhancer transgene. These transgenes were injected to generate transgenic stocks via phiC31-mediated recombination in *D. melanogaster*. We are in the process of determining whether there is transvection-mediating activity within the PRE-insulator-homologous fragment from a single species, and if there are interactions among the fragments from different species. We observe different intensities of reporter expression depending on the extent of functional conservation. Future analyses will involve detailed dissection of functional sequences. Thus, our comparative approach using these 4 species will give us an understanding of how the homolog pairing function of PREs and insulators has changed during evolution, and the mechanistic basis of those changes.

371 Investigating the post-embryonic role and regulation of *Ultrabithorax*. *A. D Buffry*[1], S Kittlemann [2], F. A Franke[1], K Hens[3], S Arif[1], A. P McGregor [1] 1) Department of Health and Life Sciences, Oxford Brookes University, Oxford, GB; 2) Sir William Dunn School of Pathology, University of Oxford, Oxford, GB; 3) Centre for Neural Circuits and Behaviour, University of Oxford, Oxford, GB.

Hox genes play fundamental roles in the patterning of animal body plans. Early expression of Hox genes in different domains along the embryonic anterior-posterior axis are responsible for determining segmental identity. However, Hox genes also function post-embryonically to specify fine scale morphology such as the size, shape and appearance of limbs and other organs in *Drosophila*. Less is known about these post-embryonic roles and their regulation but, given that changes in Hox gene expression is often correlated with the phenotypic evolution, it is essential that we investigate the spatial regulation of Hox genes at all developmental stages to fully understand their role in conferring distinct and diverse patterns. In *Drosophila Ultrabithorax (Ubx)* regulates the morphology of the T2 and T3 legs and has been implicated in variation in leg trichome patterning. We have investigated this role further by generating ATAC-seq data for T2 legs to identify a novel enhancer of *Ubx* that contributes to trichome patterning. Dissection of this putative regulatory region reveals that postembryonic regulation of *Ubx* is highly complex. To determine how this expression of *Ubx* is regulated we have carried out a yeast one hybrid screen on this novel enhancer to identify putative transcription factors. Our results will help to reveal how Hox genes are wired into the postembryonic gene regulatory networks responsible for fine-scale patterning of animal bodies.

372 The ecdysone hormone receptor directs the spatial and temporal activity of target enhancers. *C.M. Uyehara*[1,2,3,4], D.J. McKay[1,2,4] 1) Department of Biology; 2) Department of Genetics; 3) Curriculum in Genetics and Molecular Biology; 4) Integrative Program for Biological and Genome Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC.

A remarkable feature of insect development is the complete transformation of the body during metamorphosis. Decades of research have established the central role that pulses of the steroid hormone ecdysone play in promoting this process. At the genetic level, ecdysone acts through its receptor, EcR, which has been shown to induce a diverse array of transcriptional responses that vary between tissues and over time. However, although it has been known for many years that EcR promotes changes in gene expression, our understanding of how it effects these changes genome-wide remains incomplete. To investigate this ability, this project focuses on how EcR directs gene expression changes in the developing wing during the late-larval ecdysone pulse, which triggers the transition from the larval to pre-pupal stage. To determine EcR's role in promoting wing morphogenesis, we used tissue-specific RNAi to knockdown EcR throughout wing development and performed RNAseq prior to the late larval ecdysone pulse and 6hrs following this pulse. We find that EcR is required for thousands of gene expression changes genome-wide. To identify direct targets of EcR in the developing wing, we used CUT&RUN to generate genome-wide DNA binding profiles at both time points. We find that EcR binds to many thousands of sites genome-wide, and that, while a subset of its binding occurs near genes previously reported as canonical ecdysone targets, much of its binding occurs near genes that have not been previously identified as direct targets of EcR. Remarkably, many of these genes are important for wing development, specifically, suggesting that EcR's binding may be tissue-specific. We also observe that EcR binding is temporally dynamic – a subset of its binding sites are unique to each time point – but that, on the whole, its binding profile globally decreases between the two time points. To investigate the function of EcR binding, we cloned EcR binding sites into transgenic reporter constructs and find that EcR regulates enhancers from the gene *broad*, a canonical ecdysone target, and *Delta*, a non-canonical ecdysone target. Surprisingly, we find that EcR not only regulates the temporal activity of enhancers, but their spatial pattern as well. Overall, these findings demonstrate the central role EcR plays in shaping the response to ecdysone, including by directly acting at thousands of DNA regulatory elements genome-wide. In the future, we will explore the mechanism through which tissue and temporal-specific responses to ecdysone are achieved by assaying EcR binding at additional time points and in additional tissues.

373 Enhancer decommissioning during *Drosophila* wing development. *M. Niederhuber*[1,2,3,4], D. McKay[2,3,4] 1) Curriculum in Genetics and Molecular Biology; 2) Department of Biology; 3) Department of Genetics; 4) Integrative Program for Biological and Genome Sciences, University of North Carolina, Chapel Hill, NC.

Proper animal development requires cell-type specific gene expression programs to be effectively established and maintained over time. *Cis*-regulatory elements such as enhancers, in conjunction with the transcription factors that bind them, are key to this process. Enhancers function as points of integration for combinations of transcription factors, such that enhancer activity can be dramatically affected by intercellular differences in transcription factor expression. Beyond variations in factor composition, chromatin accessibility has emerged as another key component of enhancer regulation. Nucleosomal DNA is generally inhibitory to transcription factor binding and it is thought that enhancers must be first depleted of nucleosomes or "opened" to be sensitive to transcription factor input. The reverse process of "closing" or "decommissioning" enhancers is thought to be an equally important regulatory process during development, aiding in the dynamic utilization of *cis*-regulatory elements during specification of cell lineages over time. Despite its essential role, little is known about how enhancer decommissioning is accomplished. Our lab uses development of the *Drosophila* wing as an experimental system to investigate the mechanisms underlying chromatin accessibility and enhancer activity. We have recently observed that there are dramatic temporal changes in chromatin accessibility in the wing during the larval to pupal transition, and that many of these changes are systemically coordinated by the steroid hormone ecdysone. We have found that the ecdysone-induced transcription factor Eip93F (E93) is necessary and sufficient to both activate and decommission many enhancers during wing development. This raises the question of how E93 is specifically directed to decommission rather than activate enhancers, and what other factors may be involved in this process. We hypothesize that E93 is working cooperatively with other transcription factors and chromatin remodelers, and may form distinct complexes to achieve these different functions. Here we present genomic and *in vivo* reporter data demonstrating that E93 is both necessary and sufficient to decommission the $broad^{disc}$ enhancer (br^{disc}), and we describe our ongoing efforts to identify other factors that are required for enhancer decommissioning.

374 Affect of E93 DNA binding domain on chromatin accessibility in *Drosophila melanogaster* during development. *M. Savage*, M. Niederhuber, D. McKay, E. Liriano Department of Biology, UNC Chapel Hill, Chapel Hill, NC.

Spatio-temporal chromatin accessibility during early development is controlled by the interactions between transcription factors and cis-acting elements. The presence of certain transcription factors can either activate or deactivate enhancers, which ultimately affect if a region of chromatin is nucleosome depleted (open) or nucleosome rich (closed). Since unique chromatin schemes correspond to specific developmental transitions, understanding how this process is regulated is important to understanding how early embryological development in humans is controlled, and ultimately find effective measures to treat such negative interactions. I am currently investigating the transcription factor E93 in *drosophila melanogaster,* which is known to be required for both opening and closing chromatin. The goal was to determine how E93 can serve to both open and close chromatin, which would be utilizing two separate functional pathways. Since E93 has several functional domains, it is hypothesized that each domain corresponds with either the opening chromatin or closing chromatin pathway, therefore exhibiting a separation of function between the domains. The first step is creating a mutation in one of these domains and examining how enhancer activity, and therefore chromatin accessiblity, changes when ectopically expressed in half of the wing. Site directed mutagenesis was used to create a loss of function mutation in the DNA binding domain of E93, and then injected into fly embryos to create the transgenic line. I will test if E93 is sufficient in opening chromatin by ectopically expressing E93 and seeing if it affects the functioning of the enhancer tmcA-td. Tomato (which has already been proven to require E93 to function). If it is shown to be sufficient, then I will repeat this experiment but using the transgenic flies with the mutation in the DNA binding domain. If sufficiency is lost, then the DNA binding domain must be involved in opening chromatin. This was then repeated with closing chromatin. The results of this study are still currently being investigated.

375 Dynamic interplay between enhancer-promoter topology and gene activity. *H. Chen*[1,2], M. Levo[1,2], L. Barinov[3], M. Fujioka[4], J. Jaynes[4], T. Gregor[1,2] 1) Physics, Princeton University, Princeton, NJ; 2) Lewis-Signer Institute, Princeton University, Princeton, NJ; 3) Department of Molecular Biology, Princeton University, Princeton, NJ; 4) Department of Biochemistry, Thomas Jefferson University, Philadelphia, PA.

A long-standing question in metazoan gene regulation is how remote enhancers communicate with their target promoters over long distances. Combining genome editing and quantitative live imaging we simultaneously visualize physical enhancer–promoter interaction and transcription in Drosophila embryos. Enhancers regulating stripes of even-skipped expression activate transcription of a reporter gene 150kb away. We show, at the single-cell level, that activation only occurs after the enhancer comes into close proximity with its regulatory target and that upon dissociation transcription ceases almost immediately. We further identified three distinct topological conformation states involved in long-distance enhancer-promoter interaction. By measuring the transition kinetics between these topological states, we found that transcription is associated with a temporal stabilization of the enhancer–promoter proximal conformation and with a spatial compaction of the locus. Furthermore, genetically tuning insulator protein binding sites modulates kinetics of topological state transitions and transcriptional activation. Finally, the facilitated long-range activation results in transcriptional competition at the endogenous *eve* locus, causing corresponding developmental defects. In summary, our experimental system provides approaches to quantitatively study the dynamic interplay between chromatin topology and gene activation and probe the implications of long-range regulation on cellular fates.

376 Role of the Mediator complex in regulating SREBP-dependent gene expression. *X. Li*[1], M. Liu[1], J-Q. Ni[2], J-Y. Ji[1] 1) Department of Molecular and Cellular Medicine, Texas A&M University Health Science Center, College Station, TX; 2) School of Medicine, Tsinghua University, Beijing, China.

Aberrant *de novo* lipogenesis is strongly associated with a variety of human diseases, such as metabolic syndromes and cancers. The transcription factor SREBP (Sterol regulatory element-binding protein) plays a critical role in regulating the expression of the key lipogenic enzymes, such as FAS (Fatty acid synthase), ACC (Acetyl-CoA carboxylase) and ACS (Acetyl-CoA synthetase), in *Drosophila* and mammals. The homologs of SREBP have been reported to activate lipogenic gene expression by interacting with Med15, a conserved subunit of the Mediator complex in *C. elegans* and *Xenopus*. The Mediator complex is composed of 30 different subunits, including the only enzymatic subunit CDK8 (cyclin-dependent kinase 8) and its regulatory partner CycC (cyclin C). Our previous developmental genetic analyses of *cdk8* and *cycC* mutants have revealed that CDK8-CycC negatively regulates *de novo* lipogenesis by inhibiting SREBP-dependent expression of lipogenic enzymes. We have observed that CDK8-CycC can phosphorylate nuclear SREBP at a conserved threonine residue (Thr390 in *Drosophila*), thereby destabilize SREBP proteins in mammalian cells. Here we report our further analyses of the interplay among SREBP, Med15, and CDK8, as well as the physiological consequence of the SREBP phosphorylation. Our results suggest that Med15 directly interacts with the N-terminus of SREBP, and interestingly, the same region of SREBP can also directly interact with CDK8. Mutation of six essential amino acids within this region of the SREBP abolishes its binding to either Med15 or CDK8. In addition, mutation of the phosphorylation site (T390A) strongly stabilized SREBP protein, enhanced its binding to chromatin, and significantly increased the transcriptional activity in fat body. These results suggest that the dynamic interplays among subunits of the Mediator complex plays critical roles in the tight control of the SREBP-dependent lipogenic gene transcription.

377 *Drosophila* tsRNAs suppress general translation machinery *via* antisense pairing and participate in cellular starvation response. Shiqi Luo, Feng He, Junjie Luo, Shengqian Dou, Yirong Wang, Annan Guo, *Jian Lu* School of Life Sciences, Peking University, Bejing, CN.

Transfer RNA-derived small RNAs (tsRNAs) are an emerging class of small RNAs, yet their regulatory roles have not been well understood. Here we studied the molecular mechanisms and consequences of tsRNA-mediated regulation in Drosophila. By analyzing 495 public small RNA libraries, we demonstrate that most tsRNAs are conserved, prevalent and abundant in Drosophila. By carrying out mRNA sequencing and ribosome profiling of S2 cells transfected with single-stranded tsRNA mimics and mocks, we show that tsRNAs recognize target mRNAs through conserved complementary sequence matching and suppress target genes by translational inhibition. The target prediction suggests that tsRNAs preferentially suppress translation of the key components of the general translation machinery, which explains how tsRNAs inhibit the global mRNA translation. Serum starvation experiments confirm tsRNAs participate in cellular starvation responses by preferential targeting the ribosomal proteins and translational initiation or elongation factors. Knock-down of AGO2 in S2 cells under normal and starved conditions reveals a dependence of the tsRNA-mediated regulation on AGO2. We also validated the repressive effects of representative tsRNAs on cellular global translation and specific targets with luciferase reporter assays. Our study suggests the tsRNA-mediated regulation might be crucial for the energy homeostasis and the metabolic adaptation in the cellular systems.

Reference: Luo S, He F, Luo J, Dou S, Wang Y, Guo A, **Lu J*** (2018). *Drosophila* tsRNAs preferentially suppress general translation machinery via antisense pairing and participate in cellular starvation response. ***Nucleic Acids Research.*** 46(10):5250-5268.

378 Identifying chromatin modifiers that regulate stochastic Spineless expression in *Drosophila* retinas. *L. Yuan*, C. Anderson, K. Viets, R. Johnston Biology, Johns Hopkins University, Baltimore, MD.

Stochastic gene expression is important for several developmental processes, including proper immune cell fate determination, and sensory receptor diversification. The mosaic of R7 photoreceptor subtypes in the *Drosophila melanogaster* retina is controlled by the stochastic expression of the transcription factor Spineless. In the subset of R7s that express Ss Rh4 is expressed, whereas the R72 lacking Ss express Rh3. Although R7 subtype specification is random, the ratio of Rh4:Rh3 is similar among flies of the same genotype. Here, I found that long range heterochromatin repression and chromatin modifiers affect Ss expression. I examined RNAi knockdowns and loss-of-function mutants of chromatin modifiers to determine their role in Ss expression. I discovered that Trithorax Group (TrxG) protein Trx and Ash2, and Polycomb Group (PcG) protein Pho regulate stochastic Ss expression. In accordance to its H3K4 methyltransferase activity, Ash2 upregulates Ss expression. However, sharing similar function, Trx exhibited the opposite effect, and Pho, which recruits PRC2 to Polycomb Response Elements (PREs), upregulates Ss expression. I performed Ss RNA FISH in third instar larvae eye discs, where a wave of Ss is expressed in R7 precursor cells prior to terminal differentiation, and found that Trx defines the Ss expression boundaries of the wave and that Ash2 increases Ss expression levels in R7 precursors. We propose a model in which the chromatin state at the *ss* locus during differentiation dictates Ss expression levels in R7 precursors, which in turn defines the SsON:SsOFF ratio and subsequent Rh4:Rh3 ratio in adult retinas. Further, we hypothesize that the competitive nature of PcG and TrxG binding to the *ss* locus determines Ss expression levels in the precursor cells. These data suggest that chromatin modifiers play an essential role in the regulation of stochastic gene expression and identifying their function will provide insight into other stochastic gene regulation systems.

379 A tsRNA-AGO1 autoregulatory feedback loop. J. Shi[1], J. Ma[1,2], *F. He*[1,2] 1) Institute of Genetics, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China; 2) Children's Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China.

Transfer RNA-derived small RNAs (tsRNAs) are an emerging class of small regulatory RNAs. Previously we showed that Drosophila tsRNAs regulate gene expression preferentially by supressing general translation machinery in an AGO2-dependent manner. Here we uncovered that AGO1 can be targeted by 5′-tsRNA^{Asp}, a tsRNA species that is highly abundant and conserved from *Drosophila* to human, in the 3′ UTR and reduces its own mRNA level. By transfecting S2 cells with 5′-tsRNA^{Asp} mimics, we showed a significant reduction of the AGO1 expression and the increased mRNA levels of the miRNA target genes. To examine the functions of the endogenous 5′-tsRNA^{Asp}, we generated a UAS-sponge line, which expresses the non-translating RNA with repeated complementary sites and the EGFP protein. We showed that, driven by GAL4, the sponge RNAs effectively suppressed the inhibitory activity of 5′-tsRNA-Asp on the AGO1 expression. To illustrate AGO1 autoregulation in vivo, we generated mutant lines by replacing all three 5′-tsRNA^{Asp} target sites of *ago1* with scrambled sequences. Finally, our mathematical model demonstrated that tsRNAs may play a role in buffering the expression noise of the miRNA target genes. Thus, our study sheds lights on the crosstalk between the miRNA and the tsRNA repertoires.

380 Probing the role of early and transient ncRNAs into opening of the segment-specific regulatory domains of the BX-C. *F. Karch*, S. Galetti, A. Mutero, R. Maeda Dept Genetic and Evolution, Univ Geneva, Geneva, CH.

The bithorax complex (BX-C) is composed of 9 segment-specific regulatory domains that are arranged along the chromosome in the same order as the segments they specify along the antero-posterior axis of the fly. Each domain is composed of 3 types of regulatory elements. The 1st includes the “initiator” elements. Initiators contains binding sites for the gap and pair-rule genes products, sensing thereby their position along the anterior-posterior axis(AP) of the embryo to turn on the regulatory domains in the appropriate parasegment. The 2nd class of regulatory elements includes the cell type- or tissue-specific enhancers. When present in an ectopic reporter gene construct, their activities are usually not restricted along the AP axis. The 3rd class of regulatory elements are the maintenance element that respond to the Polycomb- and trithorax-group protein complexes (PREs/TREs). PREs and TREs remember the state of activity of the regulatory domains once the products of the gap and pair-rule genes has vanished. In our working model, initiator elements function to turn on the regulatory domain into the appropriate parasegment during early embryogenesis. Initiator elements function as parasegmental address, but do not function to assign parasegmental identity. Instead, parasegmental identity is provided by the cell type-enhancers. Then the PRE/TRE are recruiting

Polycomb repressive complex if the domain is off or trithorax activating complex if the domain is on. In this model, the initiator function as a domain control region, raising the question as how the initiator communicates with the cell type-specific enhancers and with the PREs/TREs. It has been long known that the segment-specific regulatory domains are transiently transcribed in discrete domains along the AP axis during early embryogenesis. Whether these transcripts play a role into domain activation or whether they result from the spurious activation of cryptic promoters due to the opening of the domains remains an open question. Using our extensive collection of internal deficiencies in the *iab-6* regulatory domain, we find that these early transcripts emanate from the initiator element. By reorganizing the orientation and the position of the initiator element within the *iab-6* domain, our results agree well with a causal role of the early ncRNAs in domain activation. But so far we failed to interfere with their production and to correlate it with a failure of domain activation.

381 How do changes in DNA lead to changes in tissue function? *G. Hanna* College of Biological Sciences, UC Davis, Davis, CA.

Some transcription factors play major roles in the testes of *D. melanogaster*. These factors help maintain the identity and function of cell types present in the stem cell niche; a collection of cells essential to the male's fertility in Drosophila.

Surprisingly, some of these transcription factors are not produced in the testes of many closely related species of flies, and we have shown that the genes encoding those factors have only gained expression in this tissue after divergence from *D. simulans.*

Since transcription factors assume important roles in extensive networks where they regulate the expression of other genes, I use the testes in Drosophila as a model to 1) Explain how transcription factors gain expression in a new tissue, 2) Study the extent of gene regulatory network rewiring in this rapidly evolving tissue, and 3) Assess how testis function changes due to rewiring of

transcription factor networks.

382 Identification of enhancer elements and factors binding to these elements in the proximal and intergenic non-coding regions of *dmyc* Gene. *J. Kharazmi* Department of Molecular Life Science, University of Zurich, Schlieren, Zurich, CH.

MYC, a key *X*-linked growth-regulating gene, integrates mitogenic signals by modulating the expression of a wide range of targets involved in cellular growth and metabolism to regulate apoptosis, proliferation, and differentiation. However, deciphering the exact role of pathways regulating the expression of *myc* at the level of transcription is still a challenge. In previous work we showed that most of the regulatory elements and factor binding sites are located within the 5' UTR and the intergenic regions of *dmyc*. In this work by further dissection of regulatory sequences into smaller truncations, I have identified nested cis-regulatory modules (CRMs), which depending on the presence or absence of each element the activity of *dmyc*-lacZ reporter changes in the developing primordial tissues. Functional Genomics experiments are under way to identify factors binding to the identified enhancer elements. These findings may provide valuable experimental data in placing *dmyc* at the nexus of pathways regulating its transcription in the early development.

383 Transcriptional silencers in *Drosophila* serve a dual role as transcriptional enhancers in alternate cellular contexts. *S.S. Gisselbrecht*[1], A. Palagi[1,2], J.V. Kurland[1], J.M. Rogers[1,3], H. Ozadam[4], Y. Zhan[4], Y. Sytnikova[1], J. Dekker[4,5], M.L. Bulyk[1,3,6] 1) Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Boston, MA; 2) Doctoral School of Life and Health Sciences, University of Nice Sophia Antipolis, 06560 Valbonne, France; 3) Committee on Higher Degrees in Biophysics, Harvard University, Cambridge, MA; 4) Program in Systems Biology, Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA; 5) Howard Hughes Medical Institute, Chevy Chase, MD; 6) Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

A major challenge in biology is to understand how complex gene expression patterns are encoded in the genome. While transcriptional enhancers have been studied extensively, few transcriptional silencers have been identified and they remain poorly understood. Here we used a novel strategy to screen hundreds of sequences for tissue-specific silencer activity in whole *Drosophila* embryos. Strikingly, almost all transcriptional silencers we identified were also active enhancers in other cellular contexts. We discovered more of such bifunctional *cis*-regulatory modules (CRMs) than were previously known across all biological systems. These elements were enriched in highly occupied target region overlap and make contact with promoters of transcriptionally repressed genes. CRM bifunctionality complicates the understanding of how gene regulation is specified in the genome and how it is read out in different cell types. Our results challenge the common practice of treating enhancers and silencers as separate classes of regulatory element and suggest that thousands or more bifunctional CRMs remain to be discovered in *Drosophila* and 10^4-10^5 in human.

384 Distinct patterns of combinatorial regulation by isoforms of the ETS activator Pointed confer specificity to retinal cell fate acquisition. *C. Wu*[1,2,3], J. Lachance[1,2,4], I. Rebay[1,2] 1) Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL; 2) Ben May Department for Cancer Research, University of Chicago, Chicago, IL; 3) USACommittee on Genetics, Genomics and Systems Biology, University of Chicago, Chicago, IL; 4) University of Chicago, Department of Human Genetics, McGill University, Montreal, Quebec, Canada.

During development, cells integrate external signals and internal information to coordinate the transition from a multipotent to a differentiated state. We study this process in the developing retina where reiterative use of the Epidermal Growth Factor Receptor (EGFR) signaling pathway directs a temporally and spatially precise sequence of cell fate transitions. Molecularly, sequential specification of the distinct photoreceptor and non-neuronal fates that make up each ommatidium of the compound eye is achieved by integrating the transcriptional input from the EGFR pathway effector Pointed (Pnt) with different combinatorial codes of other transcription factors. Published work from multiple labs has shown that the *pnt* locus encodes alternative spliced isoforms, and that a common pattern of sequential activation from one isoform to the other is required for the photoreceptor-inductive transcriptional response to EGFR signaling. Here we demonstrate that much more complicated patterns of mutual regulation and combinatorial action among Pnt isoforms accompany and likely direct different retinal cell fate transitions. Using isoform-specific fluorescent protein tags and systematic quantification of expression dynamics, we find spatio-temporally distinct patterns of Pnt isoform expression associated with different progenitor cell pools and different photoreceptor cell types. Photoreceptor cell fate specification defects associated with individual or combinatorial knockout of the different isoforms further suggest cell-type specific requirements for Pnt function. Mechanistically, isoform-specific overexpression and genetic rescue assays reveal distinct levels of Pnt transcriptional activity, suggesting much greater context-specificity to the transcriptional response downstream of EGFR than previously suspected. Overall, our work uncovers novel transcriptional and functional feedback and feedforward connections within the local Pnt isoform network that contribute significantly to the combinatorial codes that confer specificity to retinal cell fate acquisition.

385 Two distinct pathways are involved in the THO/TREX-mediated piRNA biogenesis in *Drosophila* testis. *Chulsung Park*[1], Jeongyeon Kim[1], Junho Hur[2], Yundoo Chung[1] 1) Department of Life Science, University of Seoul, Seoul, KR; 2) Department of Pathology, Kyung Hee University School of Medicine, Seoul, KR.

The THO/TREX complex is a conserved multi-subunit complex known to be required for the biogenesis of the export-competent mRNP. In *Drosophila* ovary, recently, we found that THO/TREX is loaded onto the precursors of PIWI-interacting RNAs (piRNAs), a class of small non-coding RNAs that ensure germline genomic integrity by suppressing genomic parasites such as transposons, by interacting with RDC complex. In ovary, THO/TREX is recruited onto only the dual-strand piRNA source loci in a chromatin-code dependent manner and ensures the efficient transcription of piRNA precursors. To examine whether THO/TREX and RDC have similar roles in the testis to those in the ovary, we analyzed the expression profile of transposons and piRNAs in the testis by using RNA-

sequencing. Our results showed that transposon-mapped piRNAs in the early stage germ cells (gonialblasts (GB) and spermatogoina (SG)) were preferentially affected by RDC mutation compared to those in the later stage germ cells (spermatocytes (SC)). On the other hand, THO/TREX affected piRNA biogenesis throughout the whole spermatogenesis stages. In terms of piRNA source loci, most piRNAs affected by THO/TREX or RDC were from the dual-strand clusters, suggesting that piRNA biogenesis from the dual-strand clusters in the testis has very similar mechanisms to that in the ovary. Taken together, our data suggested that two distinct pathways are involved in the THO/TREX-mediated piRNA biogenesis; the RDC-dependent pathways in the early GB/SG stage cells, and the RDC-independent pathways in the later SC stages.

386 The *Drosophila* CLAMP regulator of dosage compensation co-localizes with group of RNA binding proteins in a tissue-specific manner in both sexes. *M. Ray*, E. Larschan MCB, Brown University, Providence, RI.

Chromatin architecture plays pivotal role in determining gene expression. There are many factors known to regulate the dynamic organization of chromatin, either making it more accessible to the transcription machinery or compacting it, resulting in silencing of the locus. In *Drosophila,* CLAMP (**C**hromatin-**l**inked **a**daptor for **M**SL **p**roteins) is a zinc finger domain rich protein required for recruitment of the MSL complex to the male X-chromosome and increasing the accessibility of the single male X-chromosome, allowing it to transcribe twice as much as the female X- chromosome. However, CLAMP is present on autosomes as well, making the chromatin more accessible but does not recruit the MSL complex to these loci. To determine how CLAMP functions differently on the X-chromosome compared to the autosomes, we identified interactors of the CLAMP protein which include several RNA-binding proteins. Here, we characterize the interaction of CLAMP with a group of RNA binding proteins which were from ChIP-Mass spec studies performed using the CLAMP antibody in S2 and Kc cells. We found that in polytene chromosomes CLAMP co-localized with some of these RNA binding proteins like Squid and Syncrip at certain loci, well-distributed over both autosomes and sex-chromosomes. This co-localization was not dependent on the sex of the individuals. CLAMP distribution in different tissues at different stages was also similar between males and females. However, we identified tissue specific variation in co-localization of CLAMP with RNA binding proteins. Clamp showed similar dynamic localization as Squid in developing egg chambers of ovary, whereas Syncrip only co-localized with Clamp in tissues with some degree of polytenization but not in any other larval or adult tissues. This indicates that Clamp is likely to show differential interaction with different RNA binding proteins depending upon tissue type rather than sex of the individual.

387 Post-transcriptional control of gene expression in the early Drosophila embryo. *C.A. Smibert* Biochemistry, University of Toronto, Toronto, Ontario, CA.

Regulation of mRNA stability and translation play central roles in the early development of all animals. Nuclei within the newly formed zygote are transcriptionally silent and all early developmental processes are controlled by maternal proteins and mRNAs deposited into the egg by the mother. As such RNA-binding proteins (RBPs) that control the translation and/or stability of target mRNAs play a major role in early embryonic development.

We have previously shown that the RBP Smaug (SMG) plays a direct role in inducing the degradation of a large number of mRNAs in the early Drosophila embryo. Here we describe our work showing that the RBP Pumilio (PUM) is involved in another embryonic mRNA decay pathway. A critical target of PUM is the mRNA encoding SMG. We show that PUM-mediated degradation of smg mRNA is required to clear SMG protein from the embryo. Failure to do so results in large-scale changes to the embryo's transcriptome, as ectopic SMG inappropriately targets mRNAs for degradation. Our data also indicate that PUM functions with the TRIM-NHL RBP Brain tumor (BRAT) and Argonuate 1, the Drosophila miRNA Argonuate, to induce smg mRNA degradation. Interestingly SMG is required to induce embryonic miR expression, likely through its ability to repress target mRNA expression. Thus we propose that a regulatory loop exists that temporally regulates mRNA decay in the embryo. Specifically, SMG activates miRNA expression thereby triggering the degradation of its own mRNA through the combined action of PUM, BRAT and AGO1.

In a separate project, we are studying Drosophila Rasputin (RIN), the Drosophila homolog of the mammalian G3BP1/2 proteins. While these proteins have a well-characterized role in stress granule formation, their function outside of stress granules remains poorly understood. We have immunoprecipitated RIN and identify its target mRNAs using microarrays. RIN's target transcripts are enriched in translated and stable mRNAs, suggesting RIN might potentiate the expression of bound transcripts. Consistent with this possibility we find that tethering RIN to a reporter mRNA with a heterologous RNA-binding domain increases reporter gene expression in transfected Drosophila S2 tissue culture cells. Tethering of either human G3BP1 or G3BP2 to a reporter mRNA in S2 cells results in a similar increase. Taken together our data suggests that the G3BP family of proteins function outside of stress granules to potentiate the expression of their target mRNAs.

388 Deciphering effects of RNA editing Enzyme on metamorphosis of *Drosophila*. *Anzer Khan*, Nagraj Sambrani, Barbora Novakova, Mary A. O'Connell , Liam P. Keegan Masaryk University, Kamenice 753/5, A35/143, 625 00 Brno, Czech Republic CEITEC MU, Brno, CZ.

One of the most prevalent type of RNA editing is the conversion of adenosine to inosine in double-stranded RNAs that is mediated by adenosine deaminases acting on RNA (ADAR) enzymes. A→I RNA editing can lead to a codon change as the nucleoside inosine (I) is interpreted as guanosine (G) by ribosomes, resulting in a diversification of protein function.

The ADAR family of proteins is present in all metazoans. In *Drosophila*, a single *Adar* is present on the distal X chromosome and is an orthologue of vertebrate *ADAR2.* In spite of major progress in the identification of editing sites, little is known about the regulatory mechanism of ADAR proteins in normal development and in disease.

In this present study, we performed a genetic screen that has uncovered a novel effect of *Adar* on ecdysone signalling which is a crucial regulator of *Drosophila* development. Ubiquitous expression of *Adar* with the *act*5c-Gal4 driver results in pupal lethality with defects in ecdysis and head eversion. The lethality caused by ubiquitous expression of *Adar* can be rescued by blocking ecdysone synthesis and signalling.

Tissue-specific over-expression of *Adar* in the Prothoracic Gland (PG) with *phm*-Gal4 causes extended larval life with a long delay in pupation, with major reductions in prothoracic gland transcripts encoding the enzymes of ecdysone synthesis and signalling. These defects may be due to either aberrant RNA editing or RNA binding by ADAR protein. We hypothesize that *Adar* expression in *Drosophila* is a prerequisite to regulate ecdysone signalling during metamorphosis.

Currently, we are dissecting regulation of the ecdysone pathway by *Adar* and pursuing loss of functions studies with *Adar* RNAi lines to decipher the role of *Adar* in metamorphosis of *Drosophila*

389 Bin3 targets multiple mRNAs during *Drosophila melanogaster* oogenesis and embryogenesis. *Ryan Palumbo*, Steve Hanes Biochemistry & Molecular Biology, SUNY Upstate Medical University, Syracuse, NY.

The Bin3 RNA methyltransferase is a protein that is essential for stability of 7SK RNA. 7SK is a ncRNA that forms a scaffold for proteins including Bin3, Larp7, and HEXIM, the latter of which interacts with P-TEFb to block its catalytic activity to induce promoter-proximal pausing. Bin3 adds a methyl group to the 5' phosphate of 7SK, thus protecting it from 5'-3' exoribonucleolytic degradation and allowing for formation of the scaffold.

We have previously demonstrated that during *Drosophila* development, Bin3 plays a role in the translational regulation of *caudal* mRNA. Consequently, we wanted to know whether Bin3 associates with this and other mRNAs, and to further demonstrate that Bin3 is a translational regulator. To these ends, we used CRISPR to epitope-tag the native *bin3* gene with 3xFLAG, and performed RNA immunoprecipitation of 3xFLAG-Bin3 from ovary and embryo extracts, followed by RNA-seq. While we did not identify ncRNAs besides 7SK, surprisingly, we identified multiple mRNA targets that were verifiable by RT-qPCR. Curiously, *caudal* mRNA was not among the hits, suggesting that Bin3 may regulate *caudal* indirectly. We are currently in the process of using polysome analysis on ovary and embryo extracts from wild type and *bin3*^{4} flies to determine how *bin3* affects polysome occupancy of these mRNAs.

One of the mRNA targets from the RIP-seq experiment was *bin3* mRNA itself. Using HOMER, we identified a motif that is enriched in two regions of the *bin3* 3'UTR (termed "Bin3 Response Elements", BRE1 and BRE2), as well as in the 3'UTRs of the other mRNA targets. We have generated transgenic flies containing inducible EGFP reporters fused to either the *bin3* 3'UTR, the *bin3* 3'UTR lacking BRE1, the SV40 poly(A) signal sequence, or the SV40 poly(A) signal sequence fused to BRE1. We are using these transgenic flies to address whether the BRE confers association with 3xFLAG-Bin3, and confers translational regulation to the mRNAs by Bin3. Our work has revealed that Bin3, previously only known to associate with 7SK ncRNA, additionally targets several mRNAs, and may regulate development through translational regulation of these targets.

390 Brain-wide screen for protein and mRNA localization reveals that multiple post-transcriptional mechanisms contribute to synaptic protein enrichment. *Josh Titlow*[1], Ana Palanca[1], Jeff Lee[1], Joyce Yu[1], Mary Thompson[1], Darragh Ennis[1], David Ish-Horowicz[2], Ilan Davis[1] 1) Dept. of Biochemistry, University of Oxford, UK; 2) MRC Lab for Molecular Cell Biology, University College London, UK.

The modulation of synaptic connections in response to neuronal activity underlies learning and memory. Although activity-dependent plasticity is thought to involve local translation, it has proved remarkably difficult to identify specific mRNAs whose synaptic localization and translation are required for plasticity *in vivo*. Here, we analyzed the distribution of 200 randomly chosen mRNAs and their encoded proteins at subcellular resolution in the intact larval central nervous system (CNS), segmental nerves, and neuromuscular junction (NMJ). Using smFISH probes against the open reading frames of *Drosophila* protein-trap collections and fluorescence of the inserted protein, we visualized gene expression from transcription in the nucleus, to mRNA and protein accumulation at the synapse. We also developed novel software solutions to visualize the images for each gene with associated bioinformatic, expression and phenotype data through FlyMine. Several new principles of mRNA localization and localized translation in the nervous system emerge from our microscopy screen: 1) Cells use a common mechanism to specify transport of mRNAs into synaptic regions. Most transcripts that localize to one synaptic region (mushroom bodies, optic lobe neuropil, or sensorimotor neuropil), are present in multiple synaptic regions. 2) mRNA enrichment in synaptic regions is rare. Fewer than 15% of analyzed genes encode mRNA transcripts found in synaptic regions, and 'localized' transcripts are present there at very low copy number. 3) Protein and mRNA distributions in the nervous system correlate poorly. Over 30% of the genes analyzed exhibit strong protein enrichment in synaptic regions, despite an absence or very low abundance of mRNA molecules. Similarly, enrichment of mRNA at the post synaptic density (PSD) of the NMJ was not observed for any of the proteins with strong PSD enrichment (5% of analyzed genes). Together these results emphasize the importance of translational control and other post-transcriptional regulatory mechanisms in addition to mRNA localization for establishing the synaptic proteome.

391 Exploring a possible link between altered mRNA splicing and Nuclear Envelope Budding. Brian Jenkins[1], Sarah Neuman[2], Yunsik Kang[1], Robert Ihry[2], Alex Chang[1], Cole Lambo[1], Grace Walker-Stevenson[1], Arash Bashirullah[2], *Sean Speese*[1] 1) Department of Neurology, OHSU - Jungers Center for Neuroscience Research, Portland, OR; 2) School of Pharmacy, University of Wisconsin-Madison, Madison, WI.

Exchange of material between the nucleus and cytoplasm is critical for gene expression, and while the majority of this exchange occurs through the nuclear pore complex (NPC), recent studies have highlighted the possibility of an additional nucleocytoplasmic transport mechanism, termed Nuclear Envelope Budding (**NEB**). During this process, large RNA granules (megaRNPs), ~200nm in diameter, presumably bud through the nuclear membrane in a process strikingly similar to the envelopment/de-envelopment mechanism utilized by members of the Herpesviridae family of viruses to escape the nucleus. Our initial studies in *Drosophila* found that specific mRNA transcripts, encoding postsynaptic domain proteins, were localized to the megaRNP granules in muscle nuclei. The observation that mRNA messages found in the granules were also localized to the postsynaptic domain of the NMJ, led to the proposal that these megaRNP granules would be released from the nucleus and traffic to the synapse for local translation, however this hypothesis has yet to be directly demonstrated and the function of NEB is still unclear.

Our recent studies have uncovered a transcript that enters the NEB pathway in the *Drosophila* salivary gland at distinct developmental time points. Molecular characterization of this transcript at these times has uncovered what appears to be unregulated intron retention occurring stochastically in all introns of this message. We are currently using various FISH techniques to determine if there are retained introns in the transcripts that are assembled into megaRNPs. Our working hypothesis is that high transcriptional loads during certain developmental time points may overwhelm the splicing machinery, thereby leading to inappropriate intron retention and thus the need for NEB. Previous studies have demonstrated that disruption of core splicing components and alternative splicing factors can lead to increases in unregulated intron retention, therefore we performed a reverse genetics screen to see if disruption of various splicing regulators would activate the NEB pathway. Indeed, our initial analysis at the light and EM level suggest that altering splicing can lead to increases in budding that is morphologically similar to what has been observed during normal developmental processes. Collectively, these results suggest that nuclear envelope budding may represent a method to rid the nucleus of transcripts that are not properly spliced. In particular, we are exploring the possibility that transcripts with inappropriately retained introns may be targeted to this pathway for ultimate destruction in the cytoplasm. These studies will ultimately lead to a greater understanding of this enigmatic pathway.

392 Post-transcriptional regulation of maternally deposited transcripts during D. melanogaster oogenesis. *O.S. Omar*[1,2], A. Abdelhamid[1], E. Makowicz[1], D.P. Bratu[1,2] 1) Biology, CUNY Hunter College, New York, NY; 2) Biology, CUNY Graduate Center, New York, NY.

The life cycle of many mRNAs involves active transport and localization to specialized subcellular regions. This facilitates local protein production, which is essential for stimulus response in neurons as well as embryonic patterning during animal development. Three of these well-characterized mRNAs (*oskar, bicoid, gurken*) are crucial for *D. melanogaster* female germline development, making the fly egg an excellent model for studying mRNA life cycle events. Staufen, a double-stranded RNA binding protein, is necessary for the transport and translational de-repression of two localized maternal mRNAs (*oskar, bicoid*). Additionally, Staufen localizes to processing bodies (P-bodies), which are cytoplasmic regions where mRNA decay factors accumulate and are repositories for translationally quiescent mRNAs where transcript storage and/or decay take place. Paradoxically, the mammalian homologues of Staufen (Stau1 and Stau2) were described to destabilize transcripts through a process called Staufen-mediated mRNA decay (SMD). Nonsense-mediated mRNA decay (NMD) is a closely related process that shares some key factors with SMD, including the RNA helicase Upf1. SMD and other decay pathways have been shown to downregulate a subset of transcripts during various developmental processes, including myogenesis. While SMD has only been observed in mammalian cell culture, the high degree of *staufen* homology across species lends credence to the idea that this process may be evolutionarily conserved in *D. melanogaster*. Upon perturbation of the cytoplasmic steps of the mRNA life cycle during oogenesis, we subsequently use advanced imaging techniques in combination with relative RNA

abundance quantitation to interrogate the possible relationship of mRNA localization and turnover in eukaryotes. This relationship may have implications in the progression of germline development or occur as a consequence of stress that may compromise normal development in flies, which may hold true across species.

393 Expression of Vir-1 and Vago in Nora virus infected *Drosophila melanogaster* hemolymph. *A. Macke*, D. Carlson, K. Carlson Biology, University of Nebraska at Kearney, Kearney, NE.

The *Drosophila melanogaster* immune system serves as a valuable model to identify, study, and compare similar components found in humans. Analysis of the *D. melanogaster* immune response to viral infection can be used to inform future immunity research and applications to human innate immunity. Two *D. melanogaster* proteins, Vago and Virus-induced RNA 1 (Vir-1), have been identified as candidates for analysis due to their upregulation in response to viral infection. The role of these proteins is uncharacterized in Nora virus-infected *D. melanogaster*. Nora virus replication is localized within the gut of *D. melanogaster*, but whether or not it circulates to other organs is unknown. While the complete pathology of Nora virus is not known, a locomotor defect is under investigation in our lab. This led us to hypothesize that Nora virus, Vago, and Vir-1, are circulating in the hemolymph of Nora virus infected *D. melanogaster*, allowing for virus migration to tissues beyond the gut and a conferred immunity in other tissues. To address this, we performed Western blot analysis on whole body and hemolymph collected from Nora virus infected and uninfected control *D. melanogaster*. The western blot analysis of Nora virus infected *D. melanogaster* demonstrates the presence of Vago, Vir-1, and Nora virus capsid protein, VP4b, in the hemolymph. This new finding may provide a link to effects seen in other tissues including the possible locomotor defect. The project described was supported by grants from the National Center for Research Resources (5P20RR016469) and the National Institute for General Medical Science (8P20GM103427), a component of the National Institutes of Health.

394 The Role of Histone Demethylase in Learning and Memory in the Mushroom Body of *Drosophila melanogaster*. *C. Keung*[1,2], J. Kramer[1,2,3] 1) Department of Physiology and Pharmacology , University of Western Ontario, London, CA; 2) Division of Genetics and Development, Children's Health Research Institute, London, ON, Canada; 3) Department of Biology, University of Western Ontario, London, ON, Canada.

Intellectual disability (ID) is a neurodevelopmental disorder associated with many epigenetic regulators and chromatin modifying enzymes. ID is characterized by limitations in intellectual function and adaptive behaviour before the age of 18. Most cases of ID are caused by dominant *de novo* mutations with over 350 known dominant ID genes currently identified. Gene Ontology (GO) enrichment analysis of these dominant ID genes shows an enrichment for genes encoding proteins involved in post translational histone modification. These modifications are a critical component of the epigenome and play a role in defining gene expression patterns in different cell types. Histone modifications have also been strongly implicated in the regulation of higher brain functions, like learning and memory.

The focus of this project is on a subset of histone modifiers called histone – lysine demethylases (KDMs) that act to remove methyl groups from histone tails. So far, several KDMs have been identified to be associated with ID including *KDM1A*, *KDM5C* and *KDM6A*. Histone methylation is known to be dynamically regulated in the context of learning and memory but the function of KDMs in the brain is not well described. Here, we used *Drosophila melanogaster* to systematically test the role of KDMs in learning and memory as a proxy to better understand the molecular mechanisms that might be disrupted in ID. Mushroom body (MB) specific knockdown of individual KDMs were induced using the UAS/Gal4 expression system followed by RNAi mediated knockdown. Short and long – term memory (STM and LTM) were tested in male flies using courtship conditioning. The mating attempts of the knockdown males were observed with a previously mated female (PMF). The PMFs continually reject the male mating attempts and in response, male courting is reduced. A reduction in courting indicates learning and a lack of reduction suggests defects in memory.

So far, memory defects have been observed in the KDM knockdown of *Lid*, *UTX*, and *Su(var)3-3*. STM impairment suggests defects in development or in the cell type specific transcription profile of the MB. Whereas LTM defects may indicate a role for KDMs in memory – dependent transcriptional activation. Future directions of this project include looking at brain morphology to determine if there are any defects in the MB associated with each KDM knockdown and also to look at differential gene expression in the MB to identify candidate target genes important for memory.

395 Cisregulation of cell polarity determinants by the Retinoblastoma corepressor (RBF) during *Drosophila* development. *S. Payankaulam*, D. Arnosti Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI.

The planar cell pathway (PCP) is important for normal tissue development and function, functioning through coordinated epithelial cell polarization. Conserved throughout metazoans, PCP genes are implicated in diverse human diseases. Best understood in *Drosophila*, PCP regulates multiple developmental events, including collective cell migration in dorsal closure, ommatidial rotation during eye organization, border cell migration during oogenesis, and alignment of wing and abdominal bristles. Many studies have focused on function of PCP protein complexes, but knowledge about the transcriptional regulation of PCP proteins remains sparse. Our earlier studies revealed an unexpected role for the Rbf retinoblastoma corepressor protein, a regulator of cell cycle genes, in transcriptional regulation of PCP genes, raising the possibility of a link between cell cycle and polarity through Rbf regulation. Here we examine the effect of Rbf on wing prehair initiation by examining F actin staining during early stages of pupal development. While clonal analysis of polarity mutants in pupal wings showed both non distal origin and delay in prehair initiation, we found that overexpression of Rbf caused a delay in prehair initiation, and did not alter the location of prehair initiation. To directly test the cis regulatory role of Rbf on polarity genes, we used CRISPR to disrupt the E2F1 motifs localized under the Rbf peak on direct PCP gene targets, including the *vang* gene. Homozygous individuals are viable and show mild, but consistent polarity defects, suggesting that Rbf regulation of the gene is physiologically significant and direct. We will discuss the possibility that transcriptional regulation of polarity genes by Rbf proteins may help coordinate cell division with establishment of cellular axes, and the implications for diseases such as cancer, in which Rbf regulation is disrupted.

396 A bioinformatic screen identifies conserved genes highly enriched in the *Drosophila* antenna. *P. Mohapatra*, K. Menuz Physiology and Neurobiology, University of Connecticut, Storrs, CT.

Insects rely on their olfactory systems to find food and mates, and repellents that target their olfactory systems are widely used to prevent insect-borne diseases. However, relatively little is known about the molecules supporting odor signaling in the adult antenna beyond the identity and function of the odor receptors themselves. Here, we used a computational screen to identify candidate olfaction-related genes conserved in insects.

First, we carried out an unbiased comparison of gene expression in antennae versus gene expression in other tissues and whole bodies at multiple developmental time points using *Drosophila melanogaster* ModEncode datasets and our own RNASeq antennal transcriptome. This analysis revealed 141 antennal-enriched (AE) genes each of which is more than ten times as abundant in antennae compared to other tissues or whole bodies. Antennal enrichment was validated by quantitative PCR for a subset of the genes. As expected, many known antennal odor receptors and Odorant Binding Proteins (OBPs) were included in the set of 141 AE genes. We could identify unambiguous orthologs in distantly related insect species for ~50% of the other AE genes. Analysis of existing RNASeq antennal transcriptomes indicated that nearly all of these orthologs are expressed in the antennae of *Harpegnathossaltator, Apis mellifera, Anopheles gambiae,* and *Tribolium castaneum,* suggesting a broadly conserved role for many of these genes. We then determined the morphological classes of sensilla in which the 141 AE genes are expressed by comparing gene expression in wild-type *Drosophila* antennae to antennae from mutants that fail to

develop particular sensillar classes. Finally, functional annotation revealed that AE genes encompass many functional roles, with ciliary genes and metabolic enzymes particularly enriched. Together, our study has identified numerous promising candidate genes that may play a conserved role in insect odor signaling.

397 Characterization of somatic muscle gene, holes in muscles (Him) in drosophila. *S. McKitrick*[1], R. Cripps[1,2], T. Lovato[1] 1) Biology, University of New Mexico, Albuquerque, NM; 2) Biology, San Diego State University, San Diego, CA.

Understanding the regulatory mechanisms involved in myogenesis and the control of these developmental systems is of crucial importance when attempting to comprehend the processes which drive developmentally derived diseases. We generated a null mutant of the Him (Holes in Muscles) gene to better understand its contribution to myogenesis. Him is a myogenic repressor gene that was previously was shown to inhibit Myocyte enhancer factor-2 (MEF2) activity, and is expressed in myoblasts but not differentiating myotubes. Through this inhibition of MEF2 Him additionally is predicted to acts as a block on cell differentiation and proliferation. Using a line of CRISPR-Cas9 (III) flies and a Him sgRNA targeting plasmid, we successfully obtained a knockout mutant caused by a frameshift mutation. The Him mutant is able to persist in the homozygous state but shows distinct differences in muscles morphology leading to a reduced ability or inability to fly, as well as a lessened ability to jump. Using fluorescent staining of muscle sections we have observed that the jump muscle has a distinctly different phenotype than observed in yw control flies. Ongoing research is being currently performed to further classify this mutation. This data helps to provide insight into the mechanisms of cell development and the role Him plays as a possible regulatory agent of cell differentiation.

398 Retrotransposons mimic germ plasm determinants to promote transgenerational inheritance. *Bhavana Tiwari*[1], Paula Kurtz[1], Amanda Jones[1], Annika Wylie[1], James F. Amatruda[2,3,4], Devi Prasad Boggupalli[5], Graydon B. Gonsalvez[5], John, M. Abrams[1] 1) Cell Biology, UT Southwestern Medical Center, Dallas, TX; 2) Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA; 3) Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA; 4) Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA; 5) Department of Cellular Biology and Anatomy, Medical College of Georgia, Augusta University, 1459 Laney Walker Blvd., Augusta, GA 30912, USA.

Retrotransposons are a pervasive class of mobile elements present in the genomes of virtually all forms of life. In metazoans, these are preferentially active in the germline, which, in turn, mounts defenses that restrain their activity. Here we report that certain classes of retrotransposons ensure transgenerational inheritance by invading presumptive germ cells before they are formed. Using sensitized Drosophila and zebrafish models, we found that diverse classes of retrotransposons migrate to the germ plasm, a specialized region of the oocyte that prefigures germ cells and specifies the germline of descendants in the fertilized egg. In Drosophila, we found evidence for a "stowaway" model, whereby Tahre retroelements traffic to the germ plasm by mimicking oskar RNAs and engaging the Staufen-dependent active transport machinery. Consistent with this, germ plasm determinants attracted retroelement RNAs even when these components were ectopically positioned in bipolar oocytes. Likewise, vertebrate retrotransposons similarly migrated to the germ plasm in zebrafish oocytes. Together, these results suggest that germ plasm targeting represents a fitness strategy adopted by some retrotransposons to ensure transgenerational propagation.

399 Analyzing the chromatin landscape and gene expression regulation of the *Drosophila* histone locus. *A. Chaubal*[1], R.J. Duronio[1,2] 1) Integrative Program for Biological and Genome Sciences, University of North Carolina, Chapel hill, NC; 2) Department of Genetics, Department of Biology, Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel hill, NC.

Replication-coupled production of histone proteins during S phase via cell cycle regulated transcription and processing of histone mRNA is critical for chromatin formation and maintaining genome integrity. This coordinated gene expression event is facilitated by the association of clusters of multiple histone genes with a specialized nuclear body called the histone locus body (HLB), which contains factors necessary for transcription and 3' end-processing of histone mRNA. Although recent research has provided insight into the formation and function of the HLB, a complete understanding of how the HLB facilitates cell cycle regulated expression of histone genes is lacking. This study aims to understand the mechanisms underlying transcriptional regulation of the histone gene cluster. In *Drosophila*, there are approximately 100 copies of the histone gene unit (one copy each of *His2A, His2B, His3, His4*and *His1*) tandemly arrayed at a single locus on chromosome 2. Intriguingly, previous studies from our laboratory have shown that a single transgene containing just 12 copies of the histone unit in a synthetic gene array is sufficient to compensate for the loss of ~200 histone genes in a diploid fly. We are currently investigating the mechanism underlying this histone gene dosage compensation using a variety of molecular and genetic approaches. Additionally, we are using bioinformatic tools to analyze existing histone modification and nucleosome positioning data to understand chromatin organization at the histone gene locus. This computational analysis will further inform our *in vivo* experiments directed at studying chromatin-mediated regulation of histone gene expression.

400 Coordination of transcriptional and post-transcriptional control of cell-fate transitions. *E. Larson*[1], D. Hamm[1], H. Komori[2], C-Y. Lee[2], M. Harrison[1] 1) Department of Biomolecular Chemistry, University of Wisconsin School of Medicine and Public Health, Madison, WI; 2) Department of Cell and Developmental Biology and Life Sciences Institute, University of Michigan, Ann Arbor, MI.

Stem cells divide asymmetrically, creating an identical daughter cell and a partially differentiated progeny cell. Maintaining the precise balance between self-renewal and differentiation is necessary for proper development and when mis-regulated can lead to tumorigenesis. Transcription factor regulatory networks control stem-cell fate decisions, but these mechanisms remain unclear. Zelda (Zld), a zinc finger transcription factor, governs cell fate in the early *Drosophila* embryo by activating the zygotic genome to initiate embryonic development. Zld activity must be precisely regulated, as too much or too little is lethal to the embryo. Zld is also expressed in the developing central nervous system. We have shown in the larval brain that Zld expression is limited to the neural stem cells (neuroblasts) and that over expression of Zld leads to extra neuroblasts. Further, mis-expression of Zld in the partially differentiated progeny of the neuroblasts reprograms these cells to a stem-cell like fate. Together, these data suggest that Zld drives neuroblast self-renewal and contributes towards stem-cell maintenance. Similar to the embryo, Zld activity must be precisely regulated during neuronal differentiation and rapidly inactivated following asymmetric division. We have shown that Brain tumor (Brat) regulates Zld levels in the early embryo and in the neural stem-cell lineage, suggesting common mechanisms regulate Zld activity in both tissues. Additionally, we have identified a target gene, *deadpan,* that is activated by Zld in both the embryo and neuroblasts. Ongoing experiments will determine if all or only a subset of target genes are regulated by Zld to control cell fate at both stages of development. Together our studies of a single transcription factor, Zld, have uncovered common regulatory mechanisms controlling dramatic changes in cell fate. Elucidating the shared transcriptional and post-transcriptional mechanisms in both cellular contexts will broaden our understanding of how master regulators control the developmental potential of a cell.

401 Kinase mediated regulation of the poly(ADP-ribosyl)ating pathway. *G. Bordet*, H. Datz, C. Boyle, B. MacLeod, M. Ampofo, M. Currie, J. Harbin, A V. Tulin School of medicine & health sciences, Grand Forks, ND.

Poly(ADP-ribose) polymerase 1 (PARP1) and poly(ADP-ribose) glycohydrolase (PARG) are well known antagonistic regulators of the poly(ADP-ribose) (pADPr) metabolism, as PARP1 assembles pADPr and PARG degrades it. The accumulation and degradation of pADPr is involved in several nuclear processes, including the regulation of chromatin structure and gene expression. Aberrations in pADPr metabolism have been linked to carcinogenic transformations and progression of many types of malignant tumors, as well as the development of several neurodegenerative diseases. While the mechanisms of PARP regulation

have been exhaustively studied, owing to the existence of *Drosophila* as a model organism where both key proteins are encoded by single genes, the regulation of glycohydrolase has attracted little attention. It is commonly assumed that PARG remains perpetually active throughout the cell cycle and cleaves pADPr at a constant rate. Consequently, changes in pADPr levels have been attributed to up- and downregulation of PARP activity only. Our data strongly suggest that the regulation of PARG activity is crucial for tissue-specific and cell cycle-specific differences in poly(ADP-ribosyl)ation rates. We found that transient covalent modifications of PARG may lead to conformational changes in this protein, in turn affecting its activity. Specifically, we have found that phosphorylation of two PARG domains deactivates PARG *in vivo*, leading to accumulation of pADPr in the cells. We hypothesize that PARG protein is regulated *in vivo* by phosphorylation with CK2 kinase which controls PARG activity and intracellular distribution.

402 Study of a dual localized protein and its role in mito-nuclear communication. *S. Bhuiyan*, D. Guo, A. Tulin Biomedical Science, University of North Dakota, Grand Forks, ND.

Inter-organelle communication is vital in maintaining the homeostasis of a cell. Among the most important inter-organelle communications is the one between nucleus and mitochondria. This mito-nuclear communication is key to maintaining proper cell cycle, mitochondrial biogenesis, and ATP production. Dual localized proteins, that is mitochondrial proteins with nuclear distribution and nuclear proteins with mitochondrial distribution, are keys to understanding mechanism behind mito-nuclear communication which have been poorly understood thus far. We showed that our protein of interest, CG14850, is dual localized in both mitochondria and nucleus in Drosophila through confocal microscopy and western blot. This kind of dual localization is very rare and dual localized proteins usually take part in gene regulation and/or post translational processes. We also found CG14850 binds to active chromatin in the nucleus which suggests that it functions by regulating genes in the nucleus. Even though this protein was dual localized in early third instar larvae, we found higher presence of CG14850 in nucleus and less in mitochondria in late third instar larvae which are about to metamorphosize into pupae. This suggests dual localization of this protein is regulated by developmental and/or metabolic cues. Moreover, CG14850 no longer localizes to nucleus in Poly (ADP)-ribose glycohydrolase (PARG) mutant in late third instar larvae. PARG is known to be involved in many types of cancer and in some important processes such as DNA repair and gene regulation (both activation and repression). PARG catabolizes the hydrolysis of Poly (ADP)-ribose (PAR) modification from proteins. This suggests that CG14850's function is PAR-dependent. Therefore, we hypothesize CG14850 is involved in the mechanism of nuclear and mitochondrial coordination via gene regulation. CG14850 being present in both mitochondria and nucleus and binding to active chromatin could be the key protein maintaining mito-nuclear communication. Breakdown of this type of communication can lead to mitochondrial deregulation and/or cell cycle disruption leading to uncontrolled cell division in cancer cells. Identifying CG14850 as a key component of mito-nuclear communication will give us a new therapeutic target for cancer treatment.

403 G+C Oscillations in Genomic DNA. *W. Bender*[1], Z. Moqtaderi[1], S. Brown[2] 1) BCMP, Harvard Med School, Boston, MA; 2) Div. of Biology, Kansas State University, Manhattan, KS.

The G+C content of Drosophila genomic DNA fluctuates along the chromosome in a surprisingly regular oscillation. Signal analysis, using a continuous wavelet transform, shows a wavelength of ~ 1000 bp. The oscillation is not present in long coding regions, but it is most strong in large regulatory regions, including those of the Antennapedia, bithorax and Iroquois complexes. The high G+C regions coincide well with non-coding conserved sequences, such as enhancers. We speculate that cytosine deamination drives non-conserved sequences towards A+T preference. Similar G+C oscillation is observed in some other insect species, notably in honey bees, ants, and flour beetles, but each with a distinct wavelength. It is also apparent in the pufferfish, where it again coincides with conserved sequences. Possible reasons for the oscillations include constraints on enhancer spacing, or structural features of chromosome packing, or evolutionary balance between small insertions and deletions. We are conducting experiments and analyses that might support one of these models.

404 Complex satellite DNA variation within and between populations of *Drosophila melanogaster*. *Isaac Wong*, Danielle Khost, Danna Eickbush, Amanda Larracuente Biology, Larracuente lab, University of Rochester, Rochester, NY.

Satellite DNAs (satDNAs) are large blocks of tandemly repeated sequences typically found in regions of the genome with low recombination (e.g. centromeres and telomeres). SatDNAs are highly dynamic both within and between species in both copy number and in genomic location. Here we use deep short read and long read single molecule sequencing to study the patterns of satDNA divergence and polymorphism, and gain insights into the evolutionary dynamics of satDNA and the mechanisms by which they spread in genomes. We use these data to estimate the empirical copy number of complex satellite repeats within *Drosophila melanogaster* and between species of the simulans clade. We focus on two satellite families: 1) *Responder* (*Rsp*), a complex satellite repeat well known for being the target of a male meiotic drive system in *D. melanogaster*; and 2) the 1.688 family of satellites, not known to be involved in meiotic drive. While these satellites are primarily located in pericentric heterochromatin, we also find these repeats in small euchromatic blocks, primarily on the X chromosome. We use phylogenetic methods to detect rampant gene conversion and evidence for unequal crossing over within arrays of repeats. These analyses also suggest that *Rsp-like* repeats recently spread across the X chromosomes of *D. mauritiana* and *D. simulans.* We hypothesize that one mechanism by which satDNA spreads in the genome is through the integration of extrachromosomal circular DNAs that are excised during unequal exchange events within sister chromatids. We use 2D gel electrophoresis and exonuclease digestion followed by Illumina sequencing to show that satellite DNAs are abundant on extrachromosomal circles. Our results are consistent with unequal crossing over, gene conversion, and extrachromosomal circular DNA insertions shaping both the copy number and distribution of satellites in the genome, lending mechanistic insights into complex satDNA dynamics in Drosophila.

405 Elucidating the Mechanisms of PARP-1 Binding Domains. *S.J. Johnson*, C. Thomas, Y. Ji, C. Wu, M. Currie, J. Harbin, M. Ampofo, N. Lodhi, A.J. Tulin Biomedical Sciences, University of North Dakota Medical School, Grand Forks.

PARP-1 is a multidomain nuclear enzyme, its functions include DNA repair, chromatin remodeling and transcriptional regulation. It contains three major binding domains. The N-terminal DNA binding domain consists of three Zinc fingers. The middle automodification domain interacts with itself to form dimers. The C-terminal catalytic domain consists of the protein interacting WGR motif and the NAD interacting site for PARP. Parp-1 inhibition has been shown to suppress tumor growth in human cancer cells however standard methods for inhibition have off-target effects which can lead to normal cell toxicity. Classical inhibitors use NAD-mimetics which target the C-terminal catalytic domain of PARP, we show that inhibition via the H4 pathway is much more specific and limits deleterious off-target effects. Besides cancer treatment, PARP-1 inhibitors have also been shown to be useful as treatment for inflammation, circulatory shock, stroke, and myocardial infarction. However, little is known about the mechanisms by which PARP functions. PARP-1 has 19 paralogs in the human genome, the Drosophila genome contains only 1 PARP gene making it the perfect organism for our study. We have created 12 deletional isoforms for each binding domain of PARP in Drosophila. Each isoform was tagged with a YFP sequence. Drosophila salivary gland polytene chromosome immunostaining was performed to determine the location of PARP-1 recombinant isoforms on polytene chromosomes. We found that each isoform colocalizes with DNA. All isoforms that contained Zinc fingers 1 and 2 colocalized indiscriminately with DNA. However, when Zinc fingers 1 and 2 are removed, colocalization only occurs in open uncondensed chromatin. ChIP analysis was performed to determine the localization of each deletional isoform on the HSP70 loci. We found that the histone-binding domains of PARP-1 are responsible for PARP-1 localization to the promoter of the Hsp70 locus, while DNA-

binding domains target PARP-1 to areas outside of the promoter region. Next we propose to perform ChIP-seq analysis for each deletional isoform of PARP to determine which genes and cellular processes are regulated by each of PARP's binding domains.

406 Histone 3 lysine 14 is essential and required for wing patterning in *Drosophila*. Isabel Regadas, Olle Dahlberg, Roshan Vaid, *Mattias Mannervik* Dept. Molecular Biosciences, Wenner-Gren Institute, Stockholm University, Stockholm, SE.

Histone post translational modifications (PTMs) have been implicated in many biological processes, but most of this data is based on correlative studies and lacks direct evidence. Lysines in histone H3 can be both methylated and acetylated, but H3 lysine 14 (H3K14) is predominantly acetylated. To investigate the function of histone acetylation in a multi-cellular organism, we have replaced endogenous H3 with H3K14R expressed from transgenes in *Drosophila melanogaster*. We found that this H3K14R mutation is lethal for *Drosophila*, as the animals die in the second instar larval stage. Although H3K14 is essential for *Drosophila* as an organism, we observed that H3K14R is not cell lethal, as clones of homozygous mutant cells could be generated in larval imaginal discs. H3K14R mutant cells contained severely reduced H3K14 acetylation levels, showing that the histone variant H3.3 is not the major acceptor of this histone acetylation. Dorsal-ventral patterning was affected in wing discs, and wing margin phenotypes were observed in adult flies with clones of H3K14R mutant cells. This resembles phenotypes described in hypomorphs of the histone acetyltransferase Gcn5, indicating that defects in H3K14 acetylation are causing the developmental abnormalities. We suggest that H3K14 acetylation is not needed for cell survival and proliferation, but is required for developmental patterning.

407 Functional Redundancy and Feedback Regulation between Canonical Histone H3 and Variant Histone H3.3 in *Drosophila*. *E.H. Kwon*[1,7], R.L. Armstrong[2,7], T.J.R. Penke[2,7], B.D. Strahl[3,4,7], A.G. Matera[1,2,4,5,6,7], D.J. McKay[1,2,4,5,6,7], R.J. Duronio[1,2,4,5,6,7] 1) Department of Biology; 2) Curriculum in Genetics and Molecular Biology; 3) Department of Biochemistry and Biophysics; 4) Lineberger Comprehensive Cancer Center; 5) Integrative Program for Biological and Genome Sciences; 6) Department of Genetics; 7) University of North Carolina at Chapel Hill, Chapel Hill, NC.

Genome-based processes in eukaryotic cells are influenced by chromatin organization. In animal cells, chromatin contains two general types of histone proteins: canonical and variant. Canonical histones are incorporated into chromatin only during S phase of the cell cycle and are typically encoded by multigene clusters. The *Drosophila* genome has a single cluster with ~200 genes encoding each canonical histone. Variant histones are incorporated into chromatin throughout the cell cycle and are encoded by 1 or 2 genes that are not contained within the canonical histone gene clusters. Canonical and variant histones are similar in structure and can have overlapping functions, though the extent of this functional overlap is not completely understood. Variant histone H3.3 in *Drosophila* is encoded by two genes, *H3.3A* and *H3.3B*, which produce identical protein products. H3 and H3.3 are highly conserved across eukaryotes, and we hypothesize that they can compensate for one another despite being deposited in different but overlapping genomic regions.

A homozygous inviable deletion of the ~200 canonical histone genes is rescued to viability by a BAC-based transgene containing only 12 copies of canonical histone genes (*12xHWT*; Histone Wild Type). We used this histone gene replacement platform to test whether the viability of *12xHWT* flies depends on *H3.3* gene function. We found that *12xHWT* animals are not viable after mutation of both *H3.3A* and *H3.3B* and that 1 copy of *H3.3B* only partially rescues viability of *12xHWT* flies. These data indicate that H3.3 compensates for a reduction in canonical *H3* gene copy number. Furthermore, using polytene chromosome staining and western blotting with an H3.3 specific antibody, we found that total H3.3 levels and incorporation into chromatin are higher in *12xHWT* animals compared to wild type, indicating that H3.3 upregulation likely compensates for reduced canonical *H3* gene copy number.

408 Dynamics of free and chromatin-bound histone H3 during early embryogenesis. Yuki Shindo, *Amanda Amodeo* Lewis-Sigler Institute, Princeton University, Princeton, NJ.

During zygotic genome activation (ZGA), the chromatin environment undergoes profound changes including the formation of topologically associated domains, refinements in nucleosome positioning on promoters, and the emergence of heterochromatin. In many organisms, including Drosophila, ZGA is associated with the end of a period of extremely rapid, exponential cleavage divisions that are facilitated by large maternally provided pools of nuclear components. It is therefore imperative that we understand how the supply of chromatin components relative to the exponentially increasing demand affects nuclear and chromatin composition during early embryogenesis. Here, we examine the nuclear trafficking and chromatin dynamics of histones during the cleavage divisions in Drosophila using a photo-switchable H3-Dendra2 reporter. We observe that total H3-Dendra2 in the nucleus decreases with each cleavage cycle. This change in nuclear composition is due to depletion of large pools (>50%) of free protein that are present in the early cycles. We find that the per nucleus import rate halves with each cycle and construct a mathematical model in which increasing histone demand determines the dynamics of nuclear H3 supply. These changes in H3 availability correspond to a large (~40%) reduction in global H3 occupancy on the chromatin. The loss of H3 from chromatin is compensated by the increased incorporation of H3.3, and this loss can be reduced by mutations ing the H3.3 sepcific chaparone, Hira. Moreover, alterations in maternal loading of H3 and associated replication dependent histones result corrosponding changes to the timing of cell cycle slowing and the onset of transcription associated with this developmental stage.

409 Probing the function of metazoan histones with a systematic library of H3 and H4 mutants. *X. Zhang*[1], G Gao[2,1] 1) Tsinghua University, Beijing, Beijing, China; 2) ShanghaiTech University, School of Life Science and Technology, Shanghai, China.

Replication-dependent histone genes often reside in tandemly arrayed gene clusters, hindering systematic loss-of function analyses. Here we used CRISPR/Cas9 and the attP/attB double-integration system to alter the numbers and sequences of the histone genes in their original genomic context in Drosophila. As few as 8 copies of histone gene suffices to support embryo development and adult viability, whereas flies with 20 copies are indistinguishable from WT flies. Using a hierarchical assembly method, 40 alanine substitution mutations, covering all known modified residues on histone H3 and H4, were introduced and characterized. Mutations at multiple residues compromise viability, fertility, and DNA damage responses. In particular, H4K16 is necessary for expression of male X-linked genes, male viability and maintenance of ovarian germline stem cells, whereas H3K27 is essential for late embryogenesis. Finally, using simplified mosaic analysis, we found that H3R26 is required for H3K27 trimethylation. Taken together, we have developed a powerful strategy and valuable reagents for systematically probing histone functions in the fly.

410 Cell-type specific sequential ChIP-seq reveals chromatin landscape in *fru P1*-expressing neurons. *Colleen Palmateer*, Surjyendu Ray, Shawn Moseley, Michelle Arbeitman Biomedical Sciences, Florida State University, Tallahassee, FL.

A major remaining challenge in the field of neuroscience is determining how neural circuits are specified to direct and maintain innate behaviors. The *Drosophila* courtship system provides an ideal model for studying innate sex-specific behaviors, as these behaviors are known to be under the control of the sex determination hierarchy. The sex hierarchy genes *doublesex* (*dsx*) and *fruitless* (*fru*) encode sex-specific transcription factors that are required for the potential for these reproductive behaviors. Previous studies have demonstrated that Fru^M forms a complex with transcriptional cofactor, Bonus, a TIF1 homolog. This Fru^M-Bonus complex selectively recruits the chromatin modifying protein Histone deacetylase 1 (HDAC1) to masculinize neurons. Experimental evidence suggests that if this complex recruits Heterochromatin protein 1a (HP1a) instead of HDAC1, neurons are demasculinized. This indicates that differential modification of the chromatin landscape may contribute to creating and maintaining the potential for sexually dimorphic behavior. To explore this, we have developed a tool, "Chromotag", to examine chromatin modifications in a cell-type specific manner using sequential ChIP-seq. Here, we evaluate five chromatin marks: H3K27ac, H3K4me3, H3K36me3, H3K9me3, H3K27me3 to investigate *fru P1*-expressing neurons, at three developmental stages in males and

females. In addition, we have generated cell-type specific RNA-seq data sets using Translating Ribosome Affinity Purification (TRAP) to understand how these histone modifications influence gene expression. Together, this data offers insights into stage and sex-differences in the chromatin landscape and how this impacts gene expression to ultimately produce sex-specific behavior.

411 How does H4K20 modification contribute to cell proliferation and animal development? *Aaron Crain*[1], Robin Armstrong[1], Stephen Klusza[2], Brian Strahl[3,4], Daniel McKay[1,2,4,5,6], A. Gregory Matera[1,2,4,5,6], Robert Duronio[1,2,4,5,6] 1) Curriculum in Genetics and Molecular Biology; 2) Integrative Program for Biological and Genome Sciences; 3) Department of Biochemistry and Biophysics; 4) Lineberger Comprehensive Cancer Center; 5) Department of Biology; 6) Department of Genetics, University of North Carolina, Chapel Hill, NC.

DNA replication and gene expression are vital for cell proliferation and are regulated by chromatin. The histone proteins within chromatin are comprised of two general types: replication-dependent and replication-independent. Both types of histones contain N-terminal tails that are post-translationally modified to influence chromatin organization and accessibility as well as the recruitment of trans-acting factors, which impact the spatial and temporal control of DNA replication and gene expression. Methylation on the twentieth lysine of histone H4 (H4K20) has been implicated in pathways associated with DNA replication and gene expression, usually from studies that manipulate the activity of the H4K20-specifc methyltransferase, PR-Set7 (Set8). However, PR-Set7 has non-histone substrates, so some of the observed phenotypes in these studies could be due to roles of PR-Set7 other than H4K20 modification. To directly interrogate the biological impact of H4K20 modification loss *in vivo*, we combined CRISPR-mediated mutagenesis of the replication-independent *His4r* gene with a replication dependent histone gene replacement platform to engineer *Drosophila* genotypes in which all histone H4 genes are mutant. We show that animals expressing only unmodifiable H4K20 can eclose as adult flies, whereas PR-Set7 null animals cannot, indicating that modification of H4K20 is not required to complete development. We also show that the replication-independent histone His4r partially compensates for loss of modifiable replication dependent H4. To further investigate the functions of H4K20 modification in cell proliferation, we are conducting a forward genetic screen in the *Drosophila* eye to identify genes that when functionally depleted can alter the proliferation rate of H4K20A mutant cells.

412 Lysine 27 of replication-independent histone H3.3 is required for Polycomb target gene silencing but not for gene activation. M.P. Leatham-Jensen[1], C.M. Uyehara[1,2], B.D. Strahl[3], A.G. Matera[1], R.J. Duronio[1], *D.J. McKay*[1] 1) Biology, Genetics, iBGS, The University of North Carolina at Chapel Hill, Chapel Hill, NC; 2) Curriculum in Genetics and Molecular Biology, The University of North Carolina at Chapel Hill, Chapel Hill, NC; 3) Biochemistry, The University of North Carolina at Chapel Hill, Chapel Hill, NC.

Proper determination of cell fates depends on epigenetic information that is used to preserve memory of decisions made earlier in development. Post-translational modification of histone residues is thought to be a central means by which epigenetic information is propagated. In particular, modifications of histone H3 lysine 27 (H3K27) are strongly correlated with both gene activation and gene repression. H3K27 acetylation is found at sites of active transcription, whereas H3K27 methylation is found at loci silenced by Polycomb group proteins. The histones bearing these modifications are encoded by the replication-dependent H3 genes as well as the replication-independent H3.3 genes. Owing to differential rates of nucleosome turnover, H3K27 acetylation is enriched on replication-independent H3.3 histones at active gene loci, and H3K27 methylation is enriched on replication-dependent H3 histones across silenced gene loci. Previously, we found that modification of replication-dependent H3K27 is required for Polycomb target gene silencing, but it is not required for gene activation. However, the contribution of replication-independent H3.3K27 to these functions is unknown. Here, we used CRISPR/Cas9 to mutate the endogenous replication-independent H3.3K27 to a non-modifiable residue. Surprisingly, we find that H3.3K27 is also required for Polycomb target gene silencing despite the association of H3.3 with active transcription. However, the requirement for H3.3K27 comes at a later stage of development than that found for replication-dependent H3K27, suggesting a greater reliance on replication-independent H3.3K27 in post-mitotic cells. Notably, we find no evidence of global transcriptional defects in H3.3K27 mutants, despite the strong correlation between H3.3K27 acetylation and active transcription.

413 Centromere organization and evolution in the simulans clade. Ching-Ho Chang[1], Xiaolu Wei[2], Lucas Hemmer[1], Bryce Santinello[3], Ankita Chavan[3], Jason Palladino[3], Barbara Mellone[3], *Amanda Larracuente*[1] 1) Biology, University of Rochester, Rochester, NY; 2) Biomedical Genetics, University of Rochester Medical School, Rochester, NY; 3) Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT.

Centromeres have an essential function in coordinating chromosome segregation during cell divisions. Despite this conserved role, they are rich in repeats and among the most rapidly evolving regions of the genome. Because of their repetitive nature, centromeres are typically missing from genome assemblies, making it extremely difficult to study their organization and evolution. We recently combined long read single molecule sequencing, ChIPseq with the centromeric histone variant (CENP-A), and fluorescence *in situ* hybridization on chromatin fibers, to determine the detailed organization of *Drosophila melanogaster* centromeres. We discovered that *D. melanogaster* centromeres correspond to complex islands of retrotransposon-rich DNA embedded in simple satellite repeats. All *D. melanogaster* centromeres are unique but share one particular non-LTR retroelement called *G2/Jockey-3.* The satellite sequences around centromeres differ between *D. melanogaster* and its closely related species in the simulans clade. However, the sequences corresponding to the functional centromeres are unknown outside of *D. melanogaster*. We therefore used long-read sequencing from Pacific Biosciences (PacBio) to create heterochromatin-enriched genome assemblies for *D. simulans*, *D. mauritiana*, and *D. sechellia*. We identified putative centromeric sequences based on sequence organization and confirmed that they are enriched in CENP-A using ChIPseq data in *D. simulans,* and that they are indeed centromeric with fluorescence *in situ* hybridization in each species. We discovered that the simulans clade centromeres are distinct from those in *D. melanogaster*, but show similarities in composition and organization. Interestingly, *G2/Jockey-3* is also among the most CENP-A-enriched retroelements in *D. simulans*. Our study therefore reveals a striking level of conservation among otherwise rapidly evolving sequences at centromeres, suggesting that *G2/Jockey-3* may be important for centromere function and evolution.

414 Epigenetic regulation of transcription and pre-mRNA processing by histone PTMs. *H.R. Salzler*, M.P. Meers, R.L. Armstrong, K Adams, D.J. McKay, R.J. Duronio, A.G. Matera Integrative Program in Biological and Genome Sciences, UNC Chapel Hill, Chapel Hill, NC.

Histone post-translational modifications (PTMs) modulate the organization of chromatin and are hypothesized to be carriers of epigenetic information. Until recently, it has been impossible to rigorously test this premise in multicellular eukaryotes, due to the repetitive nature of histone gene clusters. We have developed a BAC-based genetic platform in *Drosophila melanogaster* allowing direct interrogation of histone residue function. Here, we outline our studies of histone H3K36 methylation function (H3K36me) in maintaining transcriptome fidelity.

Yeast studies show that mutation of H3K36 or deletion of Set2, the H3K36 methyltransferase, causes genome-wide hyperacetylation of histone H4 and aberrant transcription initiation. The prevailing view is that H4 acetylation (H4Ac) aids nucleosome dissociation, allowing transcription initiation at cryptic sites in gene bodies. However, we found that mutation of the replication dependent H3 (H3.2) genes appears to uncouple these two phenotypes. That is, H3K36R (K36R) flies exhibit global hyper-H4ac but not cryptic transcription in genes. Rather, transcription start site (TSS) profiling in K36R flies reveals cryptic initiation primarily in gene-poor regions. Studies of the mammalian Set2 ortholog, SETD2, also suggest a role for H3K36 trimethylation in regulation of splicing. However, our work mutating H3.2K36 demonstrates that this modification is neither a significant contributor to the regulation of alternative splice site choice, nor to canonical intron removal efficiency.

One explanation for differences between our results and studies of yeast H3K36 and Set2/SETD2 might be that trimethylation of histone variant, H3.3, which more closely resembles yeast H3, is the primary mediator of cryptic initiation and splicing phenotypes. Another is that Set2/SETD2 methylation of non-histone substrates may mediate these processes. Therefore we generated an H3.3BK36R mutant in a H3.3AΔ null background to probe these hypotheses. We expect comparison of H3.3BK36R, H3.3AΔ null double mutants with both Set2 mutants and H3.3BK36R, H3.3AΔ, H3.2K36R triple mutants to definitively parse these relationships.

415 Identification of topoisomerase II as a potential factor associated with *Drosophila melanogaster* F element gene expression. *B. French*, W. Leung, S. Elgin Washington University in St. Louis, St. Louis, MO.

The *Drosophila melanogaster* Muller F element (dot chromosome) is unusual because it is packaged primarily as heterochromatin, a form associated with silencing, but contains ~80 protein-coding genes. Past studies have shown that classical markers of heterochromatin (*e.g.,* HP1a, H3K9me3) are depleted at the transcription start sites (TSS) of active F element genes, which suggests that the key factors that regulate F element gene expression are located near the TSS. In order to search for these factors, we used the MEME program (http://meme-suite.org/) to identify ungapped motifs that are enriched in the sequences surrounding the TSS of F element genes. We found that the TSS of F element genes are significantly enriched in the binding sites for topoisomerase II (Top2), with 43 of 79 genes featuring at least one Top2 motif instance in the ±300bp region surrounding the TSS. Analysis of the distributions of Top2 motifs using the FIMO program shows that, along with being enriched on the *D. melanogaster* F element (49.6% of TSS), Top2 binding sites are also enriched in the TSS of heterochromatic genes located on the other autosomes (49.1% of TSS). In contrast, Top2 binding sites are not enriched in the TSS of euchromatic genes located near the base of chr3L (3.7% of TSS). Top2 is an enzyme that induces and repairs double stranded DNA breaks to remove supercoils. Previous studies have suggested that Top2 may regulate gene expression by making the TSS of genes more accessible to the transcription machinery, by regulating R-loop formation, or by impacting the accumulation and release of RNA polymerase II in polymerase pausing. However, a MEME search of the promoters of genes that exhibit high rates of polymerase pausing (as determined by GRO-seq) did not show a significant enrichment of the Top2 motif, arguing against the latter hypothesis. We plan to incorporate these bioinformatics findings into future wet bench experiments to ascertain if Top2 affects F element gene expression (collaboration with J Cantrell and E Gracheva). This analysis could provide useful insights into how Top2 plays a role in facilitating the expression of genes that reside in a heterochromatic environment. Supported by NSF grant #1431407.

416 Driving Gene Expression in the Heterochromatic Environment of the Fourth Chromosome of *D. melanogaster*. *J. Cantrell*, S. Bieser, E. Gracheva, S. Elgin Biology, Washington University in St. Louis, St. Louis, MO.

Genomes of higher eukaryotes can be divided into two fundamental and dynamic packaging subtypes: euchromatin and heterochromatin. Genes that are active in a euchromatic environment are silenced when placed adjacent to heterochromatin by rearrangement or transposition, exhibiting a characteristic Position Effect Variegation (PEV) phenotype. However, heterochromatin is not devoid of actively functioning genes. Our goal is to identify regulatory elements that facilitate transcription of heterochromatic genes. The fourth chromosome of *Drosophila melanogaster* provides an excellent model, as the ~80 genes found within this heterochromatic domain are expressed at levels that mimic those seen for euchromatic genes. Insertion of an *hsp70-white* reporter transgene (which results in a uniform red eye when present in euchromatin) into a heterochromatic region on the fourth chromosome results in PEV. We created a construct where we replaced the *hsp70* promoter with the 5′ upstream regulatory region of an active fourth chromosome gene, *Rad23*. Insertion of the *Rad23-white* transgene into the same location on the 4th chromosome switched the *white* reporter PEV phenotype to a uniform full red eye, indicating that the *Rad23* fragment is sufficient to drive strong expression of the euchromatic gene. A series of experiments with reporter constructs containing fragments of varying lengths of the *Rad23* promoter region has identified the minimal *Rad23* promoter fragment needed to drive full *white* expression as running from -100 bp to +126 bp relative to the TSS site. Full expression is dependent on a M1BP site and an unidentified site at -100 to -50 bp, as well as RAMPAGE-identified transcription start sites. To determine sensitivity to heterochromatin formation, we have created a series of constructs swapping smaller fragments of *hsp70* and *Rad23* promoters. Analysis of the resulting transgenic phenotypes, coupled with computational analyses of the promoter elements and genetic tests is identifying DNA motifs that are linked to sensitivity/resistance to suppressive heterochromatic environments.

417 Regulation of Repeat-Induced Silencing and Position-Effect Variegation by Nutrition and the TOR Pathway. *M. Howard*, A. Smiley, Z. Payne, U. Ogbonna, S. Tye, A. Arsham Biology Baccalaureate Partnership, Bemidji State University, Brooklyn Park, MN.

Eukaryotic genomes protect themselves against invasive mobile DNA using a wide range of mechanisms both before and after mobile element genomic integration. While mobile elements are thought to play an important role in driving genome evolution, repetitive regions of the genome are targeted for transcriptional silencing by heterochromatin. It is not known whether different types of repeats trigger silencing through distinct or similar mechanisms. 256 repeats of the *E. coli* lac operator sequence (LacO) can trigger silencing and variegation of a downstream *white*+ reporter construct in the adult eye. Strong variegation is observed when larvae are reared at 25°C; surprisingly this variegation is substantially suppressed by rearing at 18°C. To investigate whether other growth-inhibitory conditions suppress LacO-induced variegation we raised larvae on food with reduced-nutrient content or rapamycin. Here we show that both treatments markedly suppress variegation independently of developmental delay. Nutrient-poor food and low concentrations of rapamycin that were insufficient to delay development were nonetheless sufficient to suppress variegation, suggesting that suppression of variegation by nutrients and TOR operates through a distinct mechanism from that by low temperatures, or that they rely on a shared mechanism that does not involve developmental timing. These results suggest metabolic regulation of repeat-induced silencing and heterochromatin formation or maintenance.

418 Crossover mapping reveals a centromere effect boundary. *M. Hartmann*, J. Sekelsky University of North Carolina at Chapel Hill, Chapel Hill, NC.

During meiosis, crossovers form between homologous chromosomes, and this physical connection is essential for proper segregation of chromosomes. Crossovers are patterned along a chromosome arm so that they occur mostly in the middle of a chromosome arm, but if this crossover patterning is disrupted, aberrantly placed crossovers can lead to improper segregation and result in nondisjunction. In fact, Trisomy 21 is correlated with an increase in crossovers near the centromere. Normally, crossovers are prevented near the centromere, which is termed the centromere effect. However, little is known about the centromere effect or how organisms normally prevent these potentially harmful crossovers. In this study, we use *Drosophila melanogaster* to finely map crossovers near the centromere in order to create a comprehensive map of centromere-proximal crossovers. Until now, there were very few crossovers mapped near the centromere, so there was not a clear understanding of where centromere-proximal crossovers occurred in relation to the centromere and pericentromeric heterochromatin. Interestingly, crossover mapping revealed that a small fraction of crossovers occurred within the heterochromatic regions, and that crossover patterning showed that there is a low crossover density until a certain point when it dramatically increases to a higher crossover density on the remainder of the chromosome arm. Mathematical modeling reveals that a similar model can explain crossover density patterning on all arms. Additionally, this identified "boundary" between low and high crossover density that is presumably created by the centromere effect raises a lot of questions to be answered. Some of those questions being addressed in this study include: Is this boundary a result of the centromere effect, or is it simply a result of being adjacent to heterochromatin? Is this boundary sequence specific? Can the centromere effect extend beyond chromosomes to a spatial region of the nucleus? Preliminary results show that heterochromatin alone does not decrease crossovers in adjacent euchromatin, nor can the centromere effect exert a

force on nearby loci that are linearly located distal to the centromere. Creating a better understanding of the centromere effect will help us begin to learn how organisms normally prevent harmful crossovers near the centromere.

419 Candidate screen to identify maternal factors regulating the establishment of heterochromatin. *K.H.C. Wei*, C. Chan, D. Bachtrog Integrative Biology, University of California Berkeley, Berkeley, CA.

The establishment and maintenance of heterochromatin are required to suppress deleterious activities of repetitive elements and maintain genome integrity. In early embryonic development, heterochromatin quickly appears at repeats and pericentric regions during cellularization of the embryos at the transition between stage 4 and stage 5. At the same time, the genome shifts from utilizing maternally deposited molecules to zygotic transcription. To determine the molecular signals that initiate the formation of heterochromatin, we characterize heterochromatin establishment in embryos where we depleted the maternal mRNA of genes known to localize to and/or regulate heterochromatin. To do this, we generated females that maternally deposit Gal4 and shRNAs to knock-down the maternal mRNA of 24 candidate genes and used ChIP-Seq to profile of the heterochromatic mark H3K9me3 before (stage 4) and after (stage 5) cellularization in single embryos. Three genes show clear perturbation to the H3K9me3 profile. *HP1a* and *crol* knock-downs have significantly reduced H3K9me3 enrichment at stage 5. Interestingly, *piwi* knock-down shows a complete failure to establish heterochromatin with little to no enrichment of H3K9me3 genome-wide. This strongly suggest that piwi plays a key role in the establishment of heterochromatin, and the possibility that piRNAs regulation is required. To elucidate *piwi*'s role in heterochromatin establishment, we are generating the transcriptome of staged embryos through early development in the piwi knock-down.

420 The role of Boundary Elements and Insulator Proteins in the Functional and Topological Segmentation of the *Drosophila melanogaster* Genome. *M.R. Stadler*[1,2], M.B. Eisen[1,2] 1) MCB, UC Berkeley, Berkeley, CA; 2) Howard Hughes Medical Institute, Chevy Chase, MD.

Drosophila genomes contains numerous insulator elements that separate genes from cis regulatory elements and demarcate regions of distinct epigenetic states. We previously showed that the functional domains defined by insulators correspond to topological domains observed in high-resolution chromosome conformation capture (Hi-C) assays, and that that the boundaries between topological domains map precisely to classical genetic insulator elements. Further, we discovered a strong correspondence between topological structures and the structure of polytene chromosomes, with compacted polytene "band" regions corresponding to topological domains, and the short insulator elements between them corresponding to the decompacted "interband" regions. This association suggested a model in which fly boundary elements achieve functional and topological insulation by decompacting short regions of chromatin to produce physical separation between adjacent domains.

I will talk about unpublished data from recent experiments that establish a causal relationship between insulators and and topological boundaries. Using CRISPR/Cas9 mutagenesis to modify endogenous genetic loci, we have generated stable fly lines carrying deletions of a number of boundary elements near well-studied genes and are characterizing the effect of these deletions on topology, expression, and phenotype. We have also inserted boundary elements, identified directly from Hi-C data, into a region of the genome shown to constitute both a large polytene band and a large topological domain, and have analyzed the resulting chromosomes by both Hi-C and microscopic analysis of polytene structure. Finally, we have generated embryos that are depleted of the proteins that most strongly characterize *Drosophila* boundary elements, including CP190, BEAF-32, and dCTCF (the fly homolog of the mammalian insulator factor CTCF), and have assayed the effects of the loss of the proteins on local and global chromosome structure. Together, these experiments define and elaborate causal roles for *Drosophila* boundary elements and the proteins that bind them in organizing the structure and function of fly chromosomes.

421 The recognition of target gene transcriptional state by Polycomb-group proteins. *Elnaz Ghotbi Ravandi*, Piao Ye, Judith Benes, Richard Jones Dept of Biological Sciences, Southern Methodist University, Dallas, TX.

Polycomb-group (PcG) proteins are conserved epigenetic transcriptional regulators that maintain the transcriptional repression of their silenced target genes by altering chromatin structure. PcG proteins do not initiate transcriptional repression, but take over repression from repressive transcription factors. The molecular mechanisms by which PcG proteins initially recognize a repressed gene are not known. Initial recruitment of PcG proteins may be governed by the presence of gene-specific transcription factors or the transcriptional state of their target gene. To examine how PcG proteins distinguish a transcriptionally repressed target gene from an active gene, we constructed *Drosophila* embryos with a transcriptionally inert *giant* (*gt*), a PcG-target gene, transgene in a background in which endogenous *gt* is transcriptionally active. Chromatin immunoprecipitation (ChIP) assays of the resulting embryos show that PcG recognition of a target gene as active or repressed is not dependent on the transcriptional state of the target gene. Rather, the presence of repressive transcription factors or the absence of activators, and/or the corepressors or coactivators they recruit, may identify a target gene as repressed or active.

422 Identifying sequences required for inter-TAD transcriptional activation. *Sandip DE*[1], Natalie Gehred[1], Miki Fujioka[2], Victoria Blake[3], James Jaynes[2], Judith Kassis[1] 1) Eunice Kennedy Shriver National Institute of Child Health and Human Development, North Bethesda, MD, 20892; 2) Thomas Jefferson University, Philadelphia, PA 19107; 3) University of California, Berkeley, CA 94720.

Chromosomes are folded into topologically associating domains [TADs] that help pack long stretches of DNA inside the eukaryotic nucleus. TADs strongly favor intra-domain chromatin contacts, restricting enhancers' activity within the domain. Disruption of the TADs is associated with cancers and developmental diseases. Therefore, it is important to understand the mechanism of formation and maintenance of TADs. We study TADs that contain repressed chromatin modified by Polycomb group proteins [PcGs]. In Drosophila, DNA-elements known as Polycomb group response elements (PREs) initiate PcG domain formation by recruiting PcGs to chromatin. PREs also drive long-range interactions that are hypothesized to help the PcG domain form. The mechanism of PRE-mediated long-range interaction is largely unknown.

To better understand this, we are investigating the '*invected* [*inv*] - *engrailed* [*en*]' domain (~113kb) in Drosophila. The *inv-en* TAD contains co-regulated genes *inv* and *en,* flanked by ubiquitously expressed genes *enhancer of Polycomb* [*e(Pc)*] and *toutatis* [*tou*]. The *inv-en* domain has four constitutive PREs: two at *inv* and two at *en*. Our results show that a *en*PRE-*lacZ*–containing transgene inserted outside the *inv-en* PcG domain requires the *en*PRE to facilitate activation by the *en* enhancers. Removal of PREs from the transgene leads to complete loss of *lacZ* expression. This suggests the PREs are important for chromatin looping. Similarly, deletion of the endogenous *en*PREs causes a loss of *en*PRE-*lacZ* expression, suggesting that the *en*PREs inside the *inv-en* domain pair with the *en*PREs present in the transgene, a self-self interaction. In contrast, transgenes inserted within the *inv-en* TAD do not require the endogenous *en*PREs for *lacZ* expression. These data suggest that *en*PREs can drive long-range interactions. Additionally, our transgenic assay showed that in flies homozygous for the transgene, *lacZ* expression was variegated compared to the uniform expression of heterozygous flies. This indicates that inter-chromosomal pairing via PREs in the interphase nucleus is preferred over intra-chromosomal looping. Finally, we observed that mutation of the GAF binding motifs within the transgenic PREs affects the chromatin looping. Our findings will aid in our understanding of how developmentally important enhancers can interact with their target promoters situated far away in different TADs.

423 Identification of novel DNA binding proteins necessary for epigenetic silencing by Polycomb group proteins. Justin Davidson, Isaac Ray, *Payal Ray* Biology, Presbyterian College, Clinton, SC.

Polycomb group proteins (PcG) are a class of transcriptional regulators that mediate the epigenetic repression of genes involved in development, cellular

differentiation, and cell proliferation. Dysfunction of PcG repression has been implicated in several types of cancer. In *Drosophila melanogaster*, where PcG proteins were first identified, members of the PcG protein family form multi-protein complexes (Polycomb Repressive Complex 1 and 2), that interact with chromatin via cis-elements known as Polycomb group Response Elements (PREs). PREs range in size from several hundred to a few thousand base pairs and are made up of binding sites for multiple DNA-binding proteins. The PRE DNA-binding protein Pho (Pleiohomeotic) plays a key role in recruitment of PcG protein complexes but it does not act alone. As a first step in understanding the recruitment of PcG protein complexes to DNA, we aimed to identify all of the DNA-binding proteins important for the function of a Drosophila *engrailed* PRE. *engrailed* is an essential gene in *Drosophila* development and a well-established PcG target. Previous studies from our group have identified a 139 bp region within the *engrailed* PRE that contains binding sites for the DNA-binding proteins Pho, Spps (Sp1-like factor for Pairing Sensitive-silencing), GAF (GAGA Factor) and retains the ability to act as a PRE. This fragment also contains binding sites for two unknown proteins and mutation of these sites abrogates PRE activity in functional studies. To identify these proteins we performed a biotin-streptavidin pull-down coupled with Mass Spectrometry (MS). We identified several candidate proteins and will be presenting results from our studies that examine the involvement of these proteins in recruitment of Polycomb complexes.

424 Identifying domains of a novel zinc finger protein that are critical for dosage compensation in Drosophila melanogaster. *E. Nguyen*, E. Larschan Molecular Biology, Cell Biology & Biochemistry, Brown University, Providence, RI.

It is critical for all cells to balance gene expression across the genome to assure that protein complexes have the correct stoichiometry. Dosage compensation in the fruit fly involves upregulating the expression of the single male X-chromosome to match the protein levels produced by the two female X-chromosomes. In order to specifically upregulate the genes on the X, that chromosome must be targeted. The Male-Specific Lethal (MSL) complex singles out the X at specific DNA sequences, called MSL recognition elements (MREs). Although immunostaining has shown that the MSL complex only identifies the X-chromosome, MREs are found throughout the entire genome. Given this, DNA sequences alone are insufficient in explaining how the X is targeted. A large-scale genetic screen performed by the Larschan Lab led to the discovery of another key player, the CLAMP (Chromatin-linked Adaptor for MSL proteins) protein. This zinc-finger protein appears to act as the physical linker between DNA and the MSL complex, promoting MSL recruitment to the X. However, nothing is known about the specific protein domains of CLAMP that regulate its function.

My work seeks to fully characterize the CLAMP protein by mapping how its amino acid structure directly affects its essential function. In order to study mutated versions of the CLAMP protein, I designed a plasmid containing an RNAi-resistant CLAMP gene. This allows me to knock down endogenous CLAMP in Drosophila tissue culture cells using RNAi while preserving my own version of CLAMP. I then designed derivatives of my RNAi-resistant plasmid that contain different variations of the CLAMP protein. These plasmids each encode a mutated CLAMP that differs from wild type by only a single amino acid within each of the known zinc finger motifs. Similarly, truncations of CLAMP were designed to delete different segments of the protein that have been identified based on predictions of structured and unstructured domains. By transforming these plasmids into tissue culture cells that lack the normal CLAMP and using a luciferase reporter assay, I will test if the mutated CLAMP proteins are able to recruit MSL complex efficiently. My research will define which CLAMP domain is involved in dosage compensation and its role in MSL complex recruitment. This work will reveal new mechanisms by which transcription factors that bind all over the genome specifically recruit large transcription complexes like the MSL complex to specific genomic locations.

425 Major parts of *Drosophila* hybrid genomes don't pair correctly. *J.G. Baldwin-Brown*, N. Phadnis Department of Biology, University of Utah, Salt Lake City, UT.

The pairing of homologous chromosomes is an essential process in all eukaryotes. In meiosis, homologous chromosomes must pair to allow recombination. Although we often associate chromosome pairing with meiosis, pairing can also occur in somatic cells. In humans, somatic pairing is not typically detected in healthy cells, and often occurs in unhealthy cells, such as various cancers. In these cancers, pairing is observed repeatedly at just a few genome locations, which leads specific chromosomes to have a high rate of aneuploidy (change in the total number of chromosomes). Knowledge of the mechanisms that underlie this aberrant pairing will help to explain the mechanisms underlying all pairing.

A deep understanding of the mechanics of pairing is not possible without a system where chromosome pairing can be manipulated with ease. This study utilizes the key insight that *Drosophila* flies in general, and interspecies hybrids in particular, can serve as a powerful system to investigate long-standing and fundamental questions regarding chromosome pairing. While somatic pairing represents the disease state in humans, it is the wild type state in flies: across *Drosophila* species, somatic chromosomes are paired rather than unpaired in normal, healthy cells. Importantly, patterns of unpaired regions are observed across the genome in interspecific hybrids between *D. melanogaster* and its closest sister species, *D. simulans*. These narrow regions are highly reproducible in these interspecies crosses.

Not only are these patterns of pairing loss valuable for understanding the effects of speciation on chromosome biology, but they also open the door to precise, powerful experiments to address unsolved problems in understanding chromosome pairing broadly. I have generated the first ever genome-wide high-resolution maps of chromosome pairing in *Drosophila* using Hi-C sequencing. My analysis contrasted pairing in within-species crosses to pairing in between-species hybrids. Additionally, I investigated the relationship between non-pairing regions in hybrids and insulators and other genomic features known to be associated with pairing. This work provides new insights into the mechanistic underpinnings of not just hybrids, but all pairing cells.

426 Ribosomal DNA repeats are maintained in the *Drosophila* male germline by the retrotransposons R1 and R2. *J.O. Nelson*[1,2], Y.M. Yamashita[1,2] 1) Life Sciences Institute, University of Michigan, Ann Arbor, MI; 2) Howard Hughes Medical Institute, University of Michigan Ann Arbor, MI.

The maintenance of inherently unstable genomic regions within the germline is critical for preserving the integrity of the genome from generation to generation. The hundreds of tandem duplications of the ribosomal RNA genes that make up the ribosomal DNA (rDNA) locus are highly unstable due to their propensity for intra-chromosomal interaction that can lead to rDNA copy loss. We recently discovered that rDNA is progressively lost from male germline stem cells (GSCs) during aging, but that rDNA is quickly expanded in the GSCs of the sons that inherit these diminished loci, revealing mechanisms to maintain the rDNA locus through cyclical rDNA loss and re-expansion in the male germline. Here we show that the activity of the rDNA-specific retrotransposons *R1* and *R2* are necessary for this germline rDNA maintenance, likely through their transposition activity.

While the expression of transposable elements is normally suppressed in the germline, we found that *R1* and *R2* are derepressed in GSCs that have lost rDNA copies during aging. Despite the ability of these transposable elements to disrupt ribosomal RNA genes, we found that inhibition of *R1* or *R2*expression by RNAi surprisingly accelerated rDNA copy loss during aging and compromised GSC maintenance, indicating that the expression of these retrotransposons can benefit the host genome. *R1* and *R2* encode sequence specific endonucleases that insert new copies of the retrotransposons precisely into individual repeats of the ribosomal RNA gene, and here we find that *R2* expression creases double stranded breaks (DSB) within the rDNA locus. We had previously shown that the homologous recombination-mediated DSB repair pathway is necessary for GSC rDNA copy expansion. Sister chromatid homologous recombination at

repetitive sites can lead to recombination of misaligned repeats, causing unequal sister chromatid exchange and expanding the repetitive sequence on one chromatid. We propose that DSBs created by R1 and R2 in GSCs with reduced rDNA content leads to unequal sister chromatid exchange and rDNA copy expansion, indicating an essential function for R1 and R2 to maintain the rDNA locus within the male germline.

427 piRNA-mediated silencing of an invading transposable element evolves rapidly through abundant beneficial de novo mutations. *S. Zhang*, E. Kelleher Department of Biology and Biochemistry, University of Houston, Houston, TX.

Transposable elements (TEs) are selfish genetic entities whose mobilization can reduce host fitness by inducing deleterious mutations and inciting genome instability. TEs are therefore silenced in germline cells by small Piwi-interacting RNAs (piRNAs), which are derived from genomic regions termed piRNA clusters. Widespread horizontal transfer of TEs continuously challenges the piRNA pathway to evolve regulation of novel TE families. piRNA-mediated silencing of an invading TE is proposed to evolve predominantly through random transpositions into existing piRNA clusters, resulting in the production of novel species of piRNAs that bring the TE under host control. However, this elegant hypothesis has yet to be tested, owing to 1) the computational challenge of detecting TE insertions into repeat-rich piRNA clusters and 2) the potentially confounding contribution of epigenetic mutations, in which a non-piRNA producing insertion is converted into a new piRNA cluster though changes in chromatin state.

P-elements provide a unique opportunity to study the evolution of piRNA-mediated silencing. *P*-elements invaded *D. melanogaster* genomes around 1950, and in response, many natural populations evolved piRNA-mediated repression. We developed a novel computational approach to annotate *P*-element insertions among ~200 fully-sequenced repressive genomes comprising the *Drosophila* Genetic Reference Panel (DGRP). We further took advantage of historical collections of *P*-element free strains to differentiate insertions in ancestral piRNA clusters from putative epigenetic mutations. We discovered that >90% of DGRP genomes harbor *P*-element insertions in ancestral piRNA clusters. Furthermore, we detected no fewer than 87 independent *P*-element insertion sites in ancestral piRNA clusters, revealing that the ubiquitous repressive phenotype is underpinned by an unprecedented number of alleles. Finally, we observed that *P*-element insertions in piRNA clusters segregate at significantly higher frequency than those outside of piRNA clusters, suggesting piRNA-producing insertions are targets of positive selection. Together, our results reveal that piRNA-mediated silencing undergoes dramatic polygenic adaptation, in which a plethora of *de novo* beneficial *P*-insertions fueled the extremely rapid evolution of a ubiquitous repressive phenotype.

428 Elucidating the role of retroelement transcription in centromere identity. *A. Chavan*[1], C. Chang[2], B. Santinello[1], J. Palladino[1], X. Wei[4], N. Martins[5], J. Erceg[5], A. Larracuente[2], B. Mellone[1,3] 1) Molecular and Cell Biology, University of Connecticut, Storrs Mansfield, CT; 2) Department of Biology, University of Rochester; Rochester, NY; 3) Institute for Systems Genomics, University of Connecticut; Storrs, CT ; 4) Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY; 5) Department of Genetics, Harvard Medical School; Boston, MA.

Centromeres are essential chromosomal loci that mediate kinetochore formation and accurate chromosome segregation during cell division. In most eukaryotes, centromeres are specified epigenetically by the histone H3 variant CENP-A and are embedded in repeat-rich genomic regions composed of satellite DNA and retroelements. Although the centromere assembly pathways are understood to a great extent, the role of DNA sequence in centromere identity remains elusive. The lack of a complete assembly for centromere sequences is one of the factors that impacts our ability to investigate this question in complex eukaryotes. Recent work has suggested that individual centromere-derived transcripts are required for centromere integrity in humans, marsupials, and frogs; however, the role of centromere transcripts has not been investigated systematically. Using long-read sequencing combined with CENP-A chromatin immunoprecipitation, we discovered that *Drosophila* centromeres are composed of islands of complex repeats enriched in non-LTR retroelements that are flanked by large blocks of simple satellites. In particular, the *G2/Jockey-3* retroelement is highly enriched in CENP-A and is common to all centromeres. To begin investigating if the transcription of centromeric *G2/Jockey-3* plays a role at the centromere, we designed primers specific for *G2/Jockey-3* copies from each centromere and analyzed their expression in total RNA from embryos and testes by quantitative reverse-transcription PCR. Preliminary data show that these sequences are transcribed at the centromeres. Interestingly, this retroelement seems transcribed preferentially from a subset of centromeres. These findings provide a basis for investigating the contribution of transcription of centromere sequences in centromere identity.

429 Identification of a non-LTR retrotransposon at Drosophila centromeres. *B. Santinello*[1], C. Chang[2], A. Chavan[1], J. Palladino[1], X. Wei[3], N. Martins[4], C. Chen[2,5], J. Erceg[4], B. Beliveau[6,7], C. Wu[4], A. Larracuente[2], B. Mellone[1,8] 1) Molecular and Cell Biology, University of Connecticut, Storrs, CT; 2) Department of Biology, University of Rochester; Rochester, NY 14627 ; 3) Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY 14642 ; 4) Department of Genetics, Harvard Medical School; Boston, MA 02115 ; 5) Current address: Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD, 21287 ; 6) Wyss Institute for Biologically Inspired Engineering; Harvard Medical School, Boston, MA 02115 ; 7) Department of Systems Biology, Harvard Medical School; Boston, MA 02115 ; 8) Institute for Systems Genomics, University of Connecticut; Storrs, CT 06269.

The centromere is an essential region on chromosomes needed for the establishment of the kinetochore which turn mediates accurate chromosome segregation in mitosis and meiosis. Centromeres are defined epigenetically by the presence of the centromere-specific histone variant CENP-A. Although the role of DNA is not fully understood, increasing evidence suggests a function in centromere assembly and inheritance. Due to the repetitive nature of centromeres and the limitations of traditional sequencing technology, the detailed mapping of centromeric DNA has long remained elusive. Using ChIPs and long-read sequencing, we now show that *Drosophila melanogaster* centromeres are comprised of an island of complex DNA deeply embedded within large arrays of satellite DNA. All islands have in common a non-LTR retrotransposon called *G2/Jockey-3* that is highly enriched in CENP-A. About 63% of all *G2/Jockey-3* copies in the genome are found within the centromere, suggesting a role in centromere identity. Using immunofluorescence and fluorescence in situ hybridization (IF-FISH) we confirm cytologically that G2/Jockey-3 is present at all centromeres of *Drosophila melanogaster*, consistent with our centromeric sequencing results. Approximately 60% of the *G2/Jockey-3* copies that are found within the centromere have a 5' truncation, which suggests incomplete reverse-transcription during insertion. Among all centromeres, the Y centromere contains the most copies, many of which are full-length, while the 2nd contains a single, truncated *G2/Jockey-3*. Interestingly, *Drosophila simulans G2/Jockey-3* is also enriched in CENP-A by ChIP and we confirm it is present at a subset of centromeres by IF-FISH. We are currently examining cytologically the genomic locations of *G2/Jockey-3* in *D. melanogaster* and *D. simulans* hybrids to determine whether *G2/Jockey-3* is remobilized, which would suggest a novel role in hybrid incompatibility. Retroelements are present at the centromeres of plants, humans, mice, bats, and marsupials, suggesting these elements may play a conserved role in centromere function and evolution.

430 Meiotic sex chromosome inactivation in the *Drosophila melanogaster* male germ line. *Miriam Akeju*[1], Sharvani Mahadevaraju[2], Justin Fear[2], Brian Galleta[2], Maria Vibranovski[3], Katie Conlon[1], Brian Oliver[2], Erika Matunis[1] 1) Cell Biology, Johns Hopkins School of Medicine, Baltimore, MD; 2) National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD; 3) Department of Genetics and Evolutionary Biology, University of Sao Paulo.

The premature transcriptional silencing of sex chromosomes before meiosis is a conserved feature of spermatogenesis. Reasons for this silencing are debated, and its occurrence in Drosophila is controversial. Here, single-cell transcriptomics show that X-linked genes are strongly expressed in pre-meiotic germ cells in Drosophila testes, but their steady-state levels fall as differentiation proceeds. In contrast, levels of autosomally encoded transripts remain constant. Cytological examination corroborates these findings: active RNA Polymerase-II is present throughout pre-meiotic germ cell euchromatin, but is lost specifically from the X chromosome as differentiation proceeds. Together these results demonstrate X inactivation in the Drosophila male germline, and

suggest it is driven by genome defense and/or sexually antagonistic evolutionary forces, rather than a need to suppress recombination, which is absent in male flies.

431 An evolutionary perspective on gene expression and regulatory dynamics at the single-cell level. *Li Zhao*, Evan Witt, Sigi Benjamin-Hong, Nicolas Svetec Laboratory of Evolutionary Genetics and Genomics, The Rockefeller University, New York, NY.

Every cellular trait originated at some time point in the past and evolved under various evolutionary paths. However, it is unknown how a novel trait originates and how gene and regulatory networks spatially orchestrate the development of cell types. Identifying the processes driving and governing morphological and functional diversity and complexity is a major step towards understanding the evolution of complex life. However, our understanding of this process is still limited. To characterize the molecular genetic basis of cell clusters, we performed single-cell sequencing and RNA fluorescent in situ hybridization (FISH) on testis. We found that a set of genes that show strong spatial and temporal expression pattern at the cell type level, indicating that there is a dynamic shift of expressed and translated pathways in testis. Combined with ATAC-sequencing data, we studied whether chromatin modification changes of TFs play a role in cluster modification. Altogether, our result will help to elucidate the origination and evolution of novel regulatory circuits and their contributions to phenotypic innovation.

432 Out of the testis, into the ovary: biased outcomes of gene birth and death in *Drosophila*. *R. Assis* The Pennsylvania State University, University Park, PA.

Gene turnover is a key source of adaptive variation. Yet most evolutionary studies have focused on adaptation via gene birth, largely dismissing gene death as a mechanism that simply eradicates genetic redundancy. Here I utilize genome-scale sequence and spatial expression data to assess the evolutionary outcomes of gene birth and death in *Drosophila*. I find that duplicate genes typically possess distinct properties after birth that diverge further before death, suggesting that redundancy cannot explain a majority of gene death. Moreover, in addition to providing support for the well-known “out of the testis” origin hypothesis for gene birth, I uncover a bias toward the preferential retention of ovary-expressed genes. Therefore, I propose a novel “into the ovary” hypothesis for gene death in *Drosophila*, in which gene death may promote adaptation by salvaging genes that contribute to the evolution of female reproductive phenotypes.

433 Cajal bodies and the role of Colin in Transposable Element Regulation. *A.D. Serrano Rodriguez*[1], J. Gall[2], M. Izaguirre Sierra[1] 1) Northern New Mexico College, Espanola, NM; 2) Carnegie Institution for Science, Baltimore, MD.

The long-term goal of our research is to study the role of nuclear architecture in the eukaryotic cell. Cajal bodies are evolutionary conserved nuclear structures that have been implicated in the assembly and metabolism of several kinds of non-coding RNAs, such as the telomerase RNA component, small nuclear ribonucleoproteins (snRNPs) and small interfering RNAs (siRNAs). We will take advantage of the powerful genetic tools available in *Drosophila* to understand the role(s) of the Cajal body in the biogenesis of non-coding RNAs, with a specific focus on interaction(s) between CBs and the transposon RiboNucleoProtein (RNP) complexes at the organismal level. Coilin is an evolutionary conserved protein that is essential for CB formation. Coilin mutants flies lack CBs and preliminary data showed that coilin mutants accumulate gypsy mRNA and gypsy envelope protein. Moreover we identified significant genome wide changes in Transposable Element (TE) repression/activation. Together, our studies will elucidate the mechanism(s) of TE RNP biogenesis, repression/activation and the basic biology of the Cajal body in the eukaryotic cell.

434 Evolutionary arms races between *Segregation Distorter* chromosomes and their suppressors in American populations of *Drosophila melanogaster*. *C.-H. Chang*, D. Pascua, T. Mouton, A. M. Larracuente Department of Biology, U of Rochester, Rochester, NY.

The autosomal *Segregation Distorter* (*SD*) chromosome is a selfish coadapted gene complex that can bias its transmission by killing other sperm in spermatogenesis. *SD* chromosomes segregate at low frequencies of 1-5% in natural populations worldwide. Independent selective sweeps have recently occurred on *SD* chromosomes in European and African populations. We hypothesize that ongoing arms races between *SD* chromosomes and their suppressors shape the dynamics of different *SD* chromosomes within populations. To test our hypothesis, we surveyed *SD* with polymorphic chromosomal inversions and their genetic modifiers in American populations. We first demonstrate that *SD* chromosomes acquired four new inversions in American populations and three of these inversions are *SD*-specific. By crossing a marked *SD* chromosome to 87 DGRP inbred lines, we found that suppressors are widespread (74%) on both X and autosomes in the DGRP population, likely contributing to the low frequency of *SD* chromosomes. Our GWAS analysis indicates that multiple genetic modifiers affect the driving ability of *SD* chromosomes in this population. Two GWAS peaks are located in regions with known enhancers of *SD*. Interestingly, we found that one X chromosome with suppressors of *SD* has distinct effects on *SD* chromosomes with different inversions in the same population. Our recombination mapping reveals that a single major locus contributes to the suppressing effect of this X chromosome. Our results suggest complex epistasis between *SD* chromosomes and their suppressors: *SD* chromosomes may acquire genetic modifiers from standing variation via inversions to escape suppressors.

435 High-resolution meiotic recombination map for *Drosophila yakuba* based on whole-genome analysis of individual meiotic events. *N. Pettie*[1], A. Llopart[1,2], J. Comeron[1,2] 1) Interdisciplinary Graduate Program in Genetics, University of Iowa, Iowa City, IA; 2) Biology, University of Iowa, Iowa City, IA.

Meiotic recombination is an important and fundamental biological process present in the vast majority of species. At the same time, meiotic recombination rates and the distribution of the recombination events across the genome vary between closely related species and between different populations of the same species. Here we present the first ultra-high-resolution meiotic recombination map for *Drosophila yakuba* based on whole-genome genotyping of more than 2,500 individual wild-type meiotic events. Moreover, we studied the meiotic products from individuals from different populations of *D. yakuba*, thus allowing us to generate a ‘species’ genetic map for the five major chromosome arms rather than a map that is specific to two genotypes. We find, as expected, several polymorphic chromosomal inversions in autosomes. Consistent with previously published studies based on phenotypic markers for the *X* chromosome, we also find that the overall rate of crossovers in *D. yakuba* is higher than that in *D. melanogaster*. Notably, the *X* chromosome shows a severely reduced ‘centromere effect’ and no evidence of crossover interference. Our results provide outgroup information for genetic studies of recombination rates and localization in *D. melanogaster* and species of the *simulans clade*, and essential data to properly infer recent events of positive selection across the *D. yakuba* genome once ‘linked selection’ due to deleterious mutations is taken into account.

436 Gene capture by transposable elements in Drosophila: Cooperation or conflict? *Christopher Ellison*, Meenakshi Kagda, Weihuan Cao Genetics, Rutgers University, Piscataway, NJ.

Transposable elements (TEs) impose a mutational burden on their host genome but have also been repeatedly co-opted to serve important genomic functions. Here we describe a TE from *D. melanogaster* that has captured a fragment of a host gene involved in RNA transport. We have found that the copy number of this TE is negatively correlated with the gene's expression level across wild *D. melanogaster* strains and we present evidence that the gene is

involved in suppressing activity of this TE. We discuss whether this gene capture event represents ongoing conflict between host and TE or whether this TE has been domesticated to play a role in expression feedback.

437 Dorsal/NF-κB exhibits a dorsal-to-ventral mobility gradient in the *Drosophila* embryo. Hadel Al Asafen[1], Natalie Clark[2], Thomas Jacobsen[1], Rosangela Sozzani[2], *Gregory Reeves*[1] 1) Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC; 2) Plant and Microbial Biology, North Carolina State University, Raleigh, NC.

Morphogen-mediated patterning is a highly dynamic developmental process. To obtain an accurate understanding of morphogen gradients, biophysical parameters such as protein diffusivities must be quantified *in vivo*. The dorsal-ventral (DV) patterning of early *Drosophila* embryos by the NF-κB homolog Dorsal (Dl) is an excellent system for understanding morphogen gradient formation. Dl gradient formation is controlled by the inhibitor Cactus/IκB (Cact), which regulates the nuclear import and diffusion of Dl protein. However, quantitative measurements of spatiotemporal Dl movement are currently lacking. Here, we use scanning fluorescence correlation spectroscopy to quantify the mobility of Dl. We find that the diffusivity of Dl varies along the DV axis, with lowest diffusivities on the ventral side, and the DV asymmetry in diffusivity is exclusive to the nuclei. Moreover, we also observe that nuclear export rates are lower in the ventral and lateral regions of the embryo. Both cross correlation spectroscopy measurements and a computational model of Dl/DNA binding suggest that DNA binding of Dl, which is more prevalent on the ventral side of the embryo, is correlated to a lower diffusivity and nuclear export rate. We propose that the variation in Dl/DNA binding along the DV axis is dependent on Cact binding Dl, which prevents Dl from binding DNA in dorsal and lateral regions of the embryo. Thus, our results highlight the complexity of morphogen gradient dynamics and the need for quantitative measurements of biophysical interactions in such systems.

438 Blimp-1 is required for normal retinal differentiation. *H. Wang*, C. Morrison, J. Treisman Skirball Institute for Biomolecular Medicine and Department of Cell Biology, NYU School of Medicine, New York, NY.

The zinc finger transcriptional repressor Blimp-1 is required to prevent photoreceptors in the mammalian retina from adopting a bipolar cell fate. We find that Blimp-1 is required for the normal differentiation of multiple cell types in the *Drosophila* eye. Knocking down *Blimp-1* throughout the eye causes abnormal photoreceptor (PR) morphology, mislocalization of PR nuclei, plano-convex lenses, and premature pigmentation. The expression of a Blimp-1 protein trap is limited to cone and pigment cells at mid-pupal stages, and knocking down *Blimp-1* in cone or pigment cells reproduces some of these phenotypes. Over-expression of *Blimp-1* also disrupts PR morphology and reduces the expression of pigment cell markers. These results suggest that *Blimp-1* contributes non-autonomously to the normal differentiation of multiple retinal cell types. Blimp-1 may function antagonistically to Glass (Gl), a related zinc finger transcription factor that is autonomously required in all the cell types of the eye for their normal differentiation. Predicted binding sites for Blimp-1 are found in many Gl target genes, and *Blimp-1* expression is expanded into additional cells in *gl* mutant clones in the pupal retina. In the fat body, *Blimp-1* is regulated by ecdysone signaling and controls the timing of pupation by repressing the expression of *ftz-f1*. To determine whether Blimp-1 affects developmental timing or cell fate choices in the retina, we will make clones homozygous for a CRISPR deletion allele of *Blimp-1* and will use RNAseq to identify genes that are repressed by Blimp-1. The results will indicate whether the role of *Drosophila Blimp-1* in the retina is related to or distinct from that of its mammalian homologue.

439 Interaction between JAK STAT pathway and axial patterning genes in *Drosophila* eye development. *A. Raj*[1], N. Gogia[1], M. Kango-Singh[1,2,3], A. Singh[1,2,3,4] 1) Department of Biology, University of Dayton, Dayton, OH; 2) Premedical Program, University of Dayton, Dayton, OH; 3) Center for Tissue Regeneration & Engineering (TREND), University of Dayton, Dayton, OH; 4) Center for Genomic Advocacy (TCGA), Indiana State University, Terre Haute, IN.

Axial patterning is the fundamental process of organogenesis, which entails delineation of three distinct axes: Antero-Posterior (AP), Dorso-Ventral (DV) and Proximo-Distal (PD) axes. Any impairment in the axis formation may lead to developmental birth defects in humans and therefore, getting insight of the mechanism of axis determination is crucial for better understanding of organogenesis. In *Drosophila* eye model, DV patterning is the primary lineage restriction event. A new member of DV patterning gene, *defective proventriculus* (*dve*, a Homeobox gene), an ortholog of SATB homeobox 1 (special AT-rich sequence binding protein 1) has been identified which acts downstream of a GATA-1 transcription factor pannier (*pnr*), and upstream of *wingless (wg)* in the dorsal gene hierarchy. Unpaired (Upd), a long range secreted ligand for JAK STAT pathway, is known to promote eye development by negatively regulating Wg expression. Here we present that Upd interacts with *dve*, to regulate the patterning and growth of the developing *Drosophila* eye. We found that activation of JAK STAT pathway in *dve* expression domain results in dorsal eye enlargement and downregulation of Wg expression whereas its inactivation in Dve domain results in eye suppression phenotype and upregulating Wg expression. Our data strongly imply that Upd plays a crucial role in defining the functional domain of Dve during DV axis formation of developing eye. We will present the complex interactions between these two highly conserved pathways, *viz.,* JAK-STAT and dorsal eye fate selectors in growth and patterning of the eye.

440 Growth Regulatory Pathway collaborates with Axial Patterning Genes to regulate Patterning and Growth in *Drosophila* Eye. *N. Gogia*[1], A. Raj[1], M. Kango-Singh[1,2,3], A. Singh[1,2,3,4] 1) Department of Biology, University of Dayton, Dayton, OH; 2) Premedical Program, University of Dayton, Dayton, OH; 3) Center for Tissue Regeneration & Engineering (TREND), University of Dayton, Dayton, OH; 4) Center for Genomic Advocacy, Indiana State University, Terre Haute, IN.

An important question in developmental biology is how any three-dimensional organ develops from a single monolayer sheet of cells. In any multicellular organism, organogenesis requires crucial process of axial patterning to determine Antero-Posterior (AP), Dorso-Ventral (DV), and Proximo-Distal (PD) axes. Any deviation in these axes during development leads to congenital birth defects. We have used *Drosophila melanogaster* (*a.k.a* fruit fly), eye as our model organ (as genetic machinery is conserved between flies and humans), where DV patterning marks the first lineage restriction event and have identified *defective proventriculus* (*dve*-a Homeobox gene), an ortholog of SATB homeobox 1 (special AT-rich sequence binding protein 1 in humans), as a new member of DV patterning genes hierarchy. We have shown, that (1) *dve* acts downstream of *pannier* (*pnr*, a GATA-1 transcription factor), and upstream of *wingless (wg)*, (2) Loss-of-function (LOF) of both *dve* or *pnr* results in dramatic dorsal eye enlargements, while their Gain-of-function (GOF) suppresses the eye specific fate, (3) Furthermore, Wingless (Wg) is a downstream target of Hippo growth regulatory pathway (highly conserved) and *wg*, (which acts downstream of *dve)*, in the eye also exhibits similar eye enlargement and suppression phenotypes (upon LOF, GOF respectively) and thus has been known to play an important role in growth. Here, we present that DV patterning genes interacts with Hippo signaling to regulate their common downstream target, Wg during growth and patterning in the developing eye. We found that these two unrelated pathways of DV patterning and Hippo signaling act antagonistically to each other in the developing eye. Furthermore, activation of Hippo signaling suppresses *dve* or *pnr* expressing cells, which downregulates Wg and changes head, antennae specific fate to an eye. Additionally gain-of-function of *dve* or its vertebrate ortholog SATB1 in eye leads to similar Wg upregulation and eye suppression phenotypes. Since gain-of-function of hippo triggers cell death, we tested if by blocking cell death by using *p35* exhibits similar phenotypes. However, we found that *hpo* GOF phenotype in *dve* domain is not due to blocking cell death but by regulating retinal differentiation. We present a model where growth regulatory pathway regulates axial (DV) patterning genes expression in the developing eye of *Drosophila*. These studies present new genetic interaction between two unrelated pathways and have significant bearing on developmental mechanisms.

441 Investigating the input of Planar Cell Polarity signaling into Notch-mediated binary cell fate decisions during photoreceptor specification. *Giovanna Collu*[1], Annie Jin[1], Keith Brennan[2], Marek Mlodzik[1] 1) Cell, Developmental and Regenerative Biology, Icahn School of Medicine at

Mount Sinai, New York, NY; 2) University of Manchester, Manchester, UK.

The patterning of photoreceptors in the fly eye is a paradigm for positional information directing cell fate decisions. There is a dorso-ventral gradient of Planar Cell Polarity (PCP) signaling that is converted into a binary cell fate decision in the R3 and R4 photoreceptors within each one of the ~800 ommatidia in the eye. The R3/R4 pair of photoreceptors start out as equivalent cells. The cell that is closest to the dorso-ventral midline – the equator – has higher levels of PCP signaling through the activity of Frizzled (Fz) and Dishevelled (Dsh). This equatorial cell adopts the R3 fate, upregulates the Notch ligand Delta and directs the neighboring cell to become R4 through Notch pathway activation. It is well documented that Fz/Dsh activate Notch signaling in R4, but in order to understand the robustness of this binary fate choice, we are investigating how Fz and Dsh might inhibit Notch signaling in the R3 cell. Through gain of function experiments in the larval and adult eye, we show that Dsh can inhibit Notch signaling and R4 fate, independently of Delta upregulation. Epistasis experiments position Dsh inhibition at the level of the active transcriptional complex, downstream of Notch cleavage. We are now using biochemical assays on larval eye disc lysates to investigate the effect of Dsh on Notch pathway components.

442 Ion channel contributions to wing development in *Drosophila melanogaster*. *L.F. George*, S.J. Pradhan, D. Mitchel , M. Josey, J. Casey, G.R. Dahal, E.A. Bates Department of Pediatrics, University of Colorado - Anschutz Medical Campus, Denver, CO.

During morphogenesis, cells communicate with each other to shape tissues and organs. Several lines of recent evidence indicate that ion channels play a key role in cellular signaling and tissue morphogenesis. However, little is known about the scope of specific ion-channel types that impinge upon developmental pathways. The *Drosophila melanogaster* wing is an excellent model in which to address this problem as wing vein patterning is acutely sensitive to changes in developmental pathways. We conducted a screen of 180 ion channels expressed in the wing using loss-of-function mutant and RNAi lines. Here we identify 45 candidates that significantly impacted development of the *Drosophila melanogaster* wing. Calcium, sodium, potassium, chloride, and ligand-gated cation channels were all identified in our screen, suggesting that a wide variety of ion channel types are important for development. Ion channels belonging to the pickpocket family, the ionotropic receptor family, and the bestrophin family were highly represented among the candidates of our screen. Six ion channels with human orthologs that have been implicated in human channelopathies were also identified. Many of the human orthologs of the channels identified in our screen are targets of common general anesthetics, anti-seizure and anti-hypertension drugs, as well as alcohol and nicotine. Our results confirm the importance of ion channels in morphogenesis and identify a number of ion channels that will provide the basis for future studies to understand the role of ion channels in development.

443 Understanding the Regulation of Differential Splicing of TGF-β Receptor Baboon in the *Drosophila* Wing Imaginal Disc. *B. Seth*[2], A. Upadhyay[1], A. Peterson[1], M. O'Connor[1] 1) Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, MN; 2) Biology Baccalaureate Partnership, Bemidji State University, Brooklyn Park, MN.

TGFβ superfamily signaling factors are widely conserved in the animal kingdom and play important roles during development and disease. Signaling is initiated by peptide-ligand dimers binding to and activating cell-transmembrane receptor complexes composed of Type I and Type II receptors. Subsequently, Type I receptors phosphorylate cytoplasmic transcription factors which translocate to the nucleus and regulate specific target genes. In *Drosophila*, three ligands (*Activin-β*, *Dawdle*, and *Myoglianin*) signal through a single Type I receptor known as *Baboon* (*Babo*). The *Babo* gene produces three different splice isoforms (Babo-a, Babo-b, and Babo-c), which are hypothesized to allow for ligand selectivity. Babo-a is the only isoform expressed in the larval wing imaginal disc where it promotes disc growth and final wing size; loss of Babo-a leads to smaller wing. We hypothesize that the expression of different splice isoforms of *Babo* is regulated by specific RNA Binding proteins (RBPs) and used a GAL4/UAS RNAi expression system to knock down specific RBPs in the imaginal disc cells. Candidate RBPs were identified in cultured S2 cells in an RNAi screen for proteins that regulate *Babo* splicing. Here we tested if specific RBPs phenocopy *Babo* loss of function in the wing imaginal disc. Knockdown of ribosomal protein S3, heterogeneous nuclear ribonucleoprotein K, B52, and P-element somatic inhibitor, had no or minimal effects on adult wing size. Knockdown of hephaestus caused a 40% reduction in adult wing area in both males and females, a phenotype very similar to loss of Babo-a itself, suggesting a role for this RNA binding protein in the regulation of Babo-a splicing.

444 Wingless counteracts epithelial folding in *Drosophila* wing discs by increasing mechanical tension at basal cell edges. *L. Sui*, C. Dahmann University of Technology Dresden, Dresden, Germany.

The modulation of mechanical tension is important for sculpturing tissues during animal development, yet how mechanical tension is modulated remains poorly understood. In *Drosophila* wing imaginal discs, the local reduction of mechanical tension at basal cell edges in the central hinge region results in basal relaxation and formation of the central hinge fold. Here we show that Wingless, which is expressed in a lateral region of the hinge, promotes basal cell edge tension through increasing βPS-integrin levels to help position the central hinge fold. Overexpression of Wingless throughout the hinge region blocks central hinge fold formation. Conversely, local depletion of Wingless in the pouch region, where Wingless signal transduction is normally high, results in ectopic fold formation. Depletion of Wingless results in decreased βPS-integrin levels, decreased basal edge tension and basal cell area relaxation. Our data show that the spatial pattern of Wingless signal transduction is converted into a spatial pattern of mechanical tension important for tissue morphogenesis.

445 Defining the genetic and cellular basis of morphological diversity in Drosophila. *Ben Vincent*[1], Ana Pinharanda[2], Eden McQueen[1], Sarah Smith[1], Peter Andolfatto[2], Mark Rebeiz[1] 1) Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA; 2) Department of Biological Sciences, Columbia University, New York, NY.

The posterior lobe in the Drosophila melanogaster clade is particularly well-suited to connecting genetic variation to morphogenetic outcomes. Recent work has identified many components of the gene regulatory network controlling posterior lobe development, including patterning molecules (transcription factors and signaling molecules) and cellular effectors (cytoskeletal regulators). Furthermore, hybrids between all three species are viable and exhibit intermediate lobe morphologies, which allows for rigorous mapping of genomic regions contributing to their drastically different phenotypes. We used RNA-seq in species and hybrids to identify genes containing expression variation, with the expectation that some of these genes contribute to morphological diversity in the posterior lobe. We are currently validating strong candidates using a CRISPR-based complementation test. In parallel, we are determining how patterning molecules and cellular effectors influence the number, shape and behavior of individual cells. Through this evolutionary case study, we are connecting variation in a gene regulatory network to molecular mechanisms that dictate cell size and shape, which will help develop the posterior lobe as a premier system for systems-level studies of morphological development and evolution.

446 Identification of Akirin-interacting proteins that are critical for myogenesis. *Mary Katherine Grimes*[1], Kristina Palermino-Rowland[1,2], Scott J. Nowak[1,2] 1) Molecular and Cellular Biology, Kennesaw State University, Kennesaw, GA; 2) Master of Science in Integrative Biology Program, Kennesaw State University, Kennesaw, GA.

The specification and differentiation of muscle precursor cells, or myoblasts, by the action of the Twist mesodermal regulator is a key event in the formation of the *Drosophila* larval musculature. Myoblast population dynamics are tightly controlled by gene expression moderated by Twist to determine somatic myoblast fates. Despite the primary importance of Twist for specifying and patterning the musculature, the identities of many molecular players involved in this process remain unknown. Akirin, a highly conserved nuclear transcriptional cofactor, regulates Twist-dependent gene expression via interactions with the Brahma

chromatin remodeling complex during mesodermal specification and muscle development. Using a genetic interaction screen in *Drosophila*, we have begun to identify other Akirin interacting proteins that participate in the process of muscle specification, patterning, and development. Our screening method has determined that Akirin interacts with Mi-2, the catalytic subunit of the NuRD complex, to correctly pattern the skeletal musculature. Double heterozygous mutant embryos for *akirin* and *mi-2* demonstrate a host of deranged or misshapen muscle phenotypes. Further, genetic interactions between *akirin* and other subunits of the NuRD complex appear to display disruptions in muscle patterning, implicating the larger NuRD complex in this process. Finally, our screening process has identified other loci beyond the NuRD complex that genetically interact with *akirin* to facilitate myogenesis. Through the generation of an interactome of potential partners, we will gain crucial insight into mechanism of Akirin during myoblast specification and muscle patterning.

447 Analysis of Defective Heart Patterning in akirin Mutants. *Hayley P. Milner*[1], Austin Howard[1,2], Madie Hupp[1], Scott J. Nowak[1,2] 1) Department of Molecular and Cellular Biology, Kennesaw State University, Kennesaw, GA; 2) Master of Science in Integrative Biology Program, Department of Molecular and Cellular Biology, Kennesaw State University, Kennesaw GA.

Among the metazoans the heart is one of the earliest discrete organ structures to form during embryogenesis, in a process highly conserved across the phyla. Heart development is controlled by a cascade of factors beginning with the emergence of cardiac progenitors known as cardiomyoblasts. In Drosophila melanogaster the specification of cardiac progenitors from mesoderm, differentiation and patterning of cardioblasts, and ensuing heart formation is controlled by the recursive action of the Tinman/Nkx2-5 transcription factor, which is itself initiated by the activity of the Twist bHLH transcription factor. We have identified Akirin as a highly conserved cofactor that works with Twist to selectively regulate expression of the mef2 and tinman enhancers. akirin mutants have profoundly abnormal hearts displaying defects in heart patterning, with disrupted organization and reduced numbers of Tinman-positive cardiomyoblasts. To investigate the nature of the heart defects observed in akirin mutants, we developed a rapid live imaging assay to visualize contractions in pre-hatching embryos. Our analysis indicates that akirin mutant hearts that do in fact form either display profoundly uncoordinated contractions, or completely lack contractions in Stage 17 embryos. Taken together, these data indicate that Akirin represents a new co-regulator of the cardiac developmental pathway, and is critical for heart patterning and formation.

448 Postmitotic Myotubes Repurpose the Cytokinesis Machinery to Effect Cellular Guidance and Elongation. Shuo Yang, *A.N. Johnson* Department of Developmental Biology, Washington University School of Medicine, St Louis, MO.

For the musculoskeletal system to produce voluntary movements, contractile muscle cells must attach to the correct tendons during development. Compared to our understanding of muscle cell fate specification and differentiation, relatively little is known about the mechanisms that guide immature muscle cells, or myotubes, to the correct tendon attachment sites. We identified the allele *back seat driver* (*bsd*) in a genetic screen for regulators of myotube guidance and mapped *bsd* to a previously uncharacterized serine/threonine kinase. *bsd* mutant embryos showed normal muscle precursor cell fate specification, but during muscle morphogenesis the myotubes often attached to the incorrect tendons or failed to make tendon attachments altogether. When we expressed Bsd in the myotubes of *bsd*mutant embryos we were able to restore normal muscle morphology, arguing that the role of Bsd is cell autonomous. To understand how Bsd regulates myotube guidance, we performed AP-MS and found Bsd physically interacts with Polo kinase, a well-characterized regulator of cytokinesis. Polo directs the localization of the RacGAP Tumbleweed (Tum) and the kinesin-like protein Pavarotti (Pav) to the cytokinesis initiation complex in mitotic cells. Although a myogenic role for Polo kinase has not previously been shown, Tum and Pav are known regulators of the microtubule cytoskeleton during myotube guidance. We found that *bsd* myotubes phenocopy the microtubule defects associated with *tum* myotubes. These studies have identified a new component of the microtubule regulatory complex that directs cellular guidance. Our ongoing studies are aimed at understanding how Bsd regulates the localization and function of Polo, Tum, and Pav during muscle morphogenesis.

449 Regulation of gonad development and homeostasis by the BTB protein Ribbon. *J.C. Jemc*, M. Alvarez, S. McDonnell, L. Tinawi, D. Talbot, U. Khan, S. Moqeet Dept. of Biology, Loyola University Chicago, Chicago, IL.

During organogenesis, cells migrate, interact, proliferate, and rearrange to form an organ with proper structure. Defects in this process can lead to birth defects, disease, and even lethality. Many genes required for organ development also function to maintain homeostasis in the adult. The gonad has been proven an excellent model for studying how genes function to regulate the establishment and maintenance of organ structure and function. The Broad Complex, Tramtrack, and Bric à Brac (BTB) protein Ribbon (Rib) has previously been shown to regulate embryonic gonad development. Subsequent work has revealed that *rib* continues to be expressed in the testis during larval testis development and in the adult. In order to understand how Rib functions in later stages of development and in adult gonad homeostasis, overexpression and knockdown approaches are being utilized. *rib*overexpression in the male and female somatic cells throughout development results in severe morphological defects. Ovaries lack niche and ovariole structures, while testes are absent or significantly reduced in size. Current studies are ongoing to examine the role of Rib in the establishment of the stem cell niche and gonad structure during larval stages, as well as to identify factors with which Rib interacts to influence gonad development. In addition to overexpressing Rib throughout development, we have also limited overexpression to adult stages. In this context, somatic overexpression leads to defects in ovariole morphology in adult females, whereas testis morphology is largely normal. The effect of knocking down *rib* expression in the germline and somatic cells of males and females is currently being investigated. Understanding the role of Rib in the context of the gonad will allow us to understand how it functions in other tissues to promote organ development and homeostasis, as well as to identify the molecular mechanisms through which Rib functions. As other BTB family proteins are critical for gonad development and function, we are also exploring possibility that Rib could positively and/or negatively regulate the activity of these critical factors in the developing gonad.

450 Ecdysone-inducible *polished rice* temporally regulates fate decision of tracheal tip cells in embryonic tracheal morphogenesis. *Y. Taira*[1], H. Wada[3], S. Hayashi[3], Y. Kageyama[1,2] 1) Graduate School of Science, Kobe University, Kobe, Hyogo, JP; 2) Biosignal Research Center, Kobe University, Kobe, Hyogo, JP; 3) Laboratory for Morphogenetic Signaling, RIKEN Center for Biosystems Dynamics Research, Kobe, Hyogo, JP.

Tubular organs undergo multiple developmental processes, including invagination of an epithelial cell layer, tubular extension, branching and branch fusion, to form their complex architectures. *Drosophila* trachea is an excellent model of tubulogenesis, in which cell fate decision, branch identities and branch migration toward the proper directions are orchestrated by intercellular signals. In the dorsal branch, two types of cells are formed at the tip: one is a fusion cell defined by FGF and Wingless at the onset of branch formation, and the other one is a terminal cell that form fine tracheoles, which is specified during tubular extension in a FGF-dependent manner. In addition to these positional cues, it has been shown that the temporal ecdysone signal should regulate these developmental events, since the inhibitions of ecdysone signaling by overexpression of dominant-negative isoform of Ecdysone receptor (EcR) or loss-of-function mutation of *EcR* results in severe tracheal defects. Nevertheless, the temporal regulation of tracheal formation is still an open question.

polishe rice (*pri*) encodes four micropeptides and acts as one of the major factors in ecdysone signaling that defines the developmental timing. Previous studies have shown that *pri* mutant embryos show defective dorsal trunk dilation and loss of dorsal branch fusion during tracheogenesis. To see further details of *pri* phenotype, we performed time-lapse imaging analysis of *pri* mutants. We found that *pri* mutants show ectopic fusion of ipsilateral dorsal

branches and loss of terminal branch. Cell marker studies revealed that terminal cell marker *DSRF* was not detected at the tip of dorsal branch while the number of the cells expressing fusion cell marker *dysf* was increased in *pri* mutant embryos. Consistently, expression of *esg*, an upstream regulator of *dysf*, was also observed in multiple tip cells. Overexpression of a dominant-negative form of the Ecdysone Receptor in the tracheal cells caused occasional loss of *DSRF*, which resembles *pri* mutants, and this phenotype was almost completely rescued by *pri* overexpression. These results suggest that *pri* is an essential factor for tip cell specification, promoting temporally coordinated development of the tracheal system in an ecdysone-dependent manner.

451 Quantitative spatial gene expression in The developing *Drosophila* eye. *S. Ali*[1], S. Signor[1], K. Kozlov[2], S. Nuzhdin[1] 1) Molecular & Computational Biology, University of Southern California, Los Angeles, CA, USA; 2) Department of Applied Mathematics, St. Petersburg State Polytechnic University, St. Petersburg, 195251, Russia.

Developmental biology has struggled to quantify natural variation in populations. Technical restrictions have limited our ability to investigate both the spatial and quantitative relationships that generate variation in gene expression at the same time. We overcame these issues by using the Hybridization Chain Reaction (HCR) and rigorous statistical models to detect mRNA in 4 genes simultaneously, allowing us to compare the spatial and co-expression patterns between genotypes, sexes and species (*Drosophila melanogaster and D. simulans*). We focused on 4 conserved morphogens (*hairy, atonal, hedgehog, and Delta*) involved in the morphogenetic furrow; a wave that sweeps anteriorly through each developing eye disc to ultimately generate the repeating eye units of the compound eye. We found considerable variation in these eye disc genes between species, genotypes, and sexes. We also found that the regulatory relationship between these genes has evolved. Lastly, we show that the spatial interrelationships of these genes evolved between species in the morphogenetic furrow. This major advancement has allowed us to evaluate wild type differences in spatial and quantitative gene expression at the level of genotype, species and sex.

452 The BTB/POZ domain factor Ribbon has a dual role as the transcriptional regulator of both organ growth and morphogenesis in the embryonic epithelium. *R. Loganathan*[1], M.B. Wells[1], J.S. Lee[2], M. Slattery[2], D.J. Andrew[1] 1) Cell Biology, Johns Hopkins University, Baltimore, MD; 2) Biomedical Sciences, University of Minnesota Medical School, Duluth, MN.

Cell growth and morphogenesis are key drivers of embryogenesis. Although organogenesis, driven primarily by proliferative cell growth has received much attention, the mechanisms driving post-mitotic tissue growth and morphogenesis are understudied and poorly understood. We have focused on post-mitotic growth and morphogenesis in two epithelial tubular organs in Drosophila, the salivary gland (SG) and the trachea (TR). Phenotypic analysis of embryos mutant for the BTB/POZ domain nuclear factor Ribbon (Rib) have revealed defects in the size and shape of both organs. SG cells of *rib* mutants are 55% the size of WT and the cells are cuboidal instead of columnar. Tracheal cells of *rib* mutants are 46% the size of WT and they fail to form a dorsal trunk, the major branch of the trachea. In an unbiased, tissue-specific in vivo determination of binding sites for Rib, which shows high levels of expression in both tissues during embryonic stages, we identified 494 bound genes in the SG and 1317 bound genes in the TR. Identification of target gene function by gene ontology cluster analysis revealed that Rib bound genes are enriched in growth and morphogenetic categories in both tissues. Remarkably, Rib binding appears to be organ-specific, with only 136 bound genes shared in the two tissues. In the SG, the primary genes linked to growth control corresponded to nearly all of the 82 known ribosomal protein genes. Meanwhile, in the TR, the Rib bound genes for growth control were components of the TOR signaling pathway. In both tissues, a diverse set of morphogenetic genes were also bound by Rib and, in the SG, several of these morphogenetic regulators have been shown to also be regulated by Rib by RT-qPCR and in situ analyses. Overall, our studies indicate a dual role for Rib in the transcriptional regulation of post-mitotic organ growth and morphogenesis in the embryonic SG and TR via distinct target clusters.

453 Regulation of the Hippo pathway from two distinct subcellular regions at the apical cortex. *S.A. Tokamov*, R.G. Fehon Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL.

How organs "know" when to stop growing is a fundamental question in developmental biology. From flies to humans, the Hippo pathway is a key regulator of tissue growth and cell proliferation. When active, the Hippo pathway suppresses growth via a core kinase cascade that culminates in the phosphorylation and cytoplasmic retention of a transcriptional co-activator Yorkie. The activity of these kinases is known to be regulated by the proteins Kibra, Merlin, Expanded, and Crumbs, all of which accumulate at the junctions in epithelial cells. Although biochemical data suggest that Kibra, Expanded, and Merlin form a complex and together activate the Hippo kinase cascade, genetic studies argue instead that Kibra and Merlin function in parallel to Expanded and Crumbs. Additionally, while Kibra, Merlin, and Expanded colocalize at the junctions of epithelial cells, they do so independent of one another. Our lab has recently shown that in addition to the apical junctions, Kibra accumulates at the apical medial cell cortex in *Drosophila* wing disc epithelial cells, and that Kibra plays a key role in assembling this medial complex. We found that Kibra recruits Merlin, a scaffold protein Salvador, and the core kinases Hippo and Warts to the medial cortex, and this complex can suppress growth independent of Expanded. We also found that the apical polarity protein Crumbs suppresses Kibra's activity, possibly by sequestering Kibra at the junctions. These results uncovered a novel subcellular compartment within which Hippo pathway components assemble and function and raise several important questions. First, how is the assembly of the junctional and medial complexes differentially regulated? Second, what is the role of junctional and medial Kibra in growth control? This study investigates how Kibra is regulated at the junctional vs. the medial cell cortex and implicates the core kinase cascade in controlling Kibra localization, protein stability, and function.

454 Mob family proteins and the nuclear Dbf2-related kinase, Tricornered, are required for tube formation in the ovarian follicular epithelium. *J.C. Duhart*, R.F. Sauceda, L.A. Raftery School of Life Sciences, University of Nevada, Las Vegas, Las Vegas, NV.

We use the follicular epithelium as a model system to identify effector genes that link patterning signals to morphogenetic behaviors. In this tissue, BMP signaling specifies anterior fates, which include two dorsal domains of cells that remodel extensively to form the dorsal appendages, two blind-end, epithelial tubes that are symmetrically placed on either side of the dorsal midline. We identified *mob2* as a candidate BMP effector, by mapping the genomic location of a BMP-responsive enhancer trap, *A359-lacZ*. The *mob2* gene encodes a small non-catalytic protein of the highly conserved Mob family. Across metazoans, Mob proteins are allosteric regulators of nuclear Dbf2-related (NDR) kinases. We generated a *mob2* allele that lacks the NDR binding domain by CRISPR/Cas9-aided homologous recombination. Eggshells from $mob2^{KO}$ homozygous females had variably shortened and misshapen dorsal appendages. These data predict a model in which one or both fly NDR kinases, Warts (Wts) and Tricornered (Trc), also regulate morphogenesis of the appendage tubes. To test this prediction, we used follicle cell-specific knock-down by RNA interference, which revealed a requirement for *trc* in tube morphogenesis. Conversely, *wts* knockdown confirmed a previously identified requirement in dorsal patterning, leaving a role in morphogenesis as an open question. In this assay, Trc depletion produces fully penetrant morphogenetic defects. Of these, ~16% of the eggs exhibit severe failures of tube closure—a step that precedes the elongation step that requires *mob2*. Because the fly genome has four *mob* genes, we screened for tubulogenesis defects from depletion of each. Only *mob4* gave tubulogenesis defects, which were similar to Trc knock down. As expected, *mats* (*mob1*) depletion was similar to Wts knock down. These data reveal a role for Trc in follicular tube formation, consistent with other roles for cell shape regulation in the larval PNS and wing disc. Furthermore, they indicate a potential for differential function of Mobs during the progression of morphogenesis.

455 Genetic Architecture and cellular basis of *Drosophila* gut plasticity. *A. Bonfini*[1], A. Dobson[2], D. Duneau[3], X. Liu[1], P. Houtz[1], J. Revah[1], R. Fay[1], N. Buchon[1] 1) Entomology, Cornell University, Ithaca, NY; 2) Institute of Healthy Ageing, University College London, London, UK; 3) Paul Sabatier University -

Toulouse III, Toulouse, France.

The gut acts as the primary interface between food and an organism, but it is unclear how nutrients affect gut epithelial turnover. We found that nutrient composition can alter gut size. Guts of flies raised on a High Yeast diet (HY) were 40% bigger than ones on a High Sugar diet (HS), through increased number and size of enterocytes. HS antagonizes yeast triggered gut growth, which requires multiple nutrients, not just proteins. The gut plastically grows and shrinks in response to diet shifts. Analysis of tissue turnover and transcriptomic demonstrate that diets uncouple intestinal stem cell (ISC) proliferation from enterocyte loss. A pathway dependent of TOR, but not insulin, is required in ISCs and enterocytes to trigger HY dependent growth. Finally, we demonstrate that gut plasticity is highly variable between individuals, and GWAS allowed us to identify new genes involved in this process.

456 Identification of the *Drosophila* Tribbles conserved COP1 binding site. *C.E. Nauman*, L.L. Dobens Department of Molecular Biology and Biochemistry, University of Missouri - Kansas City, kansas city, MO.

Tribbles encodes a pseudokinase adaptor protein, which cooperatively binds a ubiquitin ligase and a target protein to direct its degradation. Members of the Tribbles protein family have conserved structural features as well as conserved roles in cell stress, metabolism, differentiation, proliferation, and cell cycle. A protein sequence (QXVP) present in the C-terminal tail of ~90% of 492 Tribbles-like proteins is critical for binding to the ubiquitin ligase COP1, however this motif is absent from *Drosophila* Tribbles (Trbl). To identify the ubiquitin ligase binding site in this region, a series of point mutations and deletions were made in a UAS-regulated Trbl transgene and misexpressed in Trbl-sensitive tissues including the migrating border cell cluster and wing. Previously, it has been shown that border cell misexpression of Trbl transgenes effectively block migration. When we misexpressed Trbl transgenes bearing either a point mutation in the putative COP1 binding site or a truncation of the entire proposed COP1 motif, we saw that migration was effectively blocked similar to WT, casting doubt on the functional role of this divergent region. In contrast, misexpression of Trbl transgenes truncated to entirely remove the C-terminal tail resulted in complete loss of Trbl activity, pointing to a requirement for the domain for full Trbl activity. Wing misexpression of Trbl blocks cell division and cell growth and we used this assay to test the activity of these same transgenes. While the COP1 site-specific mutant and truncated protein led to no strong influence on the reduced tissue size/increased cell size phenotypes characteristic of Trbl misexpression, misexpression of the MEK1 binding site point mutant or a deletion of this motif led to a significant increase in wing tissue size, to an extent exceeding even wild type wings. These data suggest that C-terminal tail has separable effects on cell proliferation, growth, and differentiation. Experiments to identify the factors that mediate the effects of the C-terminal tail are ongoing.

457 Modeling the Interactions Between Migrating Cells and their Environment During Drosophila Embryogenesis. *W. Hamilton*[1], M. Stolarska[2], A. Ismat[1] 1) Department of Biology, University of St. Thomas, Saint Paul, MN; 2) Department of Mathematics, University of St. Thomas, Saint Paul, MN.

During embryogenesis the Caudal Visceral Mesoderm (CVM) cells migrate along the Trunk Visceral Mesoderm (TVM) before forming the longitudinal visceral muscles of the midgut. These cells migrate individually and are constantly interacting with the extracellular matrix (ECM), which is a dense network of fibers and macromolecules. In order for the cells to migrate correctly, restructuring of the ECM is required. This is the role of the extracellular protease *AdamTS-A*, to cleave the connections between the cell and its environment. It has been shown that *AdamTS-A* is required to cleave the trailing edge of migrating cells when they migrate as a collective, like in the salivary gland. Therefore, our question is whether *AdamTS-A* functions the same way in cells that migrate individually as it does in cells that migrate as a collective. One way for us to gain more insight into the role of *AdamTS-A* in cell migration, a mathematical model was created to clearly visualize what is occurring in these embryos. The model allows us to clearly visualize CVM migration in wild-type as well as *AdamTS-A* loss-of-function mutants. Additionally, by examining CVM migration in *AdamTS-A* mutants and *AdamTS-A* over-expression embryos, and comparing these experiments to the model we can both improve the accuracy of the model as well as use the model to better understand the role of this extracellular protease *AdamTS-A* in CVM migration.

458 The extracellular protease *AdamTS-B* is required for proper tracheal tube formation. *E. Steinmetz*, A. Thuringer, A. Ismat Department of Biology, University of St. Thomas, Saint Paul, MN.

The *Drosophila* trachea is a highly branched network of tubular airways made of epithelial cells that go through elaborate migration, cell intercalation and morphogenetic processes throughout embryogenesis. Much work has been done on various aspects of the collective migration of these cells; however, the role of the extracellular environment and the interactions of these cells with this dynamically changing environment are less understood. The extracellular protease *AdamTS-B (CG4096)* is a member of the ADAMTS family, which is known to play an important role in cell migration. Humans encode 19 members of this protease family, while a simpler model organism, the fruit fly *Drosophila melanogaster*, encodes only three members. *Drosophila AdamTS-B*, homologous to eight human ADAMTSs, is expressed in the embryonic trachea from early to late stages of tracheal development. Embryos completely missing *AdamTS-B* display defects in tracheal branching and migration. Specifically, the lateral transverse (LT) cells are completely disorganized and misshapen, as compared to wild-type trachea. Conversely, over-expressing *AdamTS-B* throughout the trachea displayed extra long ganglionic branches (GBs), lateral group branches (LGs), extra LT branches, as well as luminal cysts throughout the LT and GB branches. Preliminary data also shows elongated tracheal cells, and cells that do not seem to have gone through proper cell intercalation. Taken together, it is clear that this extracellular protease is required for proper tracheal tube formation. Importantly, this data sheds light on the role of the extracellular matrix and its restructuring in formation of epithelial tubes.

459 Drosophila *fibulin (fbl)* plays a role in transepithelial migration of germ cells during embryogenesis. *A. Petersen*, A. Ismat Department of Biology, University of St. Thomas, Saint Paul, MN.

Drosophila *fibulin* (*fbl*) (CG31999) is an extracellular matrix (ECM) gene associated with cellular adhesion that affects cell migration. In Drosophila, *fbl* mRNA is highly expressed in the posterior midgut (PMG) epithelium specifically during stages 9 and 10 of embryogenesis. In these stages, the germ cells, which are precursors to the gonads, migrate through PMG epithelium in a process known as transepithelial migration. Once out of the PMG, the germ cells continue migrating posteriorly. We show that over-expression of *fbl* in either the PMG or the germ cells causes disruptions in the germ cell migration patterns. Initial observations display abnormal germ cell spacing and clustering within the PMG in stages 9 and 10. These findings provide evidence that Fbl may be involved in epithelial remodeling of the PMG that allows the germ cells to migrate through it. Additional work on *fbl* knockdown embryos will allow us to gain more insight into the role of *fbl* on transepithelial migration.

460 A screen for genetic modifiers of Protein Phosphatase 1 function in *Drosophila* border cell cohesion and migration. *C.F. Del Real*, Y. Chen, M. Komp, J.A. McDonald Department of Biology, Kansas State University, Manhattan, KS.

Cells can migrate collectively, in tightly- or loosely-associated groups, during tissue and organ formation, embryonic development, wound healing, and tumor metastases. *Drosophila*border cellsserve as an excellent genetically-accessable model of collective cell migration inside a developing tissue. During ovarian development, 6-8 cells form the border cell cluster, which migrate together as a cohesive cluster to reach the large oocyte at the posterior end of the egg chamber. Previous experiments from our lab have found that inhibitionof Protein Phosphatase 1 (PP1) activity, through overexpression of the endogenous (and specific) PP1 inhibitor, nuclear inhibitor of PP1 (NiPP1), caused the border cell cluster to separate into single cells and limited their ability to migrate. Further experiments demonstrated that PP1 regulates actomyosin contractility and adhesion between border cells to promote collective migration. To gain additional insights into how PP1 activity controls collective cell migration, we have performed a genetic modifier screen of the NiPP1-induced border cell phenotypes. We screened the majority of lines from the 2ndand 3rd chromosome using Bloomington Deficiency Kits, specifically looking for chromosomal

regions whose altered gene dosage either enhanced or suppressed the effects of NiPP1 on border cell cohesion or migration. With this strategy, we have now identified five distinct deficiencies that significantly enhance the NiPP1 migration defect and one deficiency that strongly enhances the NiPP1 cluster separation phenotype. We are currently mapping the relevant genetic enhancers through a combination of testing smaller overlapping deficiencies and testing for interaction with specific RNAi lines. By identifying genes that modify the NiPP1 phenotype, we will be able to determine PP1 molecular targets and pathway members. Because many *Drosophila*genes are conserved, studies on PP1 function in border cells have implications for collective cell migration in human development and disease.

461 Investigating the role of Ena in promoting cell extrusion from epithelia. *Jennifer Nwako*[1,2], Jeanne Jodoin[2], Adam Martin[2] 1) Wellesley College, Wellesley , MA; 2) Massachusetts Institute of Technology, Cambridge, MA.

Apical constriction and tissue folding are associated with cells having apical-basal polarity. In wild-type embryos, Twi and Sna expression cause cells to apically constrict, but remain in the tissue during folding. These cells then undergo epithelial to mesenchymal transitions. Using an RNAi screen, we discovered that loss of the *Drosophila* Abelson tyrosine kinase (Abl) results in premature cell extrusion of Twi/Sna- expressing cells during tissue folding. We showed that this extrusion requires the activity of Enabled (Ena), an actin binding protein that promotes actin assembly and is negatively regulated by Abl. Furthermore, we showed that Abl depletion results in the loss of apical-basal polarity and E-cadherin polarity, both hallmarks of EMT. Here, we show that Ena overexpression also promotes cell extrusion, similar to Abl depletion and that Ena becomes enriched at basolateral contacts in cells that undergo EMT. We propose that activation of Ena results in a switch from contractile, apical actin assembly to protrusive basolateral actin assembly, promoting cell escape from the epithelium. To further test this model we will determine the localization of proteins involved in leading edge actin assembly, such as the Arp2/3 complex.

462 Characterization of Border Cell Migration and Cell Polarity in a CASK Beta-isoform Knockout Fly Line. *Audrey Farthing*, MacKenzie Kaschalk, Rand Abdullatef, Jordyn Sanner, Maggie Krause, Maddison Guthrie, Elizabeth Olah, Holly Dyer, Jamie Siders Sanford Ohio Northern University, Ada, OH.

Calcium/Calmodulin-dependent Serine Protein Kinase (CASK) protein consists of two isoform classes and belongs to the MAGUK (membrane-associated guanylate kinase) protein family. The alpha isoforms code for a truncated protein possessing the PDZ, SH3, and GK domains. The beta isoforms code for the full-length protein, including an N-terminal CamK domain, two L27 domains and a HOOK domain, in addition to the canonical PDZ, SH3 and GK domains. CASK's known functional roles include providing a scaffold for the clustering of channels and receptors, and cell adhesion. Other MAGUK proteins have been found to regulate cell polarity, yet CASK's role in apical-basal polarity has not been characterized. A recent study has also implicated CASK in collective cell migration. The current study, via knockdown of CASK's beta isoform in the fruit fly (P18 line), aims to further characterize the role of CASK's beta isoforms in cell polarity and collective cell migration using Drosophila ovaries as a model system. In order to assess whether CASK plays a role in cell polarity, the amount of Dlg (CASK's known binding partner) at the basolateral membrane in the P18 fly line was compared to that of wild-type fly lines. Preliminary results, via immunohistochemistry, indicate that Dlg is upregulated in the P18 fly line in response to loss of CASK beta isoforms, suggesting that Dlg may compensate for loss of CASK. This implies a putative role for CASK in the maintenance of apical-basal polarity the Drosophila egg chamber. CASK's contribution to collective cell migration was also investigated via analysis of border cell migration (BCM) via standard border cell migration assays. BCM is a widely used model of cell movement en masse, with implications in tumor cell metastasis, inflammation and wound healing. In these assays, complete versus incomplete migration of the border cell cluster to the oocyte border is studied at stage 10 of oogenesis. Previous total RNAi knockdown of both isoforms of CASK showed that loss of CASK results in incomplete migration in ~20% of stage 10 egg chambers. It was expected that the P18 line CASK beta isoform knockdown would display incomplete migration at percentages similar to those observed via RNAi knockdown. However, preliminary results show that the P18 line has no significant downregulation of complete BCM, suggesting that the beta isoforms of CASK do not contribute to BCM. Future analysis of a CRISPR CASK KO fly line will aid in delineation of CASK's alpha isoforms putative role in border cell migration.

463 Feedback between actomyosin and microtubules stabilizes intercellular force transmission during tissue folding. *C. S. Ko*, A. C. Martin Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

Apical constriction promotes tissue folding, such as during mesoderm invagination in *Drosophila*. While the role of actomyosin is established, the function of the microtubule cytoskeleton during apical constriction is less clear. Here, we uncovered a role for microtubules in organizing the apical actomyosin cortex during *Drosophila* mesoderm invagination. We found that, similar to Myosin 2 localization, GFP-tagged Patronin (CAMSAP), a microtubule minus-end-binding protein, concentrated in the middle of the cell apex (medioapical) in apically constricting cells. The medioapical polarity of Patronin-GFP was dependent on both RhoA signaling and F-actin. In addition, we found that Patronin was required for proper RhoA polarity, suggesting a feedback loop between actomyosin and microtubules that promotes medioapical polarity in apically constricting cells. Depleting Patronin or injecting drugs that inhibit microtubule dynamics disrupted mesoderm invagination. In contrast to previous models of microtubule function in epithelia, microtubules were not required for apical-basal polarity of adherens junctions or Myosin 2. Instead, microtubules promoted the stable connection of actomyosin networks between cells. In summary, our studies of mesoderm invagination have uncovered roles for the microtubule cytoskeleton in promoting the formation of a polarized contractile machine that is stably connected to intercellular junctions.

464 Cell polarity determinant Dlg1 regulates the mechanics of tissue invagination. *M. Fuentes*, B. He Dartmouth College, Hanover, NH.

Apical constriction mediated epithelial folding provides a fundamental mechanism that converts flat epithelial sheets into multilayered tissues. It remains elusive how forces generated near the apical surface drive tissue folding in 3D. During *Drosophila* gastrulation, prospective mesoderm cells constrict apically and subsequently invaginate to form a ventral furrow (VF). Previous studies suggest that apical constriction by itself cannot fully account for invagination. To elucidate the additional requirements for tissue invagination, we performed an RNAi-based candidate screen to identify genes that specifically regulate the invagination phase of VF formation. We found that knockdown of the apical-basal polarity determinant *dlg1* results in a pronounced delay in tissue invagination without affecting apical constriction. Interestingly, the defect in VF invagination is associated with specific cell shape abnormalities in the non-constricting cells that flank the constricting domain. When apical constriction starts, the flanking cells in *dlg1* RNAi embryos become overstretched and move like waves, resembling soft, fluid-like materials. Using a magnetic tweezers-based approach to probe mechanical properties in live embryos, we found that the mutant tissue is less elastic and more prone to irreversible deformation than wild type. At the molecular level, the aberrant mechanical properties of the *dlg1* RNAi embryos are associated with an altered spatial distribution of cortical actin and actin regulators in the non-constricting cells. These observations suggest that the effectiveness of invagination depends on the mechanical integrity of the flanking cells. To directly test this idea, we employed physical and genetic approaches to disrupt the integrity of the flanking cells. During early stages of apical constriction, physical disconnection of the flanking cells from neighboring constricting cells by laser ablation or optogenetic downregulation of F-actin specifically in the flanking cells delayed VF invagination. Together, our findings suggest that robust VF invagination requires coordination between the constricting and non-constricting cells, and that this coordination is dependent on the mechanical integrity of the non-constricting cells. Moreover, our work reveals an unexpected role for Dlg1 in regulating tissue-scale mechanics during gastrulation.

465 Tissue-scale mechanical coupling reduces morphogenetic noise to ensure precision during epithelial folding. *AS Eritano*[1], CL Bromley[1], L Schütz[2], FL Wen[3], T Shibata[3], MM Sami[1], M Takeda[1], S Lemke[2], YC Wang[1] 1) Laboratory for Epithelial Morphogenesis, Center for Biosystems Dynamics Research, Kobe, JP; 2) Centre for Organismal Studies Heidelberg, University of Heidelberg, Heidelberg, Germany; 3) Laboratory for Physical Biology, RIKEN Center for Biosystems Dynamics Research, Kobe,JP.

Understanding how complex structures are created in a precise and reproducible manner is a fundamental goal in developmental biology. Although stereotypical and robust morphogenesis is a common feature in developing systems, the mechanism by which this morphological consistency is attained remains poorly understood. In particular, although gene expression profiles have been shown to be precise in the early embryo, it is unclear whether downstream mechanical processes interpret these patterning instructions with high fidelity.

Here we investigate how morphogenetic precision is achieved in the formation of the cephalic furrow (CF), combining multiscale quantitative imaging, optogenetics and numerical simulation using a 3D vertex model. The CF is an epithelial fold initiated by localized cell shortening and a classic example of morphogenetic precision. We show that the genetic circuitry that patterns the CF region produces a quantitative, combinatorial expression code of two essential transcription factors, Buttonhead (Btd) and Even-skipped (Eve), with single-cell row resolution. While the cell shortening events largely comply with the Btd/Eve positional code, they are temporally heterogeneous and only show 80% accuracy in spatial positioning. At the cellular scale, shortening is driven by myosin-dependent contractility that shrinks the apical-basal axis of the lateral interfaces between cells. At the tissue-scale, these contracting lateral interfaces are planar polarized and form supracellular cables that align cells into longitudinal rows. We propose that this mechanical coupling ensures the morphological uniformity of the furrow structure, despite inhomogeneous initiation behaviors. Our results suggest that the intrinsic noise in decoding positional information into the spatial pattern of myosin contractility can be overcome by tissue-level mechanical coupling, thereby improving morphogenetic precision.

466 Epithelial cell reintegration: the ins and outs. N. Dawney[1], T. Wilson[1], C. Cammarota[2], C. Mallie[1], *D. Bergstralh*[1,2] 1) Department of Biology, University of Rochester, Rochester, NY; 2) Department of Physics & Astronomy, University of Rochester, Rochester, NY.

Epithelial tissues perform a range of specialized functions, including secretion, absorption, and protection. All of these functions require that the component cells remain tightly packed. This is a particular challenge during development, when new cells are being added to the tissue. Work in a number of systems shows that one answer to this challenge is cell reintegration: epithelial cells can be born protruding from the sheet, then reincorporate into it. Our lab aims to understand this process. Our previous work demonstrated that reintegration in the *Drosophila* follicular epithelium relies on Fas2 and Neuroglian, homophilic adhesion molecules that promote axonal growth and pathfinding. Our model is that these and other neuronal adhesion factors coordinate with the juxtamembrane spectrin-based cytoskeleton to form an evolutionarily-conserved assembly that maintains epithelial integrity during proliferation. We are currently testing this possibility in other epithelial systems, including mammalian epithelial organoids.

467 Investigating a role for septate junction proteins in cell polarity during dorsal closure. *O. De*, R. Ward Molecular Biosciences, University of Kansas, Lawrence, KS.

Polarized epithelia establish distinct compartments within the body of multicellular organisms and regulate paracellular transport of solutes. This diffusion barrier function is executed by occluding complexes such as septate junctions (SJs) in invertebrate epithelia. Extensive studies in *Drosophila* have identified more than 20 genes required in the assembly or maintenance of SJs. Recent studies in our lab demonstrated that core SJ genes including *coracle* (*cor*), *Macroglobulin complement-related* (*Mcr*) and *Neurexin-IV* (*Nrx-IV*) are required for embryonic developmental events such as head involution and dorsal closure (DC), that occur prior to the formation of a mature SJ. However, the mechanistic role of SJ genes during embryonic morphogenesis is unknown. We hypothesize that SJ proteins regulate aspects of cell polarity that may be required for morphogenesis. Previous studies suggest a redundant role for SJ genes- *cor* and *Nrx-IV* along with *yurt* to maintain apical/basal polarity in the epidermis of late stage embryos. Whether SJ genes are independently required to maintain apical/basal polarity and if mild alterations in polarity contribute to morphogenetic defects in SJ mutants is unclear. To address this, we are using DC as a model system. DC is a mid-embryogenesis process that seals an epidermal gap. It involves elongation of the dorsal-most epidermal cells and migration of the contralateral epidermal sheets to fuse and form a seamless epithelium. Loss-of-function mutations in *cor, Mcr* and *Nrx-IV* are embryonic lethal, with a significant penetrance of DC defects. We are examining fixed tissues in SJ mutant embryos for defects in apical/basal polarity during DC. We are also attempting to rescue DC defects in SJ mutants by subtle alterations in apical/basal polarity. In addition, the elongation of epidermal cells during DC requires planar polarized expression of various molecular components in discrete patterns and previous studies indicate a role for SJ genes in planar cell polarity in pupal wing hair alignment. Whether SJ genes are required for any aspect of planar polarization during mid-embryogenesis is unknown. To address this, we are analyzing fixed tissue of SJ mutant embryos for defects in epidermal elongation and planar polarization during DC.

468 The RhoGEF Cysts couples apical polarity proteins to Rho and myosin activity at adherens junctions. *M. Pellikka*[1], J. Silver[1], F. Wirtz-Peitz[2], S. Simões[1], D. Yan[2,4], R. Binari[2], T. Nishimura[5], Y. Li[1], T. Harris[1], N. Perrimon[2,3], U. Tepass[1] 1) Cell and Systems Biology, University of Toronto, Toronto, Ontario, CA; 2) Department of Genetics, Harvard Medical School, Boston, MA, USA; 3) Howard Hughes Medical Institute, Harvard Medical School, Boston, MA, USA; 4) Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, China; 5) RIKEN Center for Biosystems Dynamics Research, Minatojima-minamimachi, Chuou-ku, Kobe, Japan.

The spatio-temporal regulation of small Rho GTPases is crucial for the dynamic stability of epithelial tissues. However, our understanding of the temporal and spatial control of RhoGTPase activity during development remains limited. To gain further mechanistic insights into the regulation of Rho GTPases in vivo we analyzed the Drosophila Rho GTPase-specific guanine nucleotide exchange factor (RhoGEF) Cysts. Cysts is the single orthologue of the mammalian paralogs p114RhoGEF, GEF-H1, p190RhoGEF, and AKAP-13. We found that Cysts is enriched at adherens junctions (AJs) and is required to maintain epithelial integrity. Loss of Cysts causes defects in epithelial organization similar to mutations in Crumbs and other apical polarity or AJ proteins. Polarity defects were first apparent early during gastrulation when Cysts is required for the formation of a circumferential AJ belt. Genetic and biochemical experiments suggest that Cyst predominantly activates Rho1 rather than Cdc42 or Rac. In addition to the RhoGEF domain, we show that the coiled-coil domain containing C-terminal region of Cyst is essential for function. Cyst recruitment to the apico-lateral cortex depends on the polarity proteins Crumbs and Bazooka/Par3 and requires multiple domains within Cyst including the C-terminal region. Interestingly, Cyst or Crumbs compromised embryos showed similar reduction in junctional myosin. Together, our findings indicate that Cyst links apical polarity proteins to Rho1 and myosin activation at AJs to support junctional and epithelial integrity in the Drosophila ectoderm.

469 *Macroglobulin complement-related* is required for *Drosophila* egg elongation . *H. Alhadyian*, D. Shoaib , R. Ward Molecular Biosciences Department, University of Kansas, KS.

Macroglobulin complement-related (Mcr) is a transmembrane protein belonging to the thioester-containing protein family (TEP) and has been implicated in host defense against pathogens. We initially identified *Mcr* in a genetic screen of mutations that dominantly enhanced a malformed leg phenotype in *broad* mutant animals, suggesting a role for *Mcr* in morphogenesis. We subsequently determined that *Mcr* is a core component of epithelial septate junctions (SJs), which are analogous to the vertebrate tight junction in providing an essential occluding function to the epithelium. Interestingly, homozygous mutations in *Mcr* are embryonic lethal with defects in developmental processes including head involution and dorsal closure that occur before the

establishment of the SJ. These data suggest a role for Mcr in morphogenesis that is independent of its role in the occluding junctions. To extend these studies, we are investigating the role of *Mcr* in egg elongation, which is one of the major morphogenetic events that occur during oogenesis. First, we examined the expression of Mcr in the ovary, and determined that Mcr is expressed in the germline stem cells and follicle cells with the most robust expression in the polar cells. We find that reducing *Mcr* level in the follicle cells beginning in early or mid-oogenesis prevents completion of egg elongation. Interestingly, the ratio of length/width for *Mcr* knock-down egg chambers starts to deviate from the wild-type egg chambers by stage 13, suggesting a requirement for Mcr later in oogenesis. By examining the morphology of the follicular epithelium of late stage egg chambers, we find that the membrane of *Mcr* knock-down follicle cells breaks apart by stage 11-14, resulting in multi-nucleated cells with irregular cell shapes. Also, *Mcr* knock-down follicle cells of early stage 14 egg chambers have reduced levels of actin filaments and βPS-integrin (myospheroid) at the basal sides, which indicates a possible role for Mcr in follicular epithelium maintenance late in oogenesis. Currently, we are using genetics approaches and live imaging experiments to understand the molecular and cellular mechanisms by which Mcr is involved in follicular epithelium maintenance and egg elongation.

470 Modifier screen identifies *Idgf3*-interacting regions that affect Dorsal Appendage formation. *Claudia Espinoza*, Celeste Berg Genome Sciences, University of Washington, Seattle, WA.

The Imaginal Disc Growth Factor (Idgf) gene family comprises six genes, *Idgf1, Idgf2, Idgf3, Idgf4, Idgf5,* and *Idgf6*. Since their discovery in 1995, the Idgfs have been linked to cell growth, cell-shape changes, cell proliferation, fly immunity, bacterial infection, and detoxification. Moreover, their human orthologs, the Chitinases-like proteins (CLPs), are upregulated in a variety of metastatic cancers and inflammatory diseases and play an important role in pathogenesis. Although, the Idgfs and CLPs clearly impact many processes, their mechanism of action is not well understood.
I am using the Dorsal Appendages (DA) of the *D. melanogaster* egg to identify genes in the Idgf pathway. Previously, we found that Idgfs are upregulated in mutants that produce aberrant DAs. Overexpression of the *Idgf3* gene alone moderately and severely disrupts DA formation in nearly 50% of laid eggs. Leveraging these findings, I asked whether partially overlapping deficiency lines that cover chromosome 3L could suppress or enhance the *Idgf3*-overexpression phenotype. By chi-square analysis, I identified 3 regions that suppressed and 4 regions that enhanced the DA phenotype. I followed up on two of the enhancing deficiencies: Df(3L)ED4674 and Df(3L)BSC449 and narrowed down the interacting regions to 5 and 21 candidate genes, respectively. Using available TRiP RNAi lines, I tested 4 of the 5 genes for the first deficiency, *CG9705*, *CG9706*, *CG9674,* and *NudC,* for *Idgf3* interaction. For the second deficiency, I tested the largest genes out of the 21 possible genes, *skd, siz, ko, Ac78c,* and *chb*. None of the tested RNAi lines resulted in a statistically significant interaction. Since RNAi efficacy depends on the level of expression and sequence of the RNAi construct, I am testing additional RNAi lines targeting the same candidate genes. Furthermore, I am narrowing down additional identified deficiencies using other overlapping deficiencies. My ultimate goal is to identify several genes in the *Idgf3* pathway and to determine their molecular mechanism of action. These studies could reveal how the Idgfs and CLPs influence tube formation and other similar processes.

471 Mapping the Interactome of the Planar Cell Polarity Protein Van Gogh by APEX-based Proximity Labeling. *S. Song*[1], L. He[2], K. Suyama[1], N. Perrimon[2,3], J. Axelrod[1] 1) Department of Pathology, Stanford School of Medicine, Stanford, CA; 2) Department of Genetics, Harvard Medical School, Boston, MA; 3) Howard Hughes Medical Institute, Boston, MA.

Planar Cell Polarity signaling (PCP) polarizes cells in epithelial sheets along an axis orthogonal to their apical-basal axis. Tissue-wide establishment of PCP is driven by multiple global cues which guide the dynamic, subcellular enrichment of PCP core proteins, which can self-assemble into mutually exclusive complexes at opposite sides of a cell. This polarization propagates throughout the whole tissue, providing a polarity axis that governs collective morphogenetic events such as the orientation of subcellular structures and cell rearrangements. Reflecting the necessity of polarized cellular behaviors for proper development and function of diverse organs, defects in PCP have been implicated in human pathologies, most notably in severe birth defects, along with conotruncal heart defects, deafness and situs inversus, etc. However, what are the local proteomics establish and maintain the asymmetric localization of PCP core proteins are still quite unclear.

Van Gogh (Vang) is one of the six core PCP proteins in *Drosophila*. We tagged Vang with an engineered peroxidase called APEX2. APEX2 can add biotin tag to Vang nearby proteins within a 5-50nm distance when cells are still alive. When Vang was asymmetrically localized, we activated APEX2 and enriched the biotinylated proteins for Mass-spec to obtain the profile of all the Vang nearby proteins. Then we performed RNAi screen against the listed genes and looked for those genes when knockdown causes PCP phenotypes. We found the protein phosphatase 1 encoding gene *pP1-87B* when knocked down causes typical PCP phenotype, which indicates pP1-87B might be one potential novel regulator in PCP signaling. Currently, we are working on the details of whether pP1-87B directly regulates Vang and/or other PCP core proteins.

472 Regulation of specific *Enhancer of split-HLH* genes by proneural factors shapes Notch output dynamics during bristle patterning in *Drosophila*. L. Couturier[1,2], K. Mazouni[1,2], F. Corson[3], *F. Schweisguth*[1,2] 1) Institut Pasteur, Dept of Developmental and Stem Cell Biology, 75015 Paris, France; 2) CNRS, UMR3738, 75015 Paris, France; 3) Laboratoire de Physique Statistique, Ecole Normale Supérieure, CNRS, Université Pierre et Marie Curie, Université Paris Diderot, 75005 Paris, France.

Adult flies exhibit a stereotyped pattern of sensory bristles on the dorsal thorax. This pattern is produced by a self-organized process in the pupal notum. Cell-cell interactions mediated by Delta-Notch direct the sequential emergence of five stripes of cells expressing the proneural factors Achaete (Ac) and Scute (Sc) and the singling out of Sensory Organ Precursor cells (SOPs) from within each of these stripes. This patterning dynamics depends on a balance between two families of transcription factors: Ac and Sc promote adoption of the neural fate while the Enhancer of split-HLH (E(spl)-HLH) factors act downstream of Notch to antagonize their activity. How this balance is dynamically regulated during patterning is not known. Here, we show that E(spl)mβ-HLH (mβ) and E(spl)m3-HLH (m3) are expressed early, prior to the proneural onset and define where proneural stripes can form. We further find that the *E(spl)md-HLH* (*mδ*) gene is cross-repressed by m3 and/or mb and that md functionally compensates for the loss of m3 and mb in *mb m3* double mutant pupae. Once proneural stripes form, three additional factors, E(spl)m7-HLH (m7), E(spl)m8-HLH (m8) and mδ, become expressed in cells with low/intermediate levels of Ac and Sc and their expression requires Ac and Sc. These late-onset factors are required for the proper spacing of SOPs within each stripe. In contrast, mβ and m3 are detected in cells with no, or low, proneural activity, and their expression does not depend on Ac and Sc. Thus, the proneural-dependent regulation of specific E(spl)-HLH factors allow for cells with intermediate levels of Ac and Sc, which are progressing towards the SOP fate, to increase the number of *E(spl)-HLH* genes responding to Notch. This amplification of the response to Notch by proneural factors contribute to patterning dynamics in the notum and likely operate in other developmental contexts

473 The Hippo Pathway is required for morphogenesis of the pupal eye. *M.W. DeAngelis*, R.I Johnson Biology Department, Wesleyan University, Middletown , CT.

The wing and eye disc are commonly used as model systems to study the importance of Hippo signaling. In contrast we have studied the role of Hippo signaling in the post-mitotic *Drosophila* pupal retina. As expected, Hippo pathway activity does affect cell survival, however when we modify Hippo signaling we

also observe severe defects in retinal patterning that are independent of cell survival. Specifically, cells fail to organize properly and thus do not occupy their correct positions within the tissue when we reduced Mask or Yki or overexpressed Wts. We also observed defects in adherens junction distribution and density, and these defects in adhesion are likely to be the cause of eye mispatterning. To determine the mechanism by which the Hippo pathway regulates adhesion, we performed RNA-sequencing of eye tissue in which Mask activity was increased or decreased. We have identified numerous (direct or indirect) Hippo pathway targets illustrating that Hippo signaling is important not only for control of tissue size, but is central for morphogenesis.

474 How larval tissues and imaginal discs exposed to juvenile hormone follow different developmental fates. *M. Yatsenko*[1,2], P. Novák[1,2], M. Jindra[1,2] 1) Department of Molecular Biology and Genetics, University of South Bohemia in ?eské Bud?jovice, Czech Republic; 2) Biology Center CAS, ?eské Bud?jovice, Czech Republic.

Drosophila larvae harbor distinct cell populations: the larval and the imaginal cells, of which the latter are progenitors of future adult body parts. During metamorphosis, larval tissues are replaced by growing and differentiating imaginal tissues. Metamorphosis is promoted by the steroid ecdysone and inhibited by juvenile hormone (JH), which stimulates larval growth. The JH signaling pathway and its target genes are poorly characterized. While in less evolved insects treatment with JH blocks metamorphosis of larvae or pupae altogether, the main effect of ectopic JH on *Drosophila* is limited to preventing adult differentiation of the abdominal histoblasts. We have found that unlike larval tissues and the abdominal histoblasts, the wing and eye imaginal discs do not express the JH receptor Gce. We hypothesize that the natural absence of functional JH receptors during normal development safeguards imaginal discs from the anti-metamorphic action of JH. Indeed, misexpression of the JH receptor in the wing and eye imaginal discs disrupted the ultrastructure of the disc tissue and led to malformation or loss of the affected adult organs. This effect provided us with an attractive model to identify genes regulated by the JH receptor that are normally silent in the imaginal discs but may be important for developmental signaling in the JH responsive larval tissues or abdominal histoblasts. To this end, we have analyzed differential gene and protein expression in wing discs misexpressing Gce. Using RNA-seq, we have found 224 mRNAs upregulated and 118 mRNAs downregulated upon misexpression of the functional JH receptor relative to control, normally developing wing discs. Among upregulated genes, most represented were structural constituents of larval cuticle; DNA-binding transcription factors were frequently downregulated. The results suggest that the JH anti-metamorphic action involves factors previously not implicated in JH signaling.

475 A feedback mechanism mediated by myosin-dependent accumulation of Rab11-vesicles reinforces apical constriction. *W. Chen*, B. He Department of biological sciences, Dartmouth College, Hanover, NH.

During tissue morphogenesis, cell shape changes driven by mechanical forces often require active regulation of intracellular trafficking to reconfigure geometrical properties and neighbor cell relations. It is not fully understood how intracellular trafficking is regulated by mechanical stimuli and how changes in trafficking in turn impact tissue mechanics. To address these questions, we investigated the behavior of Rab GTPases, the master regulators of intracellular trafficking, during apical constriction-mediated mesoderm invagination in *Drosophila*. We found that during apical constriction, the recycling endosome marker Rab11 undergoes dynamic reorganization and becomes enriched as vesicle-like puncta in the vicinity of apical myosin. The apical accumulation of Rab11 puncta does not require endocytosis but depend on basal-to-apical transport along microtubules. Interestingly, this apical accumulation of the puncta are sensitive to disruption of myosin activity. Rab11 puncta arrived at the apical domain undergo dynamic movement near the apical surface, with a fraction of them being actively recruited to and briefly retained at the adherens junctions. As a result, the distribution of Rab11 puncta along the cell-cell boundaries is nonrandom and is correlated with the position of the adherens junctions. Injection of dominant negative Rab11 proteins inhibits Rab11 puncta formation and results in fragmented apical adherens junctions, suggesting a role of Rab11 puncta in regulating cell-cell adhesion. Meanwhile, apical myosin forms ring-like structure in each constricting cell encircling the apical domain, instead of assembling into a supracellular meshwork as seen in normal embryos. These results echo the previous finding that knocking down adherens junction components results in myosin ring formation. In Rab11-inhibited embryos, the altered junction and myosin organization is associated with a reduced rate of apical constriction. Taken together, our results suggest that myosin contractility induces apical accumulation of Rab11, which serves as a feedback mechanism to facilitate apical constriction. We further propose that this feedback mechanism acts by promoting cargo delivery to the plasma membrane to reinforce the physical connection between adherens junctions and actin-myosin network.

476 Regulation of cell dynamics and tissue remodeling in response to mechanical forces. *M. Bustillo*[1,2], W. Razzell[1], J. Zallen[1] 1) HHMI/Sloan Kettering Institute, New York, NY; 2) Weill Cornell Graduate School of Medical Sciences, New York, NY.

Early morphogenesis in development is characterized by the remodeling of simple epithelial sheets into complex tissues. During body axis elongation in the *Drosophila* embryo, epithelial remodeling is driven by dynamic cell rearrangements that rapidly reshape the tissue while maintaining adhesive cell-cell contacts. Over the course of these cell movements, cells generate and experience a number of physical forces, including forces generated by contraction of the actomyosin network within cells, as well as external pulling forces from neighboring cells and tissues. While the tension experienced by these cells during rearrangement is well documented, the mechanisms by which cells respond to these mechanical forces are less clear. Using quantitative live imaging techniques in the early *Drosophila* embryo, we are exploring the molecular mechanisms by which adherens junction organization and composition are dynamically regulated by tension. Analysis of the localization, dynamics, and activity of proteins that localize to cell interfaces experiencing high tension during *Drosophila* axis elongation will provide insight into how mechanical forces are translated into biochemical signals that influence cell adhesion and epithelial remodeling.

477 The role of non-apical forces in *Drosophila* gastrulation. *A. Goldner*[1], R. Farhadifar[2], D. Needleman[2], K. Doubrovinski[1] 1) Green Center for Systems Biology, University of Texas Southwestern Medical Center, Dallas, TX; 2) Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA.

Embryonic development in animals typically begins with a single-layered ball of epithelial cells. This monolayer undergoes a folding process known as gastrulation to form additional tissue layers. Gastrulation in *Drosophila* begins with ventral furrow formation (VFF): cells along the ventral midline constrict apically, lengthen laterally, and finally shorten back to their original length as the tissue invaginates to form the ventral furrow. While the molecular mechanisms of VFF are widely studied, the physical mechanisms remain unclear. Theoretical analysis by our laboratory strongly suggests that non-apical forces are an important component of invagination. Our models of VFF show that 1) furrow formation requires active tensions in the lateral membranes, and 2) furrow formation succeeds even in the absence of basal membranes. To begin addressing the first prediction, we must determine whether lateral membrane tensions are present in embryos. We ablated the lateral membranes of ventral cells during VFF using a novel two-photon laser setup. Results indicate that lateral membrane tensions are indeed present, and are specifically upregulated in invaginating cells. We are exploring the second prediction through genetically driven RNAi knockdown of anillin, a protein involved in microfilament ring contraction during cellularization. Basal membrane formation in anillin RNAi-treated embryos is significantly delayed, yet these embryos are still capable of VFF. This supports our hypothesis that the expulsion of basal cytoplasm during apical constriction at the onset of VFF generates viscous shear forces that drag the ventral surface inwards by pulling on the lateral membranes. Based on this evidence, we believe viscous shear forces and lateral tensions should be considered among the driving forces behind tissue folding in future models of gastrulation.

478 Investigating the function of Rho1 in early embryogenesis of *Drosophila*. *H. Guo*[1], M. Swan[2], E. Wieschaus[2,3], B. He[1] 1) Department of Biological Sciences, Dartmouth College, Hanover, NH; 2) Department of Molecular Biology, Princeton University, Princeton, NJ; 3) HHMI.

The small GTPase Rho1 plays an important role in morphogenesis by controlling cell polarity, cell adhesion and actomyosin contractility. Studying the function of Rho1 in early embryogenesis of *Drosophila* has however been difficult because Rho1 is provided maternally to the embryo and eliminating maternal contribution of Rho1 inhibits egg development. Here we developed an optogenetic tool that allow us to acutely inhibit Rho1 activity in the embryo. Specifically, we used Cryptochrome2 (CRY2)-CIBN light-sensitive dimerization system to control the recruitment of a dominant negative form of Rho1 (Rho1DN) to the plasma membrane, where it inhibits the activation of endogenous Rho1. During *Drosophila* gastrulation, the mesoderm precursor cells activate non-muscle myosin II at the apical cortex through the RhoGEF2-Rho1-Rok pathway, resulting in apical constriction and subsequent invagination of the cells. Light-mediated membrane recruitment of Rho1DN results in rapid Rho1 inhibition, as evidenced by the immediate (<1 min) loss of apical myosin in the apically constricting cells. By inhibiting Rho1 at different stage of gastrulation, we identified a critical time window during apical constriction, after which inhibition of myosin contractility had little impact on mesoderm invagination. In a separate study, we used this tool to examine the immediate impact of Rho1 inhibition on apical adherens junctions (AJs) in ectodermal cells. AJs first appear as discrete, spot-like junctions (SAJs) during mid-cellularization and subsequently mature into continuous, belt-like zonula adherens (ZAs). Inhibition of Rho1 during cellularization and gastrulation does not prevent the assembly of SAJs; however, the SAJs are localized more basally than normal and never coalesce into ZAs. When Rho1 were inhibited after the formation of ZAs, the continuous, belt-like AJs rapidly reverted back to spot-like morphology, whereas the apical-basal position of the AJs is unaffected. Our results demonstrate that Rho1 activity is required for the formation and maintenance of ZAs but not SAJs. In addition, we revealed a previously unappreciated role of Rho1 in regulating the early phase of apical-basal polarity establishment in *Drosophila* embryos. The optogenetic tool we developed provides a useful approach to determine the stage- and cell-specific function of Rho1 in *Drosophila* development and allows the study of immediate tissue responses to a sudden loss of contractile forces.

479 Insight into the Molecular Mechanisms of Cell Sheet Morphogenesis: A *Drosophila* Deficiency Screen for Genes on Chromosome 2L Involved in Dorsal Closure Using a Live Imaging Approach. *S.M. Fogerson*, R. D. Mortensen, R. P. Moore, H. Y. Chiou, N. K. Prabhu, A. H. Wei, O. Jadi, K. Andoh-Baidoo, D. P. Kiehart Biology Department, Duke University, Durham, NC.

Cell sheet morphogenesis is essential for metazoan development and homeostasis of animal form – it contributes to developmental milestones including gastrulation, neural tube closure, heart and palate formation as well as tissue maintenance through wound healing. Dorsal closure occurs during *Drosophila* embryogenesis and has emerged as a model for cell sheet morphogenesis throughout phylogeny. Closure is a remarkably robust process where conserved gene expression and signaling cascades are coordinated to regulate the cellular machines that drive closure. Many of the approximately 140 known 'dorsal closure genes' were identified by screens scoring for a terminal, dorsal open cuticle phenotype, and new genes are still being identified due to advanced microscopy techniques. Thus, key genes that contribute to the kinematics and dynamics of closure may not have been identified. Here, we extend our previous study of the right arm of the 2nd chromosome (2R, Mortensen *et al*. 2018 G3: Genes|Genomes|Genetics 8:2361) to the left arm of the 2nd chromosome (2L) using the Bloomington 2L deficiency kit, a set of large deletions, which collectively remove 98.9% of the genes on 2L to identify 'dorsal closure deficiencies'. Through two crosses, we unambiguously identify embryos homozygous for each deficiency and time-lapse image cell shapes with ubiquitously expressed E-cadherin GFP for the duration of closure. Confocal images are analyzed for defects in cell shapes and tissue movements. To date we have analyzed 65% of the available deficiencies and identified embryos homozygous for 35 deficiencies with notable, diverse defects in closure. Of these, 23 have no known dorsal closure gene removed by the deficiency region. These include defects in cell shape, canthus formation and tissue dynamics. As with our previous analysis of the 2R, we anticipate further analysis of these 2L deficiencies will lead to the identification of novel 'dorsal closure genes'. Consequently, we expect to identify links between pathways and structures already known to coordinate various aspects of closure as well as new processes and pathways that contribute to closure. Supported by NSF-DGE 1644868 to SMF, SMF and RDM funded by T32 GM007184, and GM033830 and 1R35GM127059-01 to DPK.

480 Probing emergent properties in animal development with synthetic biology. *E. Goyal Gupta*, G.T. Revees Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC.

Gene regulatory networks (GRN) are complex webs of genetic interactions that control gene regulation throughout development and the life of the organisms. GRNs are hypothesized to explain emergent properties of developing tissues, such as robustness and scaling. By studying the functional interactions of these synthetic networks, we can provide a better understanding of these emergent phenomena. Therefore, our goal is to use synthetic biology and computational modeling to study how these properties can arise through the action of GRNs. The study focuses in experimentally testing the behavior of a negative feedback motif in isolation to determine its role in providing robustness. We use the *bcd* 3'UTR to express the yeast transcription activator Gal4 in an AP axis. Further, to create the negative feedback, we will design a transgene that encodes Gal80, an inhibitor of Gal4, which will respond to Gal4 signaling. Robustness of a Gal4-driven marker gene, *lacZ*, will be measured in live and fixed embryos. In conclusion, our work aims to demonstrate how in spatial systems, gene networks can produce very different outputs depending on the relative spatial domain inputs.

481 Force-dependent tendinous ECM remodeling during flight muscle Development. *W. Chu*, X. Sai, S. Hayashi Laboratory for Morphogenetic Signaling, RIKEN BDR, Kobe, JP.

Mechanical tension is critical to the myotendinous system development and maintenance. It has been known that the long-term effect of mechanical loading is required for the formation of the functional tendon-to-bone attachment in the vertebrates. Presumably through modifying the apical extracellular matrix (aECM) composition and physical properties as reported in the in vitro tenocyte culture. However, how mechanical force changes the aECM organization or composition is unclear due to lack of in vivo live imaging. To address this, we focused on the interactions between tendinous aECM proteins and mechanical tension during flight muscle development in Drosophila by live imaging. We found two Zona pellucida (ZP) domain proteins: Dumpy (Dp) and Quasimodo (Qsm) are required to the force-resistant tendon-cuticle attachments during indirect flight muscle development. Tendon cells secrete Dp to form the force-resistant fibers in the extracellular space in between the apical surface of tendon cells and pupal cuticle (as known as exuvial space). These Dp-fibers undergo remodeling in response to the tension status during indirect flight muscle development. The amount of Qsm regulates the rigidity of Dp-fibers. Interestingly, Dp and Qsm are co-localized and transported simultaneously through vesicle trafficking in the tendon cells. However, these two ZP domain proteins show different extracellular distribution patterns and physical properties. Unlike Dp-fibers, Qsm is most likely a soluble protein filled in the exuvial space as a component of molting fluid. Our study provides an excellent model to investigate the "force-dependent aECM remodeling in vivo by live imaging" and attempt to reveal how the aECM composition affects the force-balancing during Drosophila indirect flight muscle development.

482 Nebbish is a critical component of a subnetwork utilized by the Forkhead domain transcription factor Jumeau to regulate cardiac progenitor cell division. *A.J. Kump*[1,2], M. Panta[1,2], S.M. Ahmad[1,2] 1) Biology, Indiana State University, Terre Haute, IN; 2) The Center for Genomic Advocacy, Indiana State University, Terre Haute, IN.

Forkhead (Fkh/Fox) transcription factors (TFs) mediate multiple cardiogenic processes in both mammals and *Drosophila*. Our prior work has shown that the *Drosophila* Fkh gene *jumeau* (*jumu*) mediates three distinct categories of cardiac progenitor cell division—asymmetric, symmetric, and cell division at an earlier stage—by regulating Polo kinase. However, the significant enrichment of Fkh TF binding sites in the enhancers of cardiac genes suggested that *jumu* may be utilizing additional downstream target genes to regulate cardiac progenitor cell division, raising the question of whether these individual

target genes mediated every category of *jumu*-regulated cardiac progenitor cell division or a subset thereof. We used RNA-sequencing to compare genome-wide transcriptional expression profiles of flow cytometry-purified mesodermal cells from wild-type and *jumu* loss-of-function embryos and identified 1,272 putative *jumu* targets. Ongoing phenotypic analysis of a prioritized subset of these downstream targets with amorphic and hypomorphic mutations show that *jumu* transcriptionally activates at least ten other genes involved in cardiac progenitor cell divisions. Two of these genes, *nebbish* (*neb*), which encodes a kinesin, and *scraps* (*scra*), which encodes an anilin, mediate only two of the three categories of *jumu*-regulated cardiac progenitor cell division: symmetric and cell division at an earlier stage. Synergistic genetic interactions between *neb*and *jumu* demonstrate that *neb*is an integral component of a *jumu*-regulated subnetwork mediating cardiac progenitor cell divisions. We are presently investigating the hypothesis that *scra*and a third *jumu*-activated gene we identified, *pavarotti* (*pav*), which encodes another kinesin, may also function in this subnetwork since Pav has previously been shown to physically interact with both Neb and Scra. We are also using genetic interaction assays, epistasis tests, and rescue assays to assess the roles of these three genes in previously identified cardiogenic pathways.

483 Notch activates the expression of different pericardial genes using distinct permissive and instructive mechanisms in order to specify cardiac cell subtypes. *M. Panta*[1,2], A. Kump[1,2], J. Dalloul[1,2], K. Schwab[1,2], S. Ahmad[1,2] 1) Biolgoy, Indiana State University, TERRE HAUTE, IN; 2) The Center for Genomic Advocacy, Indiana State University, TERRE HAUTE, IN.

The development of a complex organ involves the specification and differentiation of diverse cell types constituting that organ. The *Drosophila* heart is comprised of two major cell types: contractile cardial cells (CCs) that constitute an inner tube and pericardial cells (PCs) that form a sheath surrounding the CCs. Our previous work showed that binding sites of Suppressor of Hairless [Su(H)], an integral transcription factor in the Notch signaling pathway, were enriched in the enhancers of genes specifically expressed in the PCs. Using *cis*- and *trans*- assays with enhancer-reporter constructs for a PC-specific gene, *Holes in muscle* (*Him*), we demonstrate that Notch signaling activates *Him* expression in PCs in a permissive manner: in the absence of Notch signaling, Su(H) forms a repressor complex with co-repressors and binds to the *Him* enhancer, repressing its transcription; upon alleviation of this repression by Notch signaling, *Him* transcription is activated. However, using identical approaches with enhancer-reporter constructs for another PC-specific gene, *Zn finger homeodomain 1* (*Zfh1*), we show that Notch signaling activates the expression of *Zfh1* in a distinctly different, instructive manner: mere alleviation of repression by preventing the binding of the Su(H) repressor complex to the *Zfh1* enhancer is not sufficient to activate transcription in PCs. Our results inidcate that, in the case of *Zfh1*, upon Notch signaling, the Notch intracellular domain must bind with Su(H) to change the Su(H) complex bound on the *Zfh1* enhancer from a repressor to an activator complex, and that this activator complex is necessary for bringing about *Zfh1* transcription. Collectively, these data show how the same feature, enrichment of Su(H) binding sites in the enhancers of PC-specific genes, can be utilized by two distinct mechanisms, one permissive, the other instructive, to contribute to the same overall goal: the specification and differentiation of pericardial cell types by activation of the pericardial gene program.

484 Forkhead domain transcription factors restrict the expression of ECM-related genes to mediate proper positioning of cardiac cells. *M. Panta*[1,2], A. Kump[1,2], Y. Chen[3], X. Wang[3], N. Jeffries[3], S. Ahmad[1,2] 1) Biolgoy, Indiana State University, TERRE HAUTE, IN; 2) The Center for Genomic Advocacy, Indiana State University, TERRE HAUTE, IN; 3) National Heart, Lung and Blood Institute, NIH, Bethesda, MD.

The development of a complex organ requires the specification of appropriate numbers of its constituent cell types as well as their correct positioning within the organ. We previously showed that Forkhead (Fkh/Fox) domain transcription factors (TFs) Checkpoint suppressor homologue (CHES-1-like) and Jumeau (Jumu) determine the correct number of different cardiac cell types by regulating cardiac progenitor cell divisions. Here we show that *CHES-1-like* and *jumu* are also required for the correct positioning of these cardiac cell types: null mutations in either gene result in the misalignment and incorrect location of cardial and pericardial cells within individual hemisegments. Using statistical analysis, we demonstrate that the positioning defects are not due solely to steric constraints caused by differing number of cardial cells in contralateral hemisgments as a consequence of cell division defects. To find the downstream targets utilized by these two Fkh TFs to bring about correct positioning, we compared genome-wide transcription expression profiles of purified mesodermal cells from wild-type embryos and Fkh mutants. Among the 2,131 target genes we identified, the heterotrimeric G protein Ggamma1; the extracellular matrix proteins (ECMs) Viking, Collagen type IV alpha 1, and Terribly reduced optic lobes; and their modulator Matrix metalloproteinase 1 were all overexpressed in Fkh mutants. Our preliminary phenotypic analysis of these specific targets suggests that the Fkh TFs bring about the correct positioning of cardiac cell types by restricting their expression: ectopic overexpression of each of these targets in the mesoderm phenocopies cardiac cell positioning defects observed in *CHES-1-like* and *jumu* loss-of-function mutants.

485 A functional screen identifying novel *Drosophila* Egf receptor targets with roles in eggshell structure and morphology. *Z. Walter*, C. Brown, N. Hudock, L. Kadlec Biology, Wilkes University, Wilkes-Barre, PA.

Drosophila epidermal growth factor receptor (Egfr) signaling plays a critical role in many aspects of development including oogenesis, embryogenesis, and proper development of wing and eye tissues. For example, during wing development, Egfr signaling helps specify vein tissues, and in the ovary Egfr signaling is known to establish the body axes during oogenesis. Microarray screens by our lab and others have identified potential downstream transcriptional targets of the Egf receptor using the Drosophila ovary as a model system. Our initial work compared gene expression in fly ovaries where the activity of the Egfr pathway was reduced (gurken mutant), wild-type (OreR), or constitutively active (CY2/λTop). We have employed a number of approaches to further investigate the expression, biological function, and mechanism of action of a subset of putative genes of interest, focusing primarily on genes of previously unknown function. A small-scale functional screen using available collections of UAS-RNAi transgenic flies and P-element insertion lines was used to investigate the possible functions of a group of these novel EGFR-responsive genes. A number of these genes have been found to play roles in normal eggshell structure and morphogenesis. Gene mutant/knockdown phenotypes include decreased chorionic integrity, shortened eggs, and various dorsal appendage malformations and in some cases decreased fertility. Mutant lines created using the CRISPR-Cas9 system have recapitulated the previously observed phenotypes, and will be used for further study and characterization of the genes.

486 Differential mechanisms of Notch activation are used in *Drosophila* spermathecal lineage specification. *W. Shen*[1], J. Sun[2] 1) Physiology and Neurobiology, University of Connecticut, Storrs, CT; 2) Institute for Systems Genomics, University of Connecticut, Storrs.

Asymmetric Notch activation has been utilized in many examples of binary-cell fate determination, which often time relies on asymmetric distribution of ligands or Notch receptor in different cellular systems. It is unknown whether differential modes of asymmetric Notch activation could occur in the same cell lineage. Here we investigated the mechanism of Notch activation in the spermathecal linage. Our previous work showed that one spermathecal gland precursor (P0) divides to give rise to a lumen epithelial precursor (LEP) and a secretory unit precursor (SUP). The SUP divides to give rise to a pIIa and a pIIb. While the pIIa divides to give rise to a basal cell (BC) and a secretory cell (SC), the pIIb differentiates into an apical cell (AC). Notch signaling is asymmetrically activated in the LEP, the pIIb, and the SC to specify their respective cell fate. We also showed that Delta (Dl), but not Serrate (Ser), is involved in asymmetric activation of Notch in the LEP; however, it is unknown how Notch is asymmetrically activated in the pIIb (vs. the pIIa) and the SC (vs. the BC). Using mosaic clone analysis, we found that neither *Dl* nor *Ser* mutant SUP clones affected the fate of ACs, BCs, and SCs. In contrast, *Dl/Ser* double mutant SUP clones switched the SC fate to the BC fate but not affected the AC fate, indicating that Dl and Ser play redundant role in activating Notch in SCs. This data also suggest that Dl and Ser may not be required in activation of Notch in the pIIb/AC, consistent with the result when *Dl/Ser*double mutant clones were generated in P0

cells. However, when *Dl/Ser* double mutant clones were generated in late larval stage, which gave rise to bigger clones, we found that AC fate was blocked. This work suggest that Dl/Ser may be more stable in precursors and multiple divisions are required to dilute the ligand in the mutant clones in order to block the Notch signaling in the pIIb/AC. Furthermore, Numb mutant clones showed a cell fate switch from the pIIa to the pIIb, while not affecting the asymmetric Notch activation in the SC/BC division, implying Numb asymmetrically regulates Notch activation in pIIa/pIIb division. All these data suggest that Dl/Ser may have different stability in different cellular environment and that different mechanisms of asymmetric Notch activation can be used in the cell lineage even only one division away.

487 Hedgehog regulates the rate of Intracellular Smoothened movement. *R. Hatori*, W. Chen, T. Kornberg University of California, San Francisco, San Francisco, CA.

Morphogen signaling regulates many aspects of development and disease. In Hedgehog (Hh) morphogen signaling, Smoothened (Smo), an atypical G-protein-coupled receptor, activates the pathway in response to Hh ligand. We fused fluorescent proteins to three components of the Hh pathway, Hh, Smo, and Cubitus interruptus (Ci), the transcription factor effector of Hh signal transduction, and monitored the dynamics of the tagged proteins expressed at physiological levels. We found an intracellular choreography of Hh signaling that is reflected in basolateral to apical movement of Smo. In cells that contained Hh, most Smo concentrated at basolateral membranes before localizing in apical, Rab7-containing endosomes. The residence time of Smo at basolateral membranes was approximately 4 hours. Not the route but the kinetics of Smo were affected in the absence of Hh, as basolateral residence time was shorter and most Smo was in apical, Rab7-containing endosomes. The fraction of Hh present in Hh-receiving cells was only 5% of Hh produced by Hh-expressing cells, suggesting that small amounts of Hh could induce the change in Smo kinetics. Of the Hh in the receiving cells, those that localize apically were in Rab7-containing endosomes. In addition, a dysfunctional mutant form of Hh distributed apically. These results suggest that basolateral pool of Hh may regulate the kinetics of Smo. These findings identify a novel intracellular route of Smo movement, whose speed is regulated by Hh. Our study suggests that, rather than the bulk distributions, the intracellular choreography of signaling proteins could serve as a direct readout of signal activation.

488 Wnk and Fray signaling in *Drosophila* . *P. Yarikipati*, A. Jenny Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY.

WNK kinases are serine-threonine protein kinases characterized by the lack of a highly conserved lysine in kinase subdomain II that usually mediates ATP binding and the catalysis of phosphoryl transfer in most other kinases. WNK kinases are well known regulators of Na+/K+/Cl- cotransporters (CCCs) controlling ion reabsorption in the kidney in mammals and the Malpighian tubules in *Drosophila*. They mostly act by phosphorylating the redundant downstream targets, SPAK (STE20/SPS1-related proline–alanine-rich kinase) and OSR1 (oxidative stress-responsive protein type 1) which in turn are stimulated by Mo25. Through a Dsh gel-shift-based screen, we have previouslyidentified a conserved role for Wnk kinase as a novel regulator of the canonical Wnt pathway. Wnt signaling plays an essential role for the development of organisms by controlling cell proliferation and polarization. Abnormalities in Wnt signaling lead to severe diseases and cancer.

We find that Wnk acts though Fray, the single fly homolog of OSR1/SPAK during the development of the fly wing. Mutants of *wnk*,*fray*, and mo25 are homozygous lethal but the cause for their lethality is unknown. Furthermore, it is unknown whether all functions of Wnk in *Drosophila*are Fray dependent. Consistent with mammalian cell culture results, Wnk interacts with Fray via a RF(x)Φ motif *in vitro*. However, using Crispr mediated genome editing *in vivo*, we found to our surprise that this motif is not required for viability. We are currently exploring the reason for this discrepancy. Using a modifier screen, we furthermore are in the process of identifying factors mediating cell size regulation by Wnk in wing cells to better understand how the Wnk pathway acts during development.

489 Evolution and functionality of *Wnt9 (DWnt4)* and *Wnt10* in *Drosophila melanogaster*. *Franziska Anni Franke*[1], Marisa Rodrigues[2], Amber Harper[1], Michaela Holzem[1], Shamma Rattan[1], Thomas Flatt[2], Alistair P. McGregor[1] 1) Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, United Kingdom; 2) Department of Biology, University of Fribourg, Fribourg, Switzerland.

Wnt signalling regulates many biological processes during animal development, including cell fate specification, division and patterning. There are thirteen subfamilies of Wnt ligands in metazoans, seven of which are represented in *Drosophila melanogaster*. Although some Wnt genes appear to have unique roles in *Drosophila*, it is thought that they can act cooperatively in Wnt landscapes in some contexts. In contrast to most *Drosophila Wnt* genes, the function of *Wnt10* is unknown, although expression of this gene is observed in the visceral, head and somatic mesoderm, gut and central nervous system during embryogenesis. We used CRISPR/Cas9 to mutate *Wnt10* but these flies appear to be fully viable and fertile with no obvious phenotypes under normal culture conditions. However, abnormal gut development can lead to the absence of microbes and hence immune deficiency since there is a known link between gut microbiota and animal health. Therefore, we have performed immune and lifespan assays on $Wnt10^{KO}$ flies. Interestingly, we observed that $Wnt10^{KO}$ mutant flies show a higher mortality after the infection of a gram-positive bacterium than controls. Further tests will show if there is differential expression levels of various AMPs after the infection of the $Wnt10^{KO}$ flies and controls. To further test the function and molecular specificity of *Wnt10* compared to the related gene *Wnt9* (*DWnt4*), we have used CRISPR/Cas9 to generate mutants of these genes with an integrated attp site. This allows the insertion of the coding region of another *Wnt* gene at the endogenous locus to test if *Wnt9* and *Wnt10* can be replaced by each other in regard to their roles. Since it is unlikely that one Wnt gene can completely functionally replace another Wnt gene, we will also introduce chimeric *Wnt9-Wnt10* ligands to identify domains responsible for the diversification of two Wnt ligands. Identifying the molecular basis of functional differences among Wnt ligands will help to better understand how this important signalling system regulates development and other processes.

490 Investigating how linker phosphorylations control Smad activity. N. Poole, V. Muradyan , *E. Eivers* Biological Sciences, California State University Los Angeles, Los Angeles, CA.

The transforming growth factor beta (TGF-β) signaling superfamily has been found to be critically involved in a multitude of biological processes from embryonic tissue specification at the beginning of an organism's life, to maintenance of tissue homeostasis in the adult. This family of proteins can be further divided into the bone morphogenetic protein (BMP) and Activin/TGF-β sub-families. The significance of this signaling superfamily is most evident in the array of diseases and developmental defects associated with dysregulated signaling in both pathway branches. The goal of this project is to investigate how TGF-β signals in rapidly dividing *Drosophila* tissues are fine-tuned at the level of Smad linker phosphorylations. Previous work has shown that phosphorylation of BMP Smad/Mad linker serines initiates a sequence of cellular events resulting in degradation of this transcription factor by the proteasome, thus revealing a cellular mechanism to control the duration of BMP signals. Here we present data identifying new roles for Smad linker phosphorylations in regulating TGF-β signals. In conclusion, our experiments aim to expand our knowledge of the signaling activities of the TGF-β pathway, by advancing our understanding of the consequences of phosphorylating Smad's linker domain.

491 *TM2D* genes in Notch signaling and Alzheimer's disease. *J.L. Salazar*[1], D. Li-Kroeger[1], O. Kanca[1], J.M. Harnish[1], S. Yamamoto[1,2,3] 1) Department of Molecular and Human Genetics, Baylor College of Medicine (BCM), Houston, TX; 2) Graduate Programs in Developmental Biology and Neuroscience, BCM, Houston, TX; 3) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX.

Genomics studies are revealing new human variants associated with Alzheimer's disease (AD) risk. In collaboration with the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) consortium, we identified a rare coding variant in *TM2D3* that increases the risk of late-onset AD (LOAD). The *Drosophila TM2D3* ortholog, *almondex* (*amx*), was one of the earliest identified neurogenic genes along with *Notch*, *Delta*, and other core Notch signaling pathway genes. While *amx* mutant flies develop normally to adulthood, all progeny of homozygous mutant females exhibit a strong neurogenic phenotype due to lateral inhibition defects during embryogenesis. We showed that wild-type human *TM2D3* can significantly suppress this phenotype of *amx* mutants while the LOAD associated variant could not (Jakobsdottir et al., 2016). An earlier epistasis study has suggested that *amx* acts at the S3 cleavage step of the Notch receptor, mediated by the γ-secretase complex. Considering the role of γ-secretase in processing the Notch receptor and in generating amyloid plaques found in AD patient brains, we hypothesize that Amx and other TM2D family members modulate γ-secretase activity.
To study the precise role of the TM2D proteins, we are generating genetic tools to dissect their roles *in vivo*. Interestingly, null mutant adults of *amx* exhibit a shortened lifespan, as well as age dependent climbing defects and decreased electroretinogram amplitudes suggestive of neuronal dysfunction. We are currently knocking out *Drosophila TM2D1 (CG10795)* and *TM2D2 (CG11103)* to further generate double and triple mutants of *TM2D* family genes to uncover potential functional redundancy between the three genes. Moreover, we found that Amx lacking the extracellular domain functions as a putative dominant negative protein that inhibits Notch signaling in several developmental contexts when overexpressed. Truncated Amx causes accumulation of Notch, similar to what we observe when γ-secretase is knocked down. These tools will help us to begin to understand the role of these proteins in Notch signaling and AD pathology.

492 HDAC1 regulates Notch signaling during Drosophila wing development . *J. Lyu*[1,2], Z Wang[1,2,3] 1) Institute of Genetics and Department of Genetics,Zhejiang University School of Medicine,Hangzhou,Zhejiang,China; 2) Division of Human Reproduction and Developmental Genetics,The Women's Hospital,Zhejiang University School of Medicine,Hangzhou,Zhejiang,China; 3) College of Life Sciences,Zhejiang University,Hangzhou,Zhejiang,China.

The Notch signaling pathway is highly conserved across different animal species and plays crucial roles in development and physiology. Regulation of Notch signaling occurs at multiple levels in different tissues and cell types. Here, we show that the histone deacetylase HDAC1 acts as a positive regulatorof Notch signaling during *Drosophila* wing development. Depletion of *HDAC1* causes wing notches on the margin of adult wing. Consistently, the expression of Notch target genes is reduced in the absence of HDAC1 during wing margin formation. We further provide evidence that HDAC1 acts upstream of Notch activation. Mechanistically, we show that HDAC1 regulates Notch protein levels by promoting Notch transcription. Consistent with this, the HDAC1-associated transcriptional corepressor Atrophin (Atro) is also required for transcriptional activation of Notch in the wing disc. In summary, our results demonstrate that HDAC1 positively regulates Notch signaling and reveal a previously unidentified function of HDAC1 in Notch signaling.

493 Characterization of Mib2 as a regulator of JAK/STAT signaling in border cell migration. *S. Trivedi*[1], M. Starz-Gaiano[2] 1) Biological Sciences, University of Maryland, Baltimore County, Baltimore, MD; 2) Biological Sciences, University of Maryland, Baltimore County, Baltimore, MD.

Collective cell migration is an essential part of many developmental processes and disease pathology, where a group of cells coordinates their signaling and movements to translocate as a cohort. Border cell migration in the Drosophila egg chamber stands as an excellent model to study collective cell migration. We identified *mindbomb2 (mib2)* as a regulator of border cell migration in egg chambers. Mib2 is an E3 ubiquitin ligase involved in Drosophila embryo muscle maintenance via Myosin regulation and a negative regulator of Signal Transducer and Activator of Transcription (STAT) activity in Drosophila cell culture. We found that *mib2* loss of function in border cells results in their delayed migration. Expression analysis by antibody supports Mib2 having a role in border cell cluster. We used domain deletion mutants to investigate how Mib2 protein structure impacts its function in border cells. Since Janus Kinase (JAK)/STAT signaling is required to specify and sustain the migratory behavior of border cell cluster, we also performed genetic interaction tests to determine Mib2's relationship with JAK/STAT signaling components. We propose that Mib2 is a JAK/STAT signaling regulator in border cells and hypothesize it may function more broadly in collective cell migration regulation.

494 Follicular tumor hotspots in *Drosophila* are determined by JAK-STAT signaling. *D. Chatterjee*, A Jevitt, T Otwell, W.M. Deng Florida State University, Tallahassee, FL.

Extensive studies in the *Drosophila* follicular epithelium provide us with powerful tools to investigate some of the earliest steps in tumorigenesis. In previous publications from our lab, we have discovered a collaborative role of the endogenous JAK-STAT signaling and tissue morphology to determine tumorigenic regions in the wing imaginal disc and the salivary gland imaginal ring tissues known as the tumor hotpots. In our studies of the follicular epithelium, we have seen similar occurrences of tumor hotspots in the terminal regions of the egg chambers. The follicular tumor hotspot phenotype is also produced in egg chambers when apicobasal polarity, cytoskeletal regulation and endosomal trafficking are disrupted, thus indicating towards a universal tumorigenic mechanism. We have disrupted the epithelial polarity determining gene *lgl* to generate tumors in the follicular epithelia. Using both tissue specific RNAi-mediated knockdowns and loss of function mutants of *lgl*, JAK-STAT dependent multilayered overgrowth is observed in the otherwise monolayered epithelia that exhibits a progressively severe phenotype over time. This multilayered growth is provided with a growth advantage due to the loss of Notch signaling mediated repression of mitosis and the growth promoting endogenous JAK-STAT signaling. We have established a deterministic role of JAK-STAT pathway components in establishing hotspot specific growth in the follicular epithelia with loss of Lgl function and is sufficient to induce growth in the coldspot regions where loss of function of Lgl alone is not sufficient to induce multilayering. Using confocal imaging, we have also shown that the multilayered cells have disrupted polarity, disrupted cell fate and seem to undergo apical delamination to invade into the germline. Our data thus suggests towards the novel occurrence of tumor hotspots in an adult tissue and aims to delineate the regulation of the tumorigenic growth in the follicular epithelia.

495 Wound-induced polyploidization is dependent on Intergrin-Hippo signaling. *R.S. Besen-McNally*, K.J. Gjelsvik, V.P. Losick MDI Biological Laboratory, Bar Harbor, ME.

A key step in tissue repair is to replace cells that have been lost or damaged by injury. One strategy occurs by restoring cell number through proliferation and another occurs by increasing cell size through polyploidization. Studies in *Drosophila* and vertebrate tissues have demonstrated that polyploid cells arise in adult tissues, at least in part, to promote tissue repair and restore tissue mass. However, the signals that cause polyploid cells to form in response to injury remain poorly understood. In the adult *Drosophila* epithelium, polyploid cells are generated by both cell fusion and endoreplication resulting in a giant polyploid syncytium that is essential for wound repair. Here we identify the β1-Integrin, Myospheroid (Mys), as an activator of wound-induced polyploidization. Mys is upregulated 2.5 fold in the wound-induced polyploid cells and epithelial specific knockdown results in a significant defect in endoreplication. In addition, we found that Mys signals through the Hippo pathway to regulate Yki targets, including *myc*. In conclusion, we found that integrin-mediated mechanotransduction is critical to initiate polyploid cell growth.

496 The Fat-regulated adaptor protein Dlish is palmitoylated and binds the growth suppressor Expanded, controling its stability and ubiquitination. *S.S. Blair*[1], X. Wang[1,2], Y Zhang[1,3] 1) Dept Integrative Biology, Univ Wisconsin, Madison, WI; 2) Beijing Key Laboratory of Biodiversity and Organic Farming, College of Resources and Environmental Sciences, China Agricultural Univ, Beijing, China; 3) Hunan Province Key Laboratory for Integrated Management of the Pests and Diseases on Horticultural Crops, Hunan Univ of Science and Technology, Hunan, China.

The *Drosophila* protocadherin Fat controls organ size through the Hippo pathway, but the biochemical links to the Hippo pathway components are still poorly

defined. We previously identified Dlish, a novel SH3 domain protein that physically interacts with Fat and the type XX myosin Dachs, and showed that Fat's regulation of Dlish levels and activity helps limit Dachs-mediated inhibition of Warts and thus Hippo pathway activity. We will first present results showing that Dlish can be palmitoylated at multiple sites, providing a mechanism for the cortical recruitment of the Dlish/Dachs complex and its sensitivity to the palmitoyltransferase Approximated. Next, we characterize a parallel growth control pathway downstream of Fat and Dlish. Using immunoprecipitation and mass spectrometry to search for novel Dlish partners, we find that Dlish binds the FERM domain growth repressor Expanded (Ex); Dlish SH3 domains directly bind sites in the Ex C terminus. We further show that, in vivo, Dlish reduces the subapical accumulation of Ex, and that loss of Dlish blocks the destabilization of Ex caused by loss of Fat. Moreover, Dlish can bind the F-box E3 ubiquitin ligase Slimb and promote Slimb-mediated ubiquitination of Expanded in vitro. Both the in vitro and in vivo effects of Dlish on Ex require Slimb, strongly suggesting that Dlish destabilizes Ex by helping recruit Slimb-containing E3 ubiquitin ligase complexes to Ex.

497 NF-κB Shapes Metabolic Adaptation by Attenuating Foxo-mediated Lipolysis in Drosophila. *M. Molaei*, C. Vandehoef, J. Karpac Department of Cellular and Molecular Medicine,Texas A&M University Health Science Center, College Station, TX, USA.

Metabolic and innate immune signaling pathways have co-evolved to elicit coordinated responses. However, dissecting the integration of these ancient signaling mechanisms remains a challenge. Using *Drosophila*, we uncovered a role for the innate immune transcription factor NF-κB/Relish in governing lipid metabolism during metabolic adaptation to fasting. This adaption requires organisms to properly balance lipid catabolism in order to modulate energy homeostasis. We found that NF-κB/Relish is required to restrain fasting-induced lipolysis, and thus conserve cellular triglyceride levels during metabolic adaptation, through specific repression of ATGL/Brummer lipase gene expression in adipose (fat body). Fasting-induced changes in Brummer expression and, consequently, triglyceride metabolism are adjusted by NF-κB/Relish-dependent attenuation of Foxo transcriptional activation function, a critical metabolic transcription factor. NF-κB/Relish limits Foxo function by influencing fasting-dependent histone deacetylation and subsequent chromatin modifications within the Bmm/ATGL locus. These results highlight that the antagonism of NF-κB/Relish and Foxo functions are crucial in the regulation of lipid metabolism during metabolic adaptation, which may further influence the coordination of innate immune-metabolic responses.

498 Dissecting domain function in Drosophila PLC-γ. *J.R. Thackeray*, E. Cojocaru, C. Naidu Biology Department, Clark University, Worcester, MA.

The Drosophila genome contains a single gene encoding a phospholipase C-γ homolog, *small wing* (*sl*). PLC-γ proteins are activated by receptor and non-receptor tyrosine kinases; in Drosophila this includes the epidermal growth factor receptor EGFR and the insulin receptor InR. Previous work in ours and other labs has shown that SL normally acts as a promoter of the activating signal from InR, whereas it acts as a negative regulator of EGFR. In addition to the phospholipase cataytic X & Y domains the protein contains several other motifs common to other signaling proteins, such as PH, EF hands, SH2, SH3 and C2. In addition, a tyrosine site essential for PLC-γ activation (via phosphorylation) has been identified in mammalian homologs. We are investigating the function of these domains/sites, using various modified genomic rescue constructs that contain the *sl* gene with point mutations designed to disrupt the functioning of single domains. We use wing blade area as a measure of signaling thru the insulin receptor, and wing venation and photoreceptor development in the adult eye to measure the effectiveness of the mutated protein on EGFR signaling. Our results suggest that the N-terminal PH and SH3 domains participate in distinct aspects of the SL-derived signal, based on complementation seen when constructs missing each domain are present in the same animal. We also find that, although PLC-γ lacking the presumed phosphorylated tyrosine is largely ineffective, they phenotypically less affected than their siblings lacking all *sl*-function.

499 Deltex at the crossroad of Notch and JNK signaling in *Drosophila*. *Debdeep Dutta*, Mousumi Mutsuddi, Ashim Mukherjee Department of Molecular & Human Genetics, Institute of Science, Banaras Hindu University, Varanasi, Uttar Pradesh, IN.

Successful cellular communication is an imperative milestone during the course of evolution from the unicellular to multicellular organism. Evolutionarily conserved cell signaling pathways, like Notch and JNK, regulate a variety of cellular events including cell fate determination, cellular differentiation, proliferation, and cell death to coordinate the developmental events. Deltex (Dx), an evolutionarily conserved cytoplasmic interactor of Notch, acts as a context-dependent-regulator of Notch signaling activity. In the present study, Dx was observed to persuade JNK-induced cell death in a dosage-dependent manner. It was also noticed that Dx can alter the activities of Eiger, the sole *Drosohpila* TNF homolog. Eiger-mediated JNK activation and cell death were found to be modulated according to the dosage of Dx. Using immunocytochemical techniques, we demonstrated that these two proteins colocalize in the cytoplasm, and the *in-vitro* GST pull-down assay indicated a possible physical interaction between these two proteins. Genetic interaction among the alleles of these two genes signifies their functional implication on wing development. Further observations suggest that Dx might help in endocytic transport of Eiger into the cytoplasmic vesicle to facilitate the signaling activities of Eiger. Additionally, our data showed that Eiger might negatively influence Notch signaling outcome. Dx was found to enhance the negative effect of Eiger over Notch pathway. In a further endeavor to investigate the molecular mechanism, we found that Notch gets colocalized with Eiger in the cytoplasm during Eiger overexpressed condition. Also, it was detected that in Dx and Eiger coexpressed condition, all three proteins namely Notch, Dx, and Eiger were colocalized in the cytoplasm. Thus, we rationale that these vesicle-like entities might serve as a site for retaining Notch to block its activity. However, our genetic analyses indicated that the Eiger-mediated JNK activation and downregulation of Notch signaling are probably two independent events that contribute their own share in the manifestation of the observed phenotypes. Altogether, our study establishes the involvement of Dx as a modulator of both Notch and Eiger-mediated signaling activities and the subsequent cell death.

500 Retinoid signaling as a Rhodopsin-1 quality control mechanism in *Drosophila melanogaster*. *B. Brown*, H.W. Huang, H.D. Ryoo Department of Cell Biology, New York University School of Medicine, New York, NY.

Proteins that are destined for the cell membrane must transit through the secretory pathway, beginning with their proper folding in the endoplasmic reticulum (ER). Excessive burden on the protein folding system of the ER contributes to a variety of human diseases, including retinitis pigmentosa (RP), a progressive retinal degenerative disorder most frequently caused by folding-defective mutations in the membrane protein Rhodopsin. These conditions are often age-related, perhaps because young cells readily activate various quality control mechanisms that reduce the levels of misfolded proteins in the ER. Our laboratory utilizes a fly model of RP to identify and characterize new regulators of protein quality control. We have recently discovered that a serine carboxypeptidase, *highroad*, is required for photoreceptor (PR) cells to efficiently degrade misfolded Rhodopsin-1 (Rh-1). Furthermore, our data suggest that *highroad* expression is regulated by cellular retinoid levels. The current understanding of the role of retinoid-regulated transcriptional events in *Drosophila* is limited, and to that end we aim to identify the mediators of *highroad* induction and other transcriptional outputs of this process. We are currently working to characterize a putative retinoid-binding protein (RBP) in *Drosophila* and determine its role in facilitating the quality control response to misfolded Rh-1. Preliminary data suggest that this putative RBP is required for retinoid-mediated induction of *highroad*. *In silico* analysis further suggests that this RBP is capable of binding a retinoid species, which we are testing through *in vitro* binding assays. Our studies hope to uncover a previously unknown role for retinoids and RBPs in *Drosophila* signal transduction.

501 Suppression of store-operated calcium entry components *dStim* and *dOrai* results in dilated cardiomyopathy. *C. E. Petersen*[1], M.J. Wolf[2], J. T. Smyth[1] 1) Uniformed Services University of the Health Sciences, Bethesda, MD; 2) University of Virginia School of Medicine, Charlottesville, VA.

Irregularities in calcium (Ca^{2+}) homeostasis greatly contribute to the pathogenesis of cardiac disease, and despite significant advances in treatment, cardiac disease continues to be a leading cause of morbidity and mortality throughout the western world. The strong correlation between disrupted Ca^{2+}handling and

heart disease highlights a pertinent need for further information regarding Ca^{2+} signaling pathways in the heart. The store operated Ca^{2+} entry (SOCE) pathway, whereby Ca^{2+} influx is triggered upon depletion of endo/sarcoplasmic reticulum (ER/SR) stores, is an essential component in the regulation of intracellular Ca^{2+} homeostasis. Recently, our lab has identified a requirement for key components of the SOCE pathway, dStim and dOrai, in *Drosophila* cardiac physiology. The comparable contractile structures and strong genetic conservation of single *dStim* and *dOrai* isoforms with human allow for targeted combinatorial genetic manipulation with meaningful comparison to vertebrate models. Using this approach, we found that heart-specific suppression of *dStim* and *dOrai* induced dilated cardiomyopathy characterized by enlarged end-diastolic and end-systolic diameters and decreased fractional shortening, suggesting an essential role for SOCE in normal heart physiology. In further support of this, SOCE suppressed animals died significantly earlier than controls. Stim and Orai suppressed hearts also exhibited highly disorganized or disrupted myofibrils, indicating that impaired cardiac function may arise due to defects in heart development, tissue remodeling, or degeneration caused by disrupted Ca^{2+} homeostasis. In support of a developmental role, animals with heart-specific *dStim* and *dOrai* suppression pupated and eclosed significantly later than controls. We are currently analyzing Ca^{2+} transients in intact larval and adult hearts to determine whether SOCE is required for contractile Ca^{2+} cycling or may have a contraction-independent signaling role involved in heart development or tissue homeostasis. Collectively, our results demonstrate an essential role for SOCE in normal cardiac physiology, and present how powerful genetic tools and *in vivo* analyses in *Drosophila* will allow us to define the mechanistic basis for this requirement.

502 An *in vivo* model of calcium signaling specificity dependent on direct association of *Drosophila* Orai calcium channels with calmodulin. *D.S. Karabasheva*, J.T. Smyth Uniformed Services University of the Health Sciences, Bethesda, MD.

Store-operated Ca^{2+} entry is a major mechanism of Ca^{2+} signaling and homeostasis in eukaryotic cells. SOCE is activated when endoplasmic reticulum (ER) Ca^{2+} stores are depleted, resulting in Ca^{2+} influx into the cell across the plasma membrane. The SOCE mechanism involves Stim proteins that function as Ca^{2+} sensors in the ER, and Orai proteins that are Ca^{2+} influx channels in the plasma membrane. A major unresolved question regarding the SOCE pathway is whether its primary function is to refill ER Ca^{2+} stores and maintain cellular Ca^{2+} homeostasis, or whether it mediates specific signaling responses independent of other Ca^{2+} mobilizing pathways. Human patients with loss of function mutations in Stim and Orai isoforms have specific disease presentations involving immunodeficiency and skeletal myopathy, supporting a role for SOCE in specific signaling paradigms. However, the molecular basis for how SOCE signals to specific Ca^{2+} response elements is poorly understood. It was recently demonstrated in tissue culture cells that human Orai1 directly associates with the major Ca^{2+} effector molecule calmodulin (CaM), and that this may underlie SOCE signaling specificity. We are currently testing this possibility *in vivo* using *Drosophila*, which express single Stim and Orai (*dStim* and *dOrai*) isoforms. Loss of function *dStim* and *dOrai* fly mutants are early larval lethal and surviving larvae fail to grow beyond first instar size, demonstrating essential functions for SOCE in flies. These phenotypes are rescued by *dOrai* transgenes. We have found that like human Orai1, dOrai from S2 cell extracts directly associates with CaM. This association is Ca^{2+} dependent, and mutations in the putative CaM binding site of dOrai disrupt the interaction. We have generated flies that express CaM binding site *dOrai* mutant transgenes, and will test whether these transgenes can rescue loss of function mutants to the same extent as the wildtype transgenes. Lack of full rescue would suggest specific SOCE signaling mechanisms that operate through associated CaM, and would set the stage for tissue and cell type specific analysis of SOCE functions signaling.

503 An evidence-based model for representing signaling pathways in FlyBase. *H. Attrill*, G. Antonazzo, N.H. Brown, FlyBase Consortium Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, Cambridgeshire, UK.

Research in *Drosophila melanogaster* was central to the discovery and elucidation of many key signaling pathways and continues to be a very active area of research in *D. melanogaster*, resulting in the identification of many new players and the molecular dissection of pathway interactions. There are many resources that present pathway models or images. These resources often represent a one-pass curation effort that, although often useful for representing the core pathway, are static and do not reflect the richness of published experimental data. FlyBase, the primary knowledgebase for *D. melanogaster* genetic research, has examined how it can capture and present up-to-date, comprehensive data on pathways; providing an alternative representation to the text-book view.

As many signaling pathways have been extensively characterised in *D. melanogaster*, we opted for a model in which the weight of the supporting experimental evidence-base is documented for each pathway member. A first pass curation of all major signaling pathways is being conducted - the pathway, evidence and source publication are captured by annotation using the Gene Ontology. These data are presented in dedicated Signaling Pathway pages which will be added to as part of on-going paper curation at FlyBase, allowing them to be continually updated. Here we illustrate how the experimental-evidence model can be used to show the support linking a gene to a pathway. We also show that using this more complete set of data, we can track how specific a component is to a given pathway and how this data can be further used to elucidate the role of pathways in larger processes. Ultimately, by using an experimentally driven representation of pathways and linking to the numerous other data types in FlyBase, the Signaling Pathway pages will provide a platform for discovery and aid hypothesis-driven research in a fast-moving field.

504 Select septate junction proteins direct ROS-mediated regulation of cardiac function in Drosophila. *H. Lim*[1], W. Wang[2], Y. Liu[1], H. Bao[1] 1) PHYSIOLOGY, UNIVERSITY OF OKLAHOMA HEALTH SCIENCES, OKLAHOMA CITY, OK; 2) INTERNAL MEDICINE, SECTION OF ENDOCRINOLOGY, UNIVERSITY OF OKLAHOMA HEALTH SCIENCES, OKLAHOMA CITY, OK.

Septate junction (SJ) complex proteins act in unison in the epithelia to provide a paracellular barrier and to maintain structural integrity. Here, we identify a novel unexpected role of two individual SJ proteins as signaling regulators of cardiac physiology. Reactive oxygen species (ROS)-p38 MAPK signaling in the non-myocyte pericardial cells (PCs) are important in maintaining normal cardiac physiology in Drosophila. However, the underlying mechanisms remain unknown. We report here that in PCs, two members of the multi-protein SJ complex, Coracle (Cor) and Kune-kune (Kune), are altered in abundance in response to manipulations of pericardial ROS-p38 signaling in PCs on proper myocardial function regulation. We further determine that Cor regulates normal Kune abundance in the PCs which in turn maintains normal cardiomyocyte Kune level that is essential for proper cardiomyocyte function. Our results reveal select SJ proteins Cor and Kune as signaling mediators of the PC-derived ROS regulation of cardiac physiology.

505 New signaling intensity-dependent regulation of the MAPK pathway revealed through an oncogenic KRAS *Drosophila* model. *J.K. Sawyer*[1], Z. Kabiri[1], R.A. Montague[1], S.V. Paramore[1], E. Cohen[2], C.M. Counter[1,3], D.T. Fox[1,2,3] 1) Department of Pharmacology & Cancer Biology, Duke University Medical Center, Durham NC; 2) Department of Cell Biology, Duke University Medical Center, Durham NC; 3) Duke Cancer Institute, Duke University Medical Center, Durham NC.

KRAS is the most frequently mutated oncogene in cancer. Paradoxically, recent work in mammalian systems revealed that rare codons lead human *KRAS* to produce less protein, activate less MAPK signaling, and to limit tumorigenicity as compared to other human Ras genes. These findings imply that, during tumor progression, KRAS modifying regulation enables *KRAS* to overcome the limits of rare codons. Work in *Drosophila* has identified numerous Ras/MAPK modifiers, but has never taken into account the cancer-relevant property of rare codons uniquely present in *KRAS,* because the single fly *Ras* gene (*Ras85D)* has a high percentage of common codons. Therefore, we developed a *Drosophila* model to identify K-Ras-specific modifiers. To do so, we created *Drosophila Ras* UAS transgenes with constitutively-active *G12V* mutations, with either all common (Ras^{CV12}) or all rare (Ras^{RV12}) codons. Both transgenes produce the classic rough

eye phenotype when driven by *sevenless-Gal4. Ras^{CV12}* eye phenotypes are ~2 fold stronger than Ras^{RV12}, which, as in mammals, mirrors observed differences in Ras protein levels. Having established Ras^{RV12} as the first *KRAS*-like *Drosophila* expression system, we then conducted a genome-wide haploinsufficiency screen using the Bloomington Deficiency Kit to identify modifiers that are unique to low-level Ras/MAPK signaling. Out of 470 deficiencies, we identified 15 deficiencies that reproducibly act to differentially modify either Ras^{CV12} or Ras^{RV12}, the majority of which enhance Ras^{RV12}. Our screen and follow-up genetic studies identified the small ribosomal subunit *Rps21* as a modifier of low-intensity Ras/MAPK signaling. Further biochemical characterization in cultured fly cells and in ovaries revealed that Rps21 is negative regulator of endogenous MAPK signaling. Finally, experimental titration of Ras signaling reveals that new Ras regulators, such as Rps21, become visible in phenotypic assays in a signal intensity-dependent manner. Our studies highlight the importance of signaling intensity as a critical component of screens for Ras modifiers. Taking intensity into account could reveal novel targets in signaling-driven tumor growth.

506 Translation, rather than transcription, is required for the initial steps of single cell wound repair. *A. Dominguez*, M. Nakamura, J. Verboon, MT Abreu-Blanco, R. Liu, J. Delrow, S. Parkhurst Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA.

On a daily basis, cells are exposed to a combination of mechanical and/or chemical stressors that compromise the integrity of the plasma membrane and underlying cortex, demanding the cell to rapidly deploy a repair mechanism or perish. This repair response is immediate, requiring each stage to occur with spatial and temporal precision in order to ensure a cohesive wound healing process. Early studies have shown that the presence of Ca^{2+} is indispensable for initiating single cell wound repair, but the early response signals of the cell that rely on this Ca^{2+} influx remain unclear. Initial response signals were proposed to be primarily transcriptional as shown by the increased expression of AP-1, a transcription factor responsible for driving the expression of cytokines and growth factors implicated to have roles in wound repair. Using the early *Drosophila* embryo, we show that while transcription does play a role in the later stages of wound repair, it is not necessary immediately after wounding. On the other hand, we find that translational activity is imperative to initiate normal wound repair dynamics, suggesting the presence of a readily available pool of mRNA and protein. Furthermore, we identified 255 genes by changes in their expression in response to laser wounding. Amongst these, we screened the fifteen most up- and the sixteen most down-regulated genes, all of which show impaired wound healing dynamics after being knocked down, suggesting a vital role in wound repair. Interestingly, we have also identified ImpL2 and Thor, terminal components of the insulin signaling (IIS) pathway, amongst the most up-regulated genes in response to wounding. We have examined other components of the IIS pathway, a number of which are recruited to the wound, further substantiating the link between cell wound repair and insulin signaling. Thus, our screen provides a foundation to explore the network of cellular pathways that may be concurrently involved in directing a stable and processive wound repair response and could serve as a basis of future work for studying diseases linked to impaired wound healing.

507 The kinesin-like protein Pavarotti functions non-canonically to regulate actin dynamics during during wound repair. *M. Nakamura*, J. Verboon, S. Parkhurst Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA.

Single cells composing tissues and organs are subjected to damage caused by daily wear and tear and environmental/physiological stresses. To survive this damage and remain functional, cells have a robust repair mechanism comprised of rapid membrane resealing/remodeling and dynamic cortical cytoskeleton repair/restoration. In the Drosophila embryo model, actin and myosin are recruited to cell wounds and their subsequent assembly into an actomyosin ring necessary for wound closure. We recently found that three RhoGEFs (RhoGEF2, RhoGEF3, and Pebble) and one RhoGAP (Tumbleweed) regulate the spatial and temporal patterns of Rho1, Rac1, and Cdc42, which are major members of Rho family GTPases and are indispensable for dynamic actin and myosin regulation. Interestingly, Pbl and Tum are also required for another actomyosin ring formation during cytokinesis to activate Rho1 at the equator of two dividing cells. During cytokinesis, Tum associates with Pavarotti (Pav), a kinesin-like protein, to form the centralspindlin complex that moves along microtubules. While Pbl, Tum, and Pav overlap spatially and regulate the Rho1 activity during cytokinesis, we find that Pbl, Pav, and Tum recruitment patterns to wounds are not identical and Pbl regulates Cdc42 in cell wound repair. Consistent with this, we also find that Tum and Pav mutants exhibit distinct phenotypes, suggesting non-canonical roles for these proteins in cell wound repair. Surprisingly, we find that Pav associates with actin and Rho family GTPases are normally recruited to wounds in Pav mutants. Thus, our results indicate that the actomyosin rings formed during cytokinesis and cell wound repair are built up differently despite their use of similar molecules. We are now investigating how Pav regulates actin dynamics to ensure robust cell wound repair.

508 Using cross-species evolutionary analysis to identify and characterize novel centrosome gene duplications. *F.C. Welsh*, R.S. O'Neill, B.J. Galletta, N.M. Rusan National Heart, Lung, and Blood Institute, NIH, Bethesda, MD.

Centrosomes are organelles that serve as the major microtubule-organizing centers of the cell and play important roles in cell division, polarity, and motility. They consist of highly structured centrioles surrounded by dynamic pericentriolar material (PCM). Basic centrosome functions are conserved across the broad eukaryotic lineage, yet the centrosome manages to tailor these diverse roles to a wide variety of cellular contexts. This raises the question: how can an organelle adapt its components to coordinate these essential functions in different tissues and organisms? One major driver of diversification is gene duplication. Genes with essential functions often cannot afford to accumulate mutations, as any one could be deleterious. However, duplication events provide an opportunity for functional innovation and/or specialization in one gene copy without compromising the function of the other. Separation of function among centrosome gene duplicates has been demonstrated in higher eukaryotes, suggesting that duplication has played a role in the specialization of centrosome components. How these adaptations arise on a shorter timescale between closely related species is not well understood. Studying centrosome genes in *Drosophila* provides an opportunity to explore the initial stages of divergence and thoroughly characterize how alterations can prove beneficial to different fly species. Taking advantage of the numerous sequenced *Drosophila* genomes, I used BLAST to investigate 20 canonical centrosome gene sequences in 12 species of *Drosophila* to identify significant variation or duplication events. I found that all centrosome genes are relatively conserved in these species. However, I identified a full-length DNA-based duplication of *spd-2* retained in *D. willistoni*. Spd-2 in *D. melanogaster* performs important roles in PCM recruitment and astral-microtubule nucleation, and is essential for duplication of the sperm centriole after fertilization. I have termed these *D. willistoni* paralogs *spd-2A* and *spd-2B*, and shown that *spd-2B* is divergent especially in the N-terminal half based on maximum likelihood analysis. Based on super-resolution SIM imaging in transfected S2 cells, both Spd-2A and Spd-2B have the same localization pattern at interphase centrioles and mitotic pericentriolar material. We have also generated *D. melanogaster* flies expressing *D. willistoni spd-2A::GFP*and *spd-2B::RFP* transgenes in order to assess localization and function of these paralogs *in vivo*. Given the multifunctionality of Spd-2 and its specialized role in sperm cells, the presence of Spd-2B may represent the initial stages of specialization of a new centrosome component, thereby providing a unique opportunity to study how the centrosome adapts to different cellular contexts.

509 Maternal RNAi screening of potential Src64 targets in actomyosin ring contraction during cellularization. *A.P.N. Bui*[1], T.Y. Carter[1,2], J.H Thomas[1] 1) Cell Biology and Biochemistry, Texas Tech Health Sciences Center , Lubbock; 2) Clinical Laboratory Science, Texas Tech Health Sciences Center , Lubbock.

The Src protein is a non-receptor tyrosine kinase associated with vascular homeostasis and cancer. Src family kinases regulate several cellular processes, including cell proliferation, morphology, motility, adhesion and cytoskeletal reorganization. Src64 is one of two Src family kinases in Drosophila. Src64 is involved in actomyosin ring contraction during Drosophila cellularization. Mutations in Src64 that reduce or eliminate Src64 protein expression, or eliminate kinase activity, cause microfilament rings to adopt irregular shapes and fail to constrict. This phenotype is thought to be caused by reduced microfilament

contractile tension in *src64* mutants. Our previous proteomics studies identified proteins that are differentially expressed in response to reduced Src64 expression levels, and our previous phospho-proteomics studies identified proteins that are differentially phosphorylated in response to the elimination of Src64 catalytic activity. These proteins likely act downstream of Src64. Maternal RNAi knockdown of Src64 produces celllularization front and microfilament ring phenotypes similar to that of the hypormorphic *src64*$^{\Delta 17}$ allele. Proteins that are associated with cell signaling or actin cytoskeleton are being tested by maternal RNAi knockdown. Embryos are assayed for cellularization front defects and microfilament rings defects. Microfilament ring phenotypes will be quantified using the microfilament circularity assay previously described. We expect those that exhibit *src64*-like defects to be involved in the Src64 signal translation pathway or to be involved in actin remodeling in response to Src64 signaling. Currently, we have evaluated 15 RNAi knockdown lines. Thus far, we have found that *Sbf* RNAi knockdown causes defects similar to s*rc64* RNAi knockdown. Sbf phosphorylation is reduced in catalytically inactive *src64* mutant embryos. Sbf has previously been implicated in cortical remodeling of Drosophila hemocytes.

510 Function of tyrosination of α-tubulin in regulating RNA transport, microtubule dynamics, and development of the ovary *in Drosophila*. *M.J. Bao*, R. Dörig, D. Beuchle, V. Paula, B. Suter Institute of Cell Biology / University of Bern, Bern, CH.

MTs have an intrinsic polarity with a minus and a plus end and they are used as tracks by kinesin motors that transport cargo towards the plus ends and by dynein motors that move towards the minus ends. Bicaudal-D proteins (BicD family members) have emerged as links that couple diverse cargos to the dynein / dynactin motor and, accordingly, the resulting motor has been termed DDB complex for dynein / dynactin / BicD complex. Through BicD, the DDB teams up with different additional proteins that are either located on the surface of the cargo or bind to the cargos. Tubulin is subjected to a special cycle of detyrosination / tyrosination, which is coserved among eukaryotes. The C-terminal Tyr of α-tubulin is cyclically removed by a vasohibin / SVBP and re-added by a Tubulin-tyrosin-ligase (TTL). McKenney and colleagues recently reported dynein / dynactin motors prefer MTs with Tyr for transport, while kinesin motors seem to prefer MTs lacking this Tyr. that MTs containing the C-term Tyr on the alpha subunit serve as entry point that positions the DDB complex properly through the MT binding-domain of p150/Glued contacting this C-terminal Tyr. Using the *Drosophila* model I therefore want to find out whether the terminal Tyr and the dynamic changes at the C-terminal end of alpha tubulin play important roles for the efficient localization of mRNAs, proteins and organelles by the DDB motor *in vivo.*

511 Functional characterization of an actin regulator, HtsRC. *J. Gerdes*, K. Mannix, A. Hudson, L. Cooley Genetics, Yale University, New Haven, CT.

In the female germline, ring canals acquire a robust actin cytoskeleton that supports the dramatic increase in ring canal lumen diameter during egg chamber development. HtsRC, a female germline specific product the *hu-li tai shao* gene, is involved with F-actin accumulation and is a substrate of the Kelch-Cullin3 E3 ubiquitin ligase. To understand the role of HtsRC, we used CRISPR gene editing to create mutations in the exon encoding HtsRC and recovered nonsense mutations that cause premature termination. Egg chambers from homozygous mutant females have ring canals that lack F-actin, while other F-actin-based structures including stage 10B cytoplasmic actin cables remain intact. *htsRC* mutant ring canals are smaller than normal and a subset of *htsRC* mutant ring canals collapse. *htsRC* mutant females produce small, malformed eggs and have decreased fecundity, indicating large ring canals are required for successful transfer of cytoplasm to the oocyte. HtsRC overexpression in the ovary stimulates an increase in ring canal diameter. Ectopic expression of HtsRC in the somatic follicle cells results in accumulation of F-actin aggregates containing HtsRC, Kelch, Filamin and Pavarotti. These results support the conclusion that HtsRC protein is necessary and sufficient for recruiting F-actin assemblies. As recombinant HtsRC is insoluble and therefore not amenable to *in vitro* F-actin biochemistry, we are leveraging the formation of HtsRC-actin aggregates to characterize the role of HtsRC in recruiting F-actin to ring canals. Using genetic mosaic analysis, we found that the ectopic F-actin aggregates do not require the Arp2/3 complex, ruling out a role in activating Arp2/3-dependent nucleation. Further analysis of HtsRC function will enhance our understanding of actin regulatory mechanisms and provide new insights into the regulation of F-actin structures.

512 Cortical myosin waves drive collective contractility and directional cytoplasmic transport. *J. Imran Alsous*[1], N. Romeo[2], J. Dunkel[2], A. Martin[1] 1) Biology, Massachusetts Institute of Technology, Cambridge, MA; 2) Mathematics, Massachusetts Institute of Technology, Cambridge, MA.

Cortical wave patterns, largely associated with actomyosin dynamics, are increasingly observed in a wide range of tissues and organisms. Regulation of their spatiotemporal patterns is critical for a variety of morphogenetic events, from embryonic compaction to peristalsis. Despite the numerous theoretical frameworks that model these dynamics, the number of tractable multicellular systems amenable for exploring these collective behaviors is limited. The *Drosophila* egg chamber – a multicellular precursor of the mature oocyte is such a system. Fruit fly eggs develop within cell lineage trees of 16 stereotypically-connected germline cells that are enveloped by an epithelium. Towards the end of oogenesis, the 15 nurse cells rapidly transfer their contents to the oocyte through cytoplasmic bridges called ring canals, in a process known as 'nurse cell dumping'. As the oocyte expands, the nurse cells regress and are cleared from the egg chamber. Previous studies have demonstrated a strong requirement for myosin in driving this bulk transport of fluid: egg chambers with germline clones of the hypomorphic *sqh1* allele fail to dump completely, resulting in dumpless oocytes and embryos that do not reach their full size. In contrast to other dumpless mutants, cortical and ring canal actin populations in such egg chambers appear normal, suggesting that failure to dump is due to lack of a force generating mechanism. How then are forces generated and distributed across the cell network to drive directional bulk fluid flow? Here we address this question using experiments and theoretical modeling. Our live imaging data of wild type and mutant egg chambers show that proper nurse cell dumping is associated with persistent cortical myosin waves that commence and terminate asynchronously across the 16-cell germline cluster. Such waves result in cell contractions and shape deformations that drive cytoplasmic flow from the nurse cells into the oocyte. To model these dynamics, we are using a modified extension of the well-known Two-Balloon experiment to describe the pattern of initiation, propagation and termination of these contractions on a 16-cell network. One prediction such a model makes is that dumping is initiated mechanically due to pressure differences between the oocyte and the nurse cell cluster – a prediction that is readily tested experimentally. The precise regulation of force generation by the actomyosin cortex is critical for ensuring proper cell and tissue shape changes across organisms; our work addresses the underlying genetic and mechanical mechanisms of such phenomena in a highly tractable experimental system.

513 Dystrophin's roles, subcellular organization, and functional network in oogenesis. *Mina Amini*, Miranda Villarreal, Srishti Goel, Andres Vidal-Gadea, Kevin Edwards Illinois State University, School of Biological Sciences, Normal, IL.

Mutations in the giant actin-membrane linker protein Dystrophin (Dys) are the cause of Duchenne Muscular Dystrophy (DMD). Designing effective muscular dystrophy therapies requires detailed understanding of the basic cellular and developmental roles of Dys and its protein isoforms. In *Drosophila,*Dys mutations are viable and produce two visible phenotypes: crossveins are detached from the longitudinal veins, and in oogenesis the developing eggs fail to elongate properly. We found these phenotypes are genetically separable, and focused on the egg chamber morphogenesis functions of Dys. A Dys-GFP protein trap is localized at the basal side of the egg chamber follicle cells, where it makes a plane-polarized striated pattern, distinct from nearby actin bundles. A mammalian Dys antibody can label a broader range of isoforms than Dys-GFP; it detects a similar but extended pattern of striations. Since each tag labels no more than one point along the protein, the pattern implies multiple copies of Dys are aligned, and two or more isoforms are coordinated in these structures. Dys mutants lose f-actin plane polarization in the follicle cells, but gain excess cell surface projections. *Dys-RNAi* eliminates both Dys-GFP and all patterned localization of anti-Dys, confirming the constructs and antibody function as expected. Germline RNAi against Dys does not trigger the typical *Dys* phenotypes, indicating Dys is

instead required in the follicle cells and/or ovary muscle. The beta-Heavy spectrin ("betaH" encoded by *karst*) is structurally related to Dys, and so we checked for redundancy between them. betaH, Dys double mutants have severe ovary defects and high lethality, unlike the single mutants, suggesting the two spectrin family members overlap in their developmental functions.

514 The forces that move nuclei are differentially affected by the LINC complex and Ensconsin. Mary Ann Collins, Lauren Alexis Coon, Riya Thomas, *Eric Folker* Biology, Boston College, Chestnut Hill, MA.

The dynamic process of nuclear movement is driven by various hypothesized forces that act upon nuclei as they translocate. Many studies have implicated actin, microtubules and nuclear envelope proteins as critical for nuclear movement during myogenesis. Yet, how each of these proteins contributes to nuclear movement is speculative. We have used quantitative live-embryo imaging and direct mechanical disruption to determine what functions several required proteins serve during nuclear movement. We focused on the directional movement of nuclei along the length of the muscle. This movement occurs after fusion and is characterized by a single cluster of nuclei breaking into two clusters of nuclei that move as a group toward either end of the muscle. The initial separation of nuclei into two clusters was blocked by the disruption of three different genes, *bocksbeutel*, *klarsicht*, and *ensconsin*. Thus, fixed embryo imaging suggests that disruption of each gene causes a similar phenotype. However, comparing the dynamics of nuclei during muscle development in *bocks*, *klar*, and *ens* mutant embryos suggested that the similar phenotypes have distinct mechanistic basis. Nuclei in *bocks*and *klar* mutants were elongated and dynamic whereas the nuclei in *ens* mutants were spherical and stationary. Additionally, in *bocks* and *klar* mutants, nuclei occasionally escaped the cluster and moved directionally to the proper cellular position. Escaper nuclei were never observed in *ens* mutants. These data suggested that the force-generating machinery and spatial cues are functional in *bocks* and *klar* mutants, but that the nuclei are actively held together. Conversely, in *ens* mutants there appeared to be an absence of force applied to nuclei. To directly test these hypotheses, we ablated individual nuclei and measured the response of the remaining nuclei. In *bocks* and *klar* mutants, after ablation the remaining nuclei rapidly recoiled indicating that they are under high tension. In *ens* mutants, the remaining nuclei did not respond to the ablation indicating that nuclei are not under tension. Together these data indicate that *bocks* and *klar* do not affect the application of force to nuclei but are necessary for nuclei to separate from their neighbors. Conversely, *ens* is necessary for the generation of microtubule-dependent force application to the nucleus. More broadly, these data indicate that similar defects in nuclear position can result from different mechanical mechanisms.

515 Dynamin bundles actin filaments to facilitate cell-cell fusion. *D.M. Lee*[1], R. Zhang[1], N. Gerassimov[2], E.H. Chen[1] 1) Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX; 2) Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD.

Cell-cell fusion is essential for fertilization and the formation and regeneration of syncytial organs, such as skeletal muscles. Using *Drosophila* myoblast fusion as a model, we have identified a conserved mechanism of cell-cell fusion, where one cell (attacking cell) invades its fusion partner cell (receiving cell) by extending finger-like protrusions at the site of cell-cell fusion, known as the fusogenic synapse. The protrusions are propelled by the Arp2/3-mediated branched actin actin polymerization. However, how the branched actin network is organized to form finger-like membrane protrusions at the fusogenic synapse is unclear. We show that dynamin, best known as a fission GTPase in endocytosis, plays a noncanonical role in cell-cell fusion through actin bundling. We found that the single *Drosophila* dynamin, *shibire (shi)*, is essential for both fusion of myoblasts in *Drosophila* embryos and cultured cells that are induced to fuse. Shi is specifically required in the attacking cell. Confocal and EM analyses revealed that the protrusions formed by the attacking cell are shorter and less invasive compared to those in wild-type embryos, suggesting that dynamin promotes the formation of long and stiff protrusions at the fusogenic synapse. Indeed, Shi protein is enriched at the fusogenic synapse, and super-resolution microscopy shows that Shi punctae are distributed along the actin bundles at the fusogenic synapse. Previously, dynamin, together with its interacting protein cortactin, has been shown to promote bundling and stabilizing actin filaments. However, whether and how dynamin directly bundles actin filaments remains controversial. Using biochemical assays, we demonstrate that purified dynamin directly binds and bundles actin by forming rings/helices along the actin bundles. Intriguingly, unlike the lipid tubule that is wrapped around at the center of dynamin helices by interacting with the inward-facing PH domain, our negative staining electron microscopy analyses showed that bundled actin filaments are locked to the periphery of dynamin helices by the outward-facing proline-rich domain (PRD) and G domain of dynamin. CryoEM analysis further confirmed that eight individual actin filaments are attached to the outer rim of the dynamin helices. In subsequent analyses of the PRD, we identified conserved positively charged amino acids that are required for actin binding, and demonstrated the requirement of these amino acids in cell-cell fusion. Taken together, these results lead to a new model of dynamin-actin interaction and have general implications in understanding how dynamin organizes actin filaments in diverse cellular processes.

516 Egalitarian binding partners, Dynein light chain and Bicaudal D, function sequentially to link mRNA to the Dynein motor. *C.H. Goldman*, R. Veeranan-Karmegam, G.B. Gonsalvez Department of Cellular Biology and Anatomy, Medical College of Georgia at Augusta University , Augusta, GA.

Establishment of polarity is essential for proper function in many cell types including epithelia, neurons, and germline cells. Defective polarity is associated with several neurodevelopmental disorders and is a hallmark of epithelial cancers. A widely conserved mechanism of polarity establishment is the localization of mRNA to specific cellular regions. Once the mRNA is localized, it is translated into protein. While it is clear that many localized mRNAs are transported to their cellular destinations along microtubule tracks, much less is known regarding the mechanism by which these mRNAs are linked to microtubule motors. The RNA binding protein Egalitarian (Egl) is necessary for the localization of several mRNAs in the *Drosophila* oocyte and embryo. In addition to binding mRNA, Egl also interacts with Dynein light chain (Dlc) and Bicaudal D (BicD). The role of Dlc and BicD in mRNA localization has remained elusive. Like Egl, both proteins are required for oocyte specification. Null mutants in these genes result in an oogenesis block, limiting functional *in vivo* analysis. In this study, we used an innovative approach to overcome the oogenesis block and to generate mid and late stage egg chambers that express mutant isoforms of Egl that are deficient for binding either Dlc or BicD. Our findings indicate that the primary function of Dlc is to promote Egl dimerization. Loss of dimerization significantly compromises the ability of Egl to bind localized mRNAs. Consequently, Egl is not bound to cargo, and as such, is not able to efficiently associate with BicD and the Dynein motor. Our results therefore define the essential steps required for assembling a localization-competent mRNP *in vivo* and linking it to a microtubule motor.

517 Endocytosis regulates Fog signaling to promote apical constriction during *Drosophila* salivary gland invagination. *T.Phuong. Le*, Vishakha Vishwakarma, SeYeon Chung Biological Sciences, Louisiana State University, Baton Rouge, LA.

The *Drosophila* embryonic salivary gland (SG) invaginates by budding to form a three-dimensional tube. Coordinated apical constriction during SG invagination is critical for proper tube shape. We previously showed that Folded gastrulation (Fog)-dependent Rho-associated kinase (Rok) accumulation in the apicomedial region of the SG cells is required for apicomedial myosin formation and clustered apical constriction near the invagination pit. Here we show that endocytic trafficking plays an important role in regulating Fog signaling during SG invagination. Key endocytic pathway molecules such as Rab5, Rab11 and Nuclear fallout (Nuf), a Rab11 adaptor and dynein heavy chain interactor, are enriched at and near the invagination pit. Furthermore, expression of a dominant-negative form of Rab5 and Rab11 in the SG and mutations of *nuf* inhibit apical constriction, suggesting a role of the endocytic pathway in apical constriction during SG invagination. Disruption of microtubules by overexpressing Spastin, a microtubule-severing protein, in the SG inhibits accumulation of

Rab11-positive vesicles near the pit, supporting the idea that Rab11 is transported to apical domain along microtubules. We further show that SGs mutant for *klarsicht* (*klar*), which encodes a putative regulator of dynein motors that transport various cargos on microtubules, show uncoordinated apical constriction. Importantly, *klar* mutant SGs fail to accumulate Rok in the apicomedial region of the cells, suggesting that endocytic trafficking might regulate apical constriction through controlling Fog signaling activity. We are currently testing our hypothesis that key components of the Fog signaling pathway, including the Fog ligand and its SG receptor(s), are trafficked by endocytic components along the microtubules to promote apical constriction during SG invagination.

518 Ykt6 mediates multiple cell functions during oogenesis in *Drosophila*. *Setse Bush*, Nancy Jo Pokrywka Department of Biology, Vassar College, Poughkeepsie, NY.

Membrane trafficking is an essential part of eukaryotic life, and defects in trafficking have been implicated in a variety of human diseases, ranging from neurological diseases to reproductive conditions. We are investigating Ykt6, an R-SNARE protein implicated in various vesicle trafficking events. In other systems, Ykt6 has been implicated in trafficking of membrane vesicles between the Golgi and ER, between the Golgi and cell surface, and in autophagosomal-lysosome fusion. Further, at least some functions of Ykt6 appear to be shared between species, as evidenced by the ability of the human Ykt6 gene to complement Ykt6 mutations in yeast. Because Ykt6 has been implicated in a variety of vesicle fusion events, we examined the role of this gene in *Drosophila* oogenesis. Oogenesis is of particular interest for several reasons. First, the cells of the egg chamber undergo significant increases in size during the five or so days that comprise a full cycle of egg production. It is likely that large amounts of endo- and exocytosis accompany these size changes, which at a minimum must accommodate the increased surface area associated with rapid cell growth. Secondly, there are a number of plasma membrane signaling events that result in dramatic intracellular rearrangements and are essential for successful oogenesis. Finally, several groups have reported that in other *Drosophila* cells, the generation and release of exosomes requires *Ykt6* function. In the current project, we characterized the role of Ykt6 in oogenesis by observing the phenotype of *Ykt6-* germline clones. Immunofluorescence was used to visualize the expression of membrane proteins, organelles, and vesicular trafficking markers in mutant egg chambers. We find that *Ykt6-* germline clones have morphological and actin defects affecting both the nurse cells and oocyte. Additionally, we examined several proteins involved in the establishment of cell polarity, and will present evidence that Ykt6 is necessary for successful signaling between the germline and follicle cells.

519 Discovery of a novel syncytium in the *Drosophila* rectal papillae. *J. King*, N. Peterson, K. Schoenfelder, B. Stormo, R. Lee, D. Fox Pharmacology and Cancer Biology, Duke University, Durham, NC.

Syncytia are tissue structures where two or more nuclei share cytoplasm. Syncytia allow for long-range sharing of metabolites, proteins, and mRNA. There are two primarily studied mechanisms of syncytium formation. One mechanism involves incomplete cytokinesis. Another mechanism involves a plasma membrane fusion pore, initiated by specialized founder cells. In contrast to these well-studied events, we have discovered a "decellularization" mechanism of syncytium formation in the Drosophila rectal papillae. Using dBrainbow, photo-activatable GFP, or dye injection, we find sharing of cytoplasm between adult papillar cells. Using time-lapse and electron microscopy, we identify a four-hour window of pupal development where papillar cell fusion occurs. Within this window, we observe de-cellularization of the papillar epithelia through the removal of basolateral, but not apical, membranes. We next performed a candidate screen to determine proteins required for syncytium formation. Knock down of proteins required for myoblast fusion pore formation and mitosis/cytokinesis in the papillar cells does not affect syncytium formation. Thus, we can rule out canonical syncytium formation mechanisms in papillar cells. Instead, our screen demonstrated that vesicle trafficking proteins including specific endosomal Rab GTPases and Shibire/Dynamin are required for syncytium formation. We further find these vesicle trafficking proteins to act in a polarized manner to facilitate removal of basolateral membrane to allow decellularization between papillar cells. Papillar cells thus represent a model for an under-studied mode of cell-cell fusion. We further speculate that such syncytium formation explains our previously observed high tolerance of chromosomal aneuploidy in this tissue.

520 A new role for the retromer complex in regulated exocytosis. *S. Neuman*, E. Terry, J. Selegue, A. Bashirullah Pharmaceutical Sciences Division, School of Pharmacy, University of Wisconsin-Madison, Madison, WI.

Proper control of intracellular trafficking is essential to ensure that proteins are delivered to the appropriate organelles. The retromer complex is a critical regulator of trafficking that is known to direct transport of specific cargo proteins from endosomes to the *trans*-Golgi network (TGN) or plasma membrane. However, a role for retromer in other intracellular trafficking pathways has not been characterized. Here, we demonstrate that retromer complex function is required for regulated exocytosis during *Drosophila* development. Our results show that the acinar cells of retromer mutant salivary glands fail to properly secrete "glue" cargo proteins in a cell-autonomous manner. We observe that retromer mutant salivary gland cells exhibit phenotypes that are consistent with known functions of the retromer complex, including dramatically enlarged late endosomes. However, we also observe that retromer mutant cells exhibit unexpected defects in the trafficking of secretory granule cargo and secretory granule membrane proteins; granule cargo and granule membrane proteins accumulate inside the enlarged late endosomes. We are using genetics and live cell imaging in salivary glands to genetically dissect this novel function of the retromer complex. Furthermore, the function of retromer in regulated exocytosis is not restricted to the salivary glands; we demonstrate that retromer mutant animals also fail to properly secrete insulin, resulting in a small body size. Overall, these results highlight a new function for the retromer complex in regulated exocytosis during *Drosophila* development.

521 Searching for subcellular mechanisms of DEG/ENaC transport in sensory neurites. *S.E. Mauthner*[1,2], W.D. Tracey[1,2] 1) Gill Center for Biomolecular Science, Indiana University Bloomington, Bloomington, IN; 2) Department of Biology, Indiana University Bloomington, Bloomington, IN.

How nociceptive neurons detect diverse, high-threshold stimuli from the environment remains an open-ended question in the field of somatosensation. The prevailing hypothesis is that these neurons must express an appropriate suite of ion channels and correctly traffick these membrane proteins to their subcellular niche for precise activity. Two members of the *Drosophila*Degenerin/Epithelial Sodium Channel (DEG/ENaC) family, Pickpocket (PPK) and Balboa (BBA), are ion channel subunits that multimerize in larval class IV multidendritic nociceptors to detect noxious mechanical forces. Using confocal microscopy, time-lapse recordings of GFP-tagged BBA (BBA::GFP) in nociceptor dendrites previously revealed that these plasma membrane proteins mislocalize to static, punctate subcellular structures in two scenarios: 1) when the ion channel subunit partner, PPK, is removed from class IV neurons and 2) when ectopically expressed in somatosensory neurons (that lack native PPK proteins). My current studies are focused on colocalization experiments testing for overlap of BBA::GFP punctae with known markers of ion channel secretory pathways. I have investigated intracellular markers for ER, ER-Golgi intermediate compartments, cis-, medial- and trans-Golgi, Golgi outposts, endocytic pathways (early, late, and recycling endosomes), degradation pathways (autophagosomes, lysosomes) and oxidative stress pathways (mitochondria, peroxisomes), and have yet to identify overlap with BBA::GFP punctae. These results suggest the possibility that BBA::GFP punctae may represent a previously unknown dendritic compartment. Future efforts to definitively identify these subcellular structures and the dendritic domain they inhabit will provide important insight into the regulation of mechanosensory channel biosynthesis and turnover.

522 Tribbles SLE: a Novel Domain Required for Proper Protein Trafficking, Turnover, and Insulin Signaling in the Larval Fat Body. *Z.J. Fischer* Molecular Biology and Biochemistry, University of Missouri-Kansas City, Kansas City, MO.

The regulation of intracellular protein trafficking and turnover must be tightly controlled and sensitive to external stimuli. In the *Drosophila* larval fat body,

protein trafficking and turnover are highly responsive to nutritional signals, which activate or inhibit the trafficking of proteins between organelles, endosomes, the nucleus, and plasma membrane to integrate insulin signaling with metabolic homeostasis during larval development. Previously, we identified Tribbles (Trbl), the founding member of the Tribbles family of pseudokinases, as a potent inhibitor of insulin signaling-mediated cell growth and anabolism in the larval fat body. Using antisera we developed, we detected Trbl protein in the cell nucleus, cytoplasm, and at the membrane in the larval fat body. Misexpression of a membrane-targeted form of Akt (myr-Akt) effectively recruited Trbl protein to the plasma membrane, suggesting a physical interaction between these two proteins. In a screen for Trbl mutants that mediate its association with Akt at the membrane we identified a mutation in the conserved SLE motif in the pseudokinase domain ($Trbl^{E286G}$), which resulted in strong mis-localization of the mutant protein to the membrane. Consistent with a role for the conserved SLE domain in membrane association via Akt, we demonstrated that dsRNA knockdown of Akt transcript in the larval fat body is sufficient to reduce $Trbl^{E286G}$ membrane accumulation and conversely $Trbl^{E286G}$ is sufficient to recruit an HA-tagged Akt to the membrane. $Trbl^{E286G}$ is more stable than WT Trbl and can recruit and stabilize endogenous Trbl at the membrane, consistent with our previous work showing Trbl multimers form in vitro. To identify pathways that regulate Trbl stabilization and association with the membrane, we mis-expressed dsRNA for key metabolic pathway regulators and found that knockdown of TSC2 homolog *gigas* in the larval fat body was sufficient to redistribute $Trbl^{E286G}$ to the lysosome. These data suggest a role for Trbl in integrating Akt and TOR signaling by trafficking between the inner leaf of the cell membrane and the lysosomal surface.

523 Investigation of sorting nexin functions in fly tissues. *T. Maruzs*[1], D. Feil-Borcsok[1], E. Lakatos[1], G. Juhasz[1,2] 1) Biological Research Centre of the Hungarian Academy of Sciences, Szeged, HU; 2) Eotvos Lorand University, Department of Anatomy, Cell and Developmental Biology, Budapest, HU.

Transmembrane proteins play essential roles in various functions of the eukaryotic cells including signaling, secretion, vesicle trafficking and fusion etc. Beside their *de novo* synthesis, recycling of these molecules contribute to the maintenance of a pool of functional proteins in the interconnected network of the **endolysosomal system**. Several protein complexes have been identified that are responsible for trafficking of transmembrane proteins either towards the lysosome for degradation or back to their original localization for **recycling**. On the recycling pathway, the endosomal **retromer**complex have a well-established role in directing a wide variety of transmembrane proteins away from the degradation route and recently, the structurally and functionally similar **retriever** complex has been identified in mammalian cells. Both complexes interact with members of the **sorting nexin (Snx) family** that are characterized by the presence of a **PX-domain** that mainly binds to PtdIns(3)P, a lipid enriched in the membrane of **early endosomes**. The mammalian sorting nexin family contains more than 30 proteins and subgroups of the family display different domain structures that enable the different Snx proteins to interact with a variety of binding partners. Flies have 10 *snx* genes and many of them are poorly characterized. Using various fly tissues, we aim to explore the functions of sorting nexins in flies and their relations to retromer and retriever complexes. Our results show that Snx3 and Snx6 have retromer-dependent functions in **autophagy** through the retrograde trafficking of lysosomal enzyme receptors in the hepatocyte-like **larval fat body cells** while Snx21 and Snx25 are required to maintain a fully functional **endolysosomal system** in the highly endocytic **garland nephrocytes**. Ongoing investigation of the tissue-specificity and subcellular localization of the fly sorting nexins will enable us to understand their relations to retromer and retriever complexes and their functions in the trafficking of particular transmembrane proteins.

524 Effects of cellular lipid droplet allocation on lipid droplet consumption and *Drosophila* embryogenesis. *M.D. Kilwein*, M.A. Welte Biology, University of Rochester, Rochester, NY.

In the past decade, lipid droplets have emerged as an exciting, disease relevant topic of research. Droplet biology is intrinsically linked to fat metabolism, which is in turn connected to a multitude of human diseases including diabetes and obesity. Despite the obvious medical importance, much of the cellular biology of lipid droplets is incompletely characterized. A conserved, but poorly studied aspect of lipid droplet biology is the recruitment of microtubule motor proteins Kinesin and Dynein to the surface of droplets. These motor proteins move droplets to new intracellular locations in a dynamic and nutrient dependent manner, but it is not clear what the role of this positioning plays within the cell.

It is in *Drosophila* embryos that we have the greatest mechanistic understanding of lipid droplet transport and positioning. Prior to cellularization, lipid droplets are transported rapidly along microtubules. In embryos mutant for *klar*, individual lipid droplets move with dramatically reduced speed and for shorter travel distances, resulting in misallocation of lipid droplets to the yolk cell post cellularization. We show now, using live imaging, that *Jabba* mutant embryos have a strikingly similar misallocation of lipid droplets post cellularization, but through a completely different mechanism. While in *klar*mutants mislocalization arises only by the end of cellularization, in *Jabba* mutants droplets are aggregated with each other as well as glycogen granules and are abnormally displaced to the embryo interior already by cycle 10. We hypothesize that aggregates make poor transport cargo and thus cannot efficiently reach the embryo periphery. Intriguingly, in *Jabba* mutant embryos, the rare, unaggregated lipid droplets move at wild type speed, in stark contrast to *klar* mutants.

Using Nile Red staining, TLC, and biochemical measurements of triglycerides, we find that both *klar* and *Jabba* mutant embryos fail to consume a portion of the misallocated lipid droplets, leaving them with elevated triglyceride levels at hatching. *Jabba* mutants display a pronounced hatching delay, possibly because they do not have full access to their lipid-droplet-stored energy reserves. Consistent with this hypothesis, misallocation, failed triglyceride consumption and hatching delay are solely due to lack of maternal Jabba and preliminary experiments indicate that *klar* mutants display a similar hatching delay.

525 Comparing the roles of Rab8 and Rab10 in regulating basement membrane secretion in the *Drosophila* follicular epithelium. *K. Sy*, A. Zajac, S. Horne-Badovinac Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL.

Basement membranes (BMs) are dense, sheet-like extracellular matrices that line the basal surface of polarized epithelial cells. They provide substrates for cell adhesion, facilitate cell-cell signaling, and act as barriers against unwanted cell migration, such as cancer metastasis. During development, BM networks can undergo modification and shape tissue morphogenesis. Despite their importance, the process of how BMs are built and modified through the secretion of newly synthesized BM protein is poorly understood. The *Drosophila* egg chamber provides a model system in which to study the polarized secretion of newly synthesized BM proteins. The egg chamber is a developing tissue in the ovary and is composed of an inner germ cell cluster surrounded by a layer of polarized epithelial cells called follicle cells (FCs). FCs make and secrete all major BM proteins to their basal surface, resulting in a BM that encases the entire egg chamber. The proper secretion of new BM protein first requires correct sorting and trafficking of vesicles containing the BM cargo. Rabs are small GTPases that are often considered master regulators of membrane traffic. They are activated by GEF proteins, which facilitate the transition of Rabs into a GTP-bound, active state. Currently, two Rabs, Rab8 and Rab10, have been identified as regulators of BM secretion. Here, we compare the roles of Rab8 and Rab10 in directing BM transport. Overexpression of Rab8 modifies the BM in an opposite manner than overexpression of Rab10, indicating that Rab8 and Rab10 may define separate BM secretory pathways. We also show that Strat, a putative GEF for Rab8, may also act as a holdase chaperone to regulate both Rab8 and Rab10 stability. Loss of Strat results in a drastic reduction of both Rab8 and Rab10. This work provides a deeper understanding of how two Rab-GTPases may act in concert to direct similar, yet distinct, secretion pathways and how these Rabs may be regulated in other ways than their GTPase cycle.

526 Properties of released and expectorated/solidified secretory Sgs-glue from larval salivary glands of Drosophila. *R. Farkas*[1], D. Benova-Liszekova[1], L. Mentelov[1,2], M. Beno[1], K. Babisova[1,2], L. Trusinova[1], B. Chase[3] 1) Laboratory of Developmental Genetics, Institute of Experimental Endocrinology,

Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia; 2) Department of Genetics, Faculty of Science, Comenius University, Bratislava, Slovakia; 3) Department of Biology, University of Nebraska, Omaha, NE, USA.

The Golgi-derived large secretory vesicles/granules of Drosophila salivary glands (SGs) constitute the components of the salivary glue secretion (Sgs). The Sgs represents a highly special and unique extracellular composite glue matrix that has not been identified so far outside of Cyclorrhaphous Dipterans. For over the half the century the only major and unambiguously documented function of the larval salivary glands was to produce a large amount of mucinous glue-containing secretory granules that, when released during pupariation, serves to affix the freshly formed puparia to a substrate. Besides initial biochemical characterization of the Sgs proteins and cloning of their corresponding Sgs genes, there is very little known about additional properties and functions of the Sgs glue. Here we report the observations on the fine SEM-ultrastructure of the Sgs glue released into to the lumen of SGs, and after it has been expectorated and solidified into the external environment. Surprisingly, in contrast to expectations, it appears to be highly structured bioadhesive mass with internal spongious to trabecular infrastructure, reflecting the state of its hydratation. We also found that in addition to its cementing properties, it is capable of highly efficient glueing and trapping microorganisms, and thus serve potentially very important immune and defense role. Moreover, our data also uncovers internal structure of the secretory duct, most proximal portion of the SG via which Sgs-glue is released into pharynx and then expectorated, and we describe organization and fine ultrastructure of taenidia, highly specialized circumferential ring-like extracellular matrix components on inferior side of these tubes that serve to reinforce them during secretory process. (Supported by the VEGA 2/0103/17, COST ENBA-CA15216 grant, EEA & NFM Norwegian Fund # SK-0086/3655/2009/ORINFM, MVTS-32060600/EC-INSTRUCT-FP7-211252 grant and APVV-16-0219 grant to R.F.).

527 Autophagy reduces reactive oxygen species in neurodegeneration caused by dihydroceramide accumulation. *Franca Tsu-Yi Su*, Fei-Yang Tzou, Yu-Lian Yu, Chih-Chiang Chan Graduate Institute of Physiology, National Taiwan University, Taipei, Taiwan.

Neurodegeneration is the progressive and irreversible loss of neurons that affects an individual's neuronal-related activities with no immediate solution to date. Therefore, it is crucial to understand the underlying causes of the diseases. Here, we have generated a *Drosophila* infertile crescent (*ifc*) null mutant to investigate its role in neuronal maintenance. *ifc* is the *Drosophila* homolog of the human dihydroceramide (dhCer) desaturase, which maintains sphingolipid homeostasis by converting dhCer to ceramide (Cer) in the sphingolipid *de novo* synthesis. In our study, we found that *ifc*-KO leads to activity dependent neurodegeneration in the eye due to dhCer accumulation; reducing the amount of dhCer rescued the neurodegenerative phenotype. *ifc*-KO animals are homozygous lethal, but further investigation into this phenomenon revealed that the lethality phenotype can be rescued cell non-autonomously by expressing Ifc in neuronal or muscle tissues. We also found that Ifc is mainly localized in the endolysosomal compartments, in addition to an increase in the level of reactive oxygen species (ROS) in *ifc*-KO eyes. Interestingly, activating autophagy can reduce the level of ROS and rescue the neurodegenerative phenotype. These results strongly suggest that *ifc* maintains sphingolipid balance and modulates the level of ROS, both of which are pivotal to neurodegeneration. Further evidence in the interplay between *ifc* and ROS regulation will be presented. These findings serve to increase the knowledge base for our understanding of the causes of neurodegenerative diseases and its therapeutic potentials.

528 Regulation of Mitochondrial Network Organization in Drosophila Muscles. *P. Katti*[1], B. Glancy[1,2] 1) National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD; 2) National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD.

The organization and dynamics of mitochondrial networks are highly important for tissue function, particularly in energetic tissues like muscles. The organization of mitochondrial networks differs depending on muscle type; however, the regulatory mechanisms underlying the mitochondrial organization in the different muscle types remain uncharacterized. In order to characterize the factors that regulate the muscle type-specific organization of mitochondrial networks, we used the fibrillar and tubular muscles of *Drosophila melanogaster* as the model system. We found Drosophila muscles have distinct mitochondrial network organization in the different muscle types, namely the fibrillar indirect flight muscles (IFM) and the tubular tergal depressor of the trochanter (TDT or jump) muscles and leg muscles. The IFMs possess large mitochondria, aligned in parallel and densely packed between the myofibers similar to mammalian cardiomyocytes. The mitochondrial network of the tubular TDT muscles comprises thin, long, reticular mitochondria arranged parallel to the axis of the muscle fibers. Interestingly, we show for the first time that the tubular muscles of the leg contain two types of mitochondrial networks; mitochondria arranged in parallel and those arranged in a grid-like manner similar to mammalian oxidative fibers. To identify the factors that determine the fiber type-specific mitochondrial organization, we misexpressed *spalt major* (*salm)*, a major regulator of fiber-type specificity in Drosophila muscles. As expected, the knock down of *salm* in the fibrillar IFM caused fiber-type switching to tubular muscles. *salm* knockdown also resulted in a change in the mitochondrial organization to a grid-like network. Importantly, the mitochondrial network in the converted IFM resembled that of the tubular leg muscle and not that of the tubular jump muscle. Our findings indicate that the determination of mitochondrial network organization is downstream and in the same regulatory pathway as the specification of muscle fiber type; however, the mitochondrial network organization might be regulated independent of fiber type specification, downstream of salm.

529 Clueless is a novel stress-responsive ribonucleoprotein particle. *K.M. Sheard*[1,2], S.A. Thibault-Sennett[1], R.T. Cox[1,2] 1) Biochemistry and Molecular Biology, Uniformed Services University, Bethesda, MD; 2) Molecular and Cell Biology Program, Uniformed Services University, Bethesda, MD.

Mitochondria require products of mitochondrial DNA and nuclear DNA to function properly, and mutations and dysregulation of either product gives rise to mitochondrial disorders and neurodegeneration. The mechanisms which regulate import of nucleus-encoded mitochondrial proteins are not well-characterized. We have demonstrated that the protein Clueless is required for mitochondrial function and regulation; however, we do not yet know the molecular mechanism of how Clu family members perform these functions.

The nucleus-encoded gene *clueless (clu)* is important for properly functioning mitochondria. *Drosophila clueless* mutants have direct and systemic mitochondrial dysfunctions including reduced ATP levels, mitochondria with abnormal morphology and clustered localization, drastically shortened lifespans, flight muscle defects, and sterility. Clu is a ribonucleoprotein which binds mRNAs bound for import into the mitochondria, ribosomes present at the mitochondrial outer membrane, and the membrane transport proteins TOM20 and Porin. Clu exists in cytoplasmic, mitochondria-associated aggregates ("particles") in healthy cellular conditions. Clu particles are reminiscent of ribonucleoprotein (RNP) granules: cytoplasmic, non-membranous aggregates which function in temporal and spatial post-transcriptional regulation of their mRNA cargos. Upregulation of RNP granules is well-characterized in cellular stress responses, where regulation of transport, translation, and stability of mature mRNAs is an acute response to cellular stress. In contrast, Clu particles are no longer visible under mitochondrial, oxidative, nor nutritional stress though there is not a corresponding decrease in Clu protein levels. This finding signifies that the response to stress is specific to Clu particles. Based on this finding, our goal is to characterize Clu particles as a new ribonucleoprotein granule by exploring Clu particle dynamics and behavior in stressed and unstressed conditions in order to determine the composition and nature of Clu particles and how they impact mitochondrial function and post-transcriptional regulation of mitochondria-bound, nucleus-encoded proteins.

530 Inhibition of Mortalin/Hsc70-5 induces pexophagy through increasing of peroxisomal reactive oxygen species. *H. Kim*[1,4], D. Jo[2], A. Kim[4], E. Yeom[4], K. Yu[4], S. Choe[3], D. Cho[2], K. Lee[4] 1) School of Biological Sciences, College of Natural Sciences, Chungbuk National University, Cheongju, KR; 2) Graduate School

of East-West Medical Science, Kyung Hee University, Yongin, KR ; 3) Department of Microbiology, Wonkwang University School of Medicine, Iksan, KR; 4) Hazard Monitoring BNT Research Center, KRIBB, Daejeon, KR.

Quality control of peroxisomes is essential for cellular homeostasis. However, mechanism underlying pexophagy is largely unknown. This study identified mortalin as a novel pexophagy regulator. Down-regulation of mortalin increased autophagy activation but decreased the number of peroxisomes in vitro and in vivo. The loss of peroxisomes by mortalin depletion was reversed in p62-deficient cells. In mortalin deficient cells, level of peroxisomal ROS was enhanced, while treatment of ROS scavenger blocked pexophagy. Importantly, reconstitution of mortalin mutants associated with Parkinson's disease failed to rescue the loss of peroxisomes, whereas wild-type mortalin inhibited pexophagy in mortalin-depleted cells. Additionally, knockdown of mortalin decreased peroxisomes in Drosophila and the PD-associated mortalin mutants also failed to rescue the loss of peroxisomes in mortalin-depleted flies. Taken together, our findings suggest that loss of mortalin enhances pexophagy by increasing the level of peroxisomal ROS and PD-associated mortalin mutants are linked with PD.

531 Actin regulation during *Drosophila* oogenesis: complex interplay between prostaglandins, lipid droplets, and ER. *J.M. Thomalla*[1], M.S. Giedt[2], T.L. Tootle[2], M.A. Welte[1] 1) Biology, University of Rochester, Rochester, NY; 2) Anatomy and Cell Biology, University of Iowa Carver College of Medicine, Iowa City, IA.

Lipid droplets (LDs) are ubiquitous organelles with critical roles in fat storage, protein sequestration, and energy metabolism. During *Drosophila* oogenesis, LDs accumulate massively in nurse cells and undergo various interactions with the endoplasmic reticulum (ER). We find that both ER and LDs are highly mobile and vary morphologically between nurse cells and over development. ER and LDs appear to reciprocally influence each other: morphological alterations occur in both organelles when either ER or LD resident proteins are mutated, such as the ER localized Pxt or the LD localized proteins LSD-2 and Jabba. Pxt, a cyclooxygenase-like enzyme, is responsible for the production of prostaglandins (PGs), potent lipid-signaling molecules. In *Drosophila* egg chambers, loss of Pxt results in breakdown of cortical actin and actin bundles, as do – to a lesser extent – loss of LSD-2 or Jabba. Genetic interaction analysis reveals that Pxt and Jabba act in the same pathway. Although PGs are known to be critical for building and maintaining the actin cytoskeleton, the relationship between ER, PGs, LDs, and actin remains unclear, e.g., PGs produced in the ER might regulate the LD proteome which in turn remodels actin. Our ongoing analysis points to a crucial role of triglycerides, the major lipid component of LDs. Abolishing either triglyceride production (*midway* mutants) or turnover (*brummer* mutants) is sufficient to cause cortical actin breakdown. Our working hypothesis is that LDs provide a substrate for Pxt to then produce the PGs necessary to regulate the actin cytoskeleton. We are also exploring whether abnormal ER-LD interactions might affect substrate availability. These studies are among the first connecting lipid droplets and ER to actin cytoskeletal regulation and are uncovering a novel pathway by which PGs and LD/ER proteins regulate actin dynamics.

532 The endoplasmic reticulum membrane protein Jagunal displays mitotic spindle defects in Drosophila Neurblasts. *A.A Martinez Peraza*[1], S. Beyler[1,2,3], B. Riggs[1,2,3] 1) Department of Biology, San Francisco State University, Daly City, CA; 2) National Human Genome Research Institute, NIH, Bethesda, MD; 3) National Science Foundation, NSF, Alexandria, VA.

The segregation of DNA into daughter cells during cell division has been established, however it is still unclear how segregation of organelles such as the endoplasmic reticulum (ER) are inherited during cell division. Recent studies have shown that the ER divided asymmetrically during early Drosophila embryo development suggesting a mechanism of inheritance. Furthermore, the asymmetric partitioning of ER relies on the highly conserved ER membrane protein Jagunal (Jagn). Inhibition of Jagn also displayed a significant delay in mitotic progression during gastrulation. In order to investigate a mitotic role for Jagn, we performed real time analysis of mitosis in Drosophila neuroblasts. Here we report neuroblasts deficient for Jagn, also show a significant delay or arrest during mitosis. In addition, RNAi inhibition of Jagn displayed defects in the mitotic spindle formation in dividing neuroblasts at metaphase. These findings suggest that Jagn may play a role in connections between the ER and mitotic spindles during mitosis. Understanding the role of the Jagunal protein and its involvement in mitosis and microtubule assembly will help identify a conserved mechanism towards ER inheritance during cell division.

533 The axonal trafficking of ER-retained proteins: Insights into how the ER pervades the axon. *N. Ruggiero*, T. Krzystek , S. Gunawardena State University of New York at Buffalo,UB, Buffalo, NY.

Recent work supports the presence of endoplasmic reticulum (ER) throughout the axon in the form of narrow tubules, known as axonal ER. However, the function of the axonal ER remains unclear. Previously, we found that KDEL (an ER-retained peptide motif) -containing vesicles showed robust bi-directional motility within axons *in vivo*. However, Reticulon-like-1 (RTNL-1), an ER transmembrane protein responsible for axonal ER tubule curvature, was distributed uniformly but showed little motility within axons. Here we test the hypothesis that molecular motors kinesin or dynein influences the motility and distribution of ER proteins KDEL and RTNL-1. Using Drosophila genetics and high-resolution epifluorescent microscopy, coupled with a custom particle tracking software, we analyzed the motility dynamics of KDEL-RFP or RTNL-1-YFP in the context of 50% reductions in Kinesin-1, Kinesin-2, Kinesin-3, or Dynein. We found that 50% reductions of either kinesin or dynein signficantly disrupted the bi-directional motility of KDEL-RFP in larval axons, indicating that KDEL-containing vesicle compartments used molecular motors for axonal transport. In contrast, 50% reduction of either kinesin or dynein did not influence the distribution of RTNL-1-YFP within the larval axons, indicating that RTNL-1 is not directly linked to motors. Taken together our work suggests that vesicles containing KDEL use molecular motors for movement within the axons while other ER proteins do not. Future work will test how other ER-retrained proteins are moved within the axon as well as elucidate the identity of these KDEL-containing vesicle compartments and their role within the axon and synapse.

534 Screening for secretion genes in fat body cells. *Lingjian Zhou*, Xutong Xue, Min Liu, Zhi Feng, Jose Pastor-Pareja School of Life Sciences, Tsinghua University, Beijing, CN.

Secretion of collagen has been a focus of interest for cell biologists in recent years because collagen trimers are too large and rigid to fit into the COPII vesicles mediating transport from the endoplasmic reticulum (ER) to the Golgi. Collagen-specific mechanisms to create enlarged ER-to-Golgi transport carriers have been postulated, including cargo loading by conserved ER exit site (ERES) protein Tango1. We conducted in the past an RNAi screening for genes involved in collagen secretion. In this screening, we found 88 gene hits for which the knockdown produced intracellular accumulation of Collagen IV in the larval fat body, the main source of matrix proteins in the larva. Among these hits, only two affected collagen secretion specifically: *PH4αEFB* and *Plod*, encoding enzymes known to mediate posttranslational modification of collagen in the ER. Every other intracellular accumulation hit turned out to affect general secretion, consistent with the notion that secretion of collagen does not use a specific mode of vesicular transport, but the general secretory pathway. Analyzing now the results of our screening from the perspective of general secretion, we found that most hits caused cargo retention inside the ER. However, we also found a category of hits in which cargo is retained in post-Golgi structures. Characterization of these hits reveals additional control points beyond ER-to-Golgi transfer, the most regulated step in general secretion.

535 Subcellular localization of the Golgi kinase Four-jointed in *Drosophila* development. *H.O. Ishikawa*, T. Okada, A. Nakazawa, Y. Kurihara, Y. Keira Graduate School of Science, Chiba University, Chiba, JP.

The atypical cadherin Fat acts as a receptor for a signaling pathway that regulates growth, gene expression, and planar cell polarity in *Drosophila* development. Another atypical cadherin Dachsous (Ds) acts as a Fat ligand, and Fat and Ds bind to each other heterophilically. Genetic studies in *Drosophila*identified the *four-jointed* (*fj*) gene as a regulator of Fat signaling. Fj resides in the Golgi and phosphorylates the cadherin domains of Fat and Ds. Fj-mediated phosphorylations promote the ability of Fat to bind to its ligand Ds and inhibit the ability of Ds to bind Fat. Golgi complex is a stack of cis-, medial-,

and trans-cisternae, and the cisternae are present as dispersed stacks (units) in *Drosophila* cells. To investigate subcellular localization of Fj, Fj tagged with the V5 epitope (Fj:V5) was expressed. Fj:V5 was localized to the medial-Golgi cisternae in cultured S2 cells and also in vivo. Moreover, Fj:V5 was localized to a subset of Golgi units in the cells of wing imaginal discs, but was not in S2 cells. The number of Fj-localizing Golgi units in the cells of the wing imaginal discs were scored at each developmental stage from the first instar larval stage through the pupal stage. The result showed that the number of Fj-localizing Golgi units increased during the third instar larval stage. This observation raises the possibility of property change of the Golgi units in the cells of the wing imaginal discs during *Drosophila* development.

536 ESCRT components differentially regulate *Drosophila* lymph gland hematopoiesis. *A. Ray*[1], M. Inamdar[1,2] 1) Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India; 2) The Institute of Stem Cell Biology and Regenerative Medicine, Bangalore, India.

Blood cell homeostasis is achieved by controlled hematopoiesis. This requires a complex interplay of multiple signaling pathways to regulate progenitor maintenance and differentiation in response to cell intrinsic and external cues. Cargo sorting and transport through the vesicular system, mediated by the well-conserved ESCRT (Endosomal Sorting Complex Required for Transport) machinery, is especially crucial for regulating protein turnover and downstream signaling. Aberrant ESCRT function results in accumulation of ubiquitinated cargoes and uncontrolled signaling. However, ESCRT-mediated regulation of hematopoiesis is underexplored. Recent reports highlight the importance of endosomal control of *Drosophila* hematopoiesis. Tissue-specific regulator like the OCIA domain containing protein Asrij interacts with and controls the generic trafficking protein ADP ribosylation factor 1 (ARF1) to maintain blood cell progenitors. We show that Asrij regulates ubiquitinated cargo level and expression of some ESCRT components in the lymph gland suggesting an active role for ESCRTs in hematopoiesis. Though uniformly expressed, ESCRT components had distinct effects on progenitor maintenance and lineage choice. By functional analysis in lymph gland blood cell progenitors, we map the role of specific ESCRT components in spatially distinct progenitor sub-populations. ESCRT-mediated receptor down-regulation is required to prevent ectopic Notch signaling activation in *Drosophila*. We show that ESCRT components differentially regulate Notch intracellular domain (NICD) trafficking in blood progenitors and their depletion leads to precocious crystal cell differentiation. Using *Drosophila* hematopoiesis as a model, our study reveals distinct roles for individual components of ESCRT machinery which are supposed to have a generic function in cargo sorting. Similar mechanisms for fine spatial control of blood cell homeostasis may operate in vertebrates and await identification.

537 Spatiotemporal dynamics of endosome tubulation during *Drosophila* cellularization. *Samuel Reed*, Wei Chen, Bing He Biological Sciences, Dartmouth College, Hanover, NH.

Endosome-derived membrane tubules have been observed in various eukaryotic cell types and are thought to mediate retrograde trafficking towards the Golgi or the plasma membrane. Endosome tubulation has been implicated in multiple cellular functions such as protein sorting and phagosome maturation, but how it is regulated in vivo has been largely unexplored. Using deep-tissue live imaging, we found that during *Drosophila* cellularization, elongated tubules labeled by the late endosomal marker Rab7 emanate from Rab7-positive granules near the yolk and protrude towards the periphery of the embryo. Individual tubules undergo dynamic growth and shrinkage, with an average lifetime of 75 ± 19 seconds in WT embryos. Sorting nexin 3 (SNX3), a PX-domain containing protein regulating retrograde trafficking, colocalizes with Rab7 on the tubules. Tubulation events show specific spatial and temporal patterns. Temporally, most events occur 10 to 35 minutes after the onset of cellularization. Spatially, the occurrence of tubulation is strongly biased to the ventral side of the embryo, being 3.1 ± 1.2 times more prevalent on the ventral side than on the dorsal side. We found this differential localization was controlled by the Dorsal signaling pathway. In *dorsal* RNAi embryos, with repressed ventral cell fate, the frequency of endosome tubulation on the ventral side was reduced to a level comparable to that on the dorsal side of the wild-type embryo. Conversely, in ventralized embryos expressing constitutively active Toll receptors (*Toll10b*), the frequency of tubulation events on the dorsal side became comparable to that seen on the ventral side. In a targeted RNAi screen, we identified several endosomal proteins that regulate tubulation. Rab7 GEF (HOPS complex subunit Vps39) and Rab7 GAP (TBC1D15) are both required for endosomal tubulation, suggesting that tubulation depends on Rab7 GTP/GDP cycle. Knocking down another HOPS complex subunit Vps41 also abolished endosome tubulation. Interestingly, the retromer component Vps26 appears to negatively regulate endosome tubulation, as knocking down *vps26* resulted in an increase in tubulation on both the dorsal and ventral sides of the embryo. Together, our results demonstrate that endosome tubulation during cellularization is under precise spatiotemporal regulation, which is controlled by dorsal-ventral patterning and differentially regulated by proteins involved in endosomal trafficking.

538 Investigating Rab5 partitioning during mitosis in Drosophila neuroblast. *B. K. Morin*[1,3], M. Gbenedio[1,3], B. Riggs[1,2,3] 1) National Human Genome Research Institute, NIH, Bethesda, MD; 2) National Science Foundation, NSF, Alexandria, VA; 3) San Francisco State University, SFSU, San Francisco, CA.

During mitosis, chromosomes are aligned and partitioned into two newly formed daughter cells, however less understood is the partitioning and inheritance of organelles during cell division. Previous studies have shown that the endoplasmic reticulum (ER) is inherited during cell division, but it is unclear how other endomembrane systems like the early endosomes are partitioned during mitosis. Here, we used a YFP-Rab5 (a GTPase) to follow early endosome dynamics during mitosis. We show that changes in GTPase activity displayed mislocalization of Rab5 along the mitotic spindle early in mitosis. In addition, we found that microtubules are necessary for correct partitioning of Rab5 along the mitotic spindle early in mitosis. Taken together, we found that Rab5 distribution during mitosis, relies on the microtubule network. Future directions will examine how Rab5 moves relative to chromosome separation and its distribution after cell fate determination. Giving rise to a more detailed understanding of the role endosomes play during mitotic events.

539 Characterization of Lysosome Associated Membrane Protein, Lamp-1, in *Drosophila melanogaster*. *N.Y. Chaudhry*, A. Riaz, L. Ambrosio, G. MacIntosh Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, IA.

While Lamp1 has been used extensively as a lysosomal marker in *Drosophila melanogaster*, the actual function of this protein within the lysosomal membrane in flies remains elusive. Vertebrates possess two orthologs of Lamp1, LAMP1 and LAMP2, and additionally, LAMP2 has 3 different splice variant. LAMP2A has been implicated in chaperone-mediated autophagy, and LAMP2B deficiency cause Danon disease, characterized by cardiomyopathy and myopathy and mental retardation, in humans. Deletion or knock-down of both *LAMP1* and *LAMP2* in mice result in embryo lethality. We hypothesize that Drosophila can be a good model to study lysosomal defects and Danon disease. Thus, we characterized a loss of function mutant for Lamp1 in Drosophila. Unlike the deletion of *LAMP1*/*LAMP2* in vertebrates, lack of Lamp1 activity in flies does not result in a lethal phenotype, and mutant flies seem to develop normally. Analysis of cellular morphology in *lamp1*-KO flies showed accumulation of Lysotracker-red (LTR) positive puncta, suggesting an increase in autophagic vacuoles in fat body tissue. These results warrant further investigation into the putative role of Lamp1 in lysosomal activity in *Drosophila melanogaster*.

540 A Modular Mechanism Mediates the Interchromosomal Association of *Drosophila* Chromocenters. *Madhav Jagannathan*[1], Ryan Cummings[1,2], Yukiko Yamashita[1,2] 1) Life Sciences Institute, Ann Arbor, MI; 2) HHMI, University of Michigan.

A central principle underlying the ubiquity and abundance of satellite DNA repeats in eukaryotes has remained poorly understood and they are frequently dismissed as 'junk' DNA. In a recent study using *Drosophila* and mouse, we have proposed that satellite DNAs are required to encapsulate the entire chromosomal complement of a species within a single nucleus, a universal feature of eukaryotes. This function of satellite DNAs relied upon their ability to form chromocenters, widely observed DNA-dense foci within interphase nuclei, which we showed are formed by proteins that bind specific satellite DNAs on multiple chromosomes and bundle them together. Moreover, disruption of chromocenters resulted in the formation of micronuclei, DNA damage and a loss of cellular viability. Here we show that a 'modular' network mediates chromocenter formation, where interactions between two sequence-specific satellite

DNA-binding proteins, D1 and Prod, bound to their cognate satellite DNAs, brings the full complement of *Drosophila melanogaster* chromosomes into the chromocenter. *D1 prod* double mutants die during embryogenesis, exhibiting enhanced phenotypes associated with chromocenter disruption, revealing the universal importance of satellite DNAs and chromocenters. Taken together, we propose that interactions between chromocenter modules, consisting of satellite DNA binding proteins and their cognate satellite DNA, package the *Drosophila* genome within a single nucleus.

541 Modifier screen to identify p160 coactivator, Taiman, interacting genes in *Drosophila* oogenesis. *Chueh Wen Wang*, Anna C.-C. Jang Biotechnology and Bioindustry Sciences, National Cheng Kung University, Tainan, TW.

Steroid hormone plays a significant role in temporal control of animal development, which regulates its downstream targets by steroid hormone receptors and the p160 coactivators. SRC (Steroid hormone Receptor Coactivator) gene family is overexpressed in numerous cancers including ovarian and breast cancers. However the molecular mechanism that SRC genes promote cancer metastasis has not been well established. Therefore, a group of migratory cells called border cells that are originated from the follicle epithelia of *Drosophila* egg chambers are applied as a model to study the role of SRC gene in cell migration. Taiman (Tai) is the only one SRC gene in *Drosophila*, which was identified from a forward genetic screen for the migration defect of border cells. Tai is composed of bHLH, PAS, LXXLL and C-terminal poly-Q domains that interact with hormone receptor complex through LXXLL domain and modulate the level steroid hormone signaling by bHLH. Overexpression of tai (ΔB) causes not only hyper-activation of steroid hormone signaling but also migration defect. To further uncover its mechanism, we carried out modifier screen to seek for any mutation that enhanced or suppressed Tai (ΔB) dependent defects in border cell migration. 209 deficiency lines provided by the public stock were analyzed for their effects on border cells in expression of pUAST-tai (ΔB) at stage 9-10 of oogenesis under the control of slboGAL4. To use this strategy that screened 209 deficiency lines to seek any of them to enhance or suppress migration defect. Currently, there are 48 lines displaying suppression phenotype and 74 lines showing enhancement. We will further characterize the top 10 enhancers and suppressors to identify which genes are responsible for the migration defect that we observed in the screen.

542 The p38 MAP kinase is critical for rapid embryonic wound closure. *G. Scepanovic*[1,2], R. Fernandez-Gonzalez[1,2,3,4] 1) Cell and Systems Biology, University of Toronto, Toronto, CA; 2) Ted Rogers Centre for Heart Research, University of Toronto, Toronto, CA; 3) Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, CA; 4) The Hospital for Sick Children, Toronto, CA.

Embryonic wounds are repaired rapidly, with no inflammation or scarring, in a process driven by collective cell movements. Upon wounding, actin and the molecular motor non-muscle myosin II become polarized in the cells adjacent to the wound and accumulate at the wound edge. Actomyosin polarization results in the assembly of a contractile cable that coordinates cell movements around the wound to drive rapid wound closure. Although the actomyosin cable is required for rapid wound closure, it is not strictly necessary for repair. Therefore, other forces must play a role in wound healing. We used the *Drosophila* embryo, which is amenable to live imaging, as well as genetic, pharmacological and biophysical manipulations, to investigate alternative mechanisms of embryonic tissue repair. Using quantitative image analysis, we found that the cells around wounds in *Drosophila* embryos were compressed as the wound expanded, and regained part of their apical surface area as the wounds closed. Interestingly, the p38 MAP kinase has been implicated in cell size maintenance *in vitro* and in embryonic epithelial cells *in vivo*. Immunostaining of wounded embryos revealed that p38 was specifically activated in the cells adjacent to wounds. Both genetic and pharmacological inhibition of p38 resulted in defects in wound closure. More specifically, knock down of p38b, but not p38a or p38c, disrupted tissue repair. Strikingly, actomyosin polarization and force generation at the wound edge were not affected by p38 inhibition. The defect in wound closure upon p38 inhibition was associated with a reduced recovery of the surface area of the cells around the wound, further suggesting that cell size control may be important for wound closure. We further confirmed that cell size regulation is required for wound closure by treating embryos with rapamycin, an inhibitor of the serine/threonine kinase mTOR, which also regulates cell size. mTOR inhibition resulted in defective wound repair, without affecting actomyosin dynamics. Together, our data suggest that p38 is activated in response to cell compression upon wounding, thus promoting cell growth in a response that may be crucial for embryonic wound closure. We are currently investigating if p38 could affect other cytoskeletal and adhesive structures important for cell migration, such as actin-based protrusions and focal adhesions; and if ectopic activation of p38 can accelerate wound repair.

543 A modifier screen provide insight into Rap1-mediated group polarity in collective cell migration. Yu-Chiuan Chang[1,2], *Jhen-Wei Wu*[1], Yi-Chi Hsieh[1], Tzu-Han Huang[1], Anna C.-C. Jang[1] 1) Biotechnology and Bioindustry Sciences, National Cheng Kung University, Tainan, TW; 2) Institute of Biomedical Sciences, National Sun Yat-sen University, Kaohsiung, TW.

In collective cell migration, a common feature in embryonic development, wound healing, and cancer metastasis, cells move in a tightly or loosely associated group. The directional protrusions orient motile cells in response to external cues and such directionality is achieved by coordinated polarity among the migrating cohort. However, the mechanism by which polarized protrusions are coordinated remains mysterious. Border cells are derived from the follicle epithelium of egg chambers and migrate collectively along guidance cues, making it a well-defined model to decipher how cells orchestrate actin cytoskeleton to form protrusion to migrate. Here we report that Rap1 is required to polarize border cells to extend forward protrusions. Suppressing Rap1 activity impedes border cell migration and cellular protrusion, but conversely, hyperactivation of Rap1 by overexpressing $Rap1^{V12}$leads to misoriented protrusions and loss of polarization in actin dynamics. Furthermore, the individual cell mobility highly depends on Rap1 activity. To unfold the molecular mechanism, we performed a modifier screen to look for genes that enhance or suppress the migration phenotypes induced by UAS-$Rap1^{V12}$. 158 deficiency lines were examined and two candidates, uncovering *hpo* and *mats*, significantly enhanced $Rap1^{V12}$-mediated migration defect. Systematic analysis shows that heterozygosity of the core kinase cassette of the Hippo signaling pathway, including *hpo*, *mats*, *sav* or *wts*, exaggerates such phenotypes. Further study with genetic, biochemical and molecular biologic approaches concludes that Rap1 negatively regulates the Hippo pathway to polarize directional protrusions in collective cell migration. In addition, we also took advantage of P-insertion and UAS-dsRNA lines from public resources to identify genes responsible for the migration phenotype caused by the top candidate lines in combination with $Rap1^{V12}$.

544 Genetic studies link *garz* to Abl-mediated cell migration during development. *S. C. Macon*, T. L. Stevens Biology, Randolph-Macon College, Ashland, VA.

The formation of organs and tissues in multicellular organisms relies on the ability of cells to migrate and change shape. The constantly rearranging actin cytoskeleton, which lies underneath the cell membrane, is a major force that drives cell shape changes. One key protein that regulates the actin cytoskeleton is Abl. Mutations in *abl*, whether hyper-active (*Bcr-Abl*) or loss-of-function, cause defects in actin structure as well as cell migration. At least some of Abl's effects are through its target Ena, but Abl pathways remain incompletely characterized. By identifying new components of Abl signaling pathways, we will gain a better understanding of the mechanisms by which Abl regulates actin structure and actin-based processes. Recently, our laboratory identified *garz*, a gene that encodes a guanine nucleotide exchange factor, as a dominant modifier of defects in cell migration associated with expression of Bcr-Abl. In *Drosophila*, Garz is essential for morphogenetic events including salivary gland development and epithelial expansion. It has previously been shown that mutations in *garz* cause defects in dorsal closure, but Garz had not previously been linked to Abl. We found that two *garz*alleles suppressed, while a third allele enhanced, phenotypes associated with expression of activated Abl. Furthermore, our preliminary findings suggest that mutant alleles of *Arl1* and *Rab6,* genes that have previously been linked to *garz*, also suppress phenotypes associated with overexpression of Abl. Taken together, these results suggest that Garz, along with at least a

subset of its known partners, function with Abl in mediating cell migration during development. Future studies will examine the mechanisms by which these genes interact.

545 A targeted RNAi screen identifies conserved cell-cell junction genes required for border cell collective migration. *N. Kotian*[1], K. Hylen[1], J.D. Lathia[2], J.A. McDonald[1] 1) Division of Biology, Kansas State University, Manhattan, KS; 2) Department of Cellular and Molecular Medicine, Lerner Research Institute,Cleveland Clinic, Cleveland, OH.

Collective cell migration is a dynamic process, fundamental not only to wound healing, immune response and embryogenesis but also to tumor invasiveness. A critical question in collective cell migration is how these cells establish and maintain the cell-cell junctions to stay together and communicate amongst cells of the collective. The relatively simple border cells from the Drosophila ovary are an excellent genetic model system to study in vivo collective cell migration and invasion. The 6-10 border cells migrate collectively to the large oocyte at the posterior end of the developing egg chamber, the functional subunit of the ovary. Recently, in collaboration with the Lathia lab (Cleveland Clinic), we demonstrated that patient-derived glioblastoma cancer cells can undergo collective cell invasion. An RNAi screen in border cells was designed to target conserved cell junction genes associated with glioblastoma patient survival. This screen revealed five candidate genes with consistent migration defects -α-Catenin, Dachsous, Lachesin, Roughest and Symplekin. RNAi for α-catenin, a component of the Adherens junction (Cadherin Catenin Complex) displayed strongest migration defects and the cluster also split apart. Validation using time lapse video of α-catenin RNAi egg chambers showed splitting of the border cell cluster along the path of migration. Knocking α-catenin down specifically in border cells or in polar cells also caused the cluster to split. Further, knocking down β-Catenin in the cluster, another component of CCC, also showed splitting of the border cell cluster. Our current work involves looking at the other members of this complex and mutant alleles of the other candidate genes to confirm the phenotypes observed in the screen. This will reveal the mechanisms that govern collective cell migration through cell-cell junctions.

546 *Drosophila* Snazarus regulates a dynamic lipid droplet sub-population beneath the cell surface of fat body adipocytes. Rupali Ugrankar[1], *Jade Bowerman*[1], Hanaa Hariri[1], Brett Collins[2], Steve Jean[3], Mike Henne[1] 1) Dept of Cell Biology, UT Southwestern Medical Center, Dallas, TX; 2) Institute for Molecular Bioscience, The University of Queensland, St. Lucia, Austr; 3) Dept of Anatomy and Cell Biology, University of Sherbrooke, Quebec, Canada.

Lipid droplets store high-energy lipids and can be mobilized during an energy crisis to sustain life. How cells functionally organize their lipid stores to maintain metabolic homeostasis remains a fundamental biological question. *Drosophila melanogaster* is an excellent model to investigate lipid biology as the fly shares many metabolic commonalities with mammals. The *Drosophila* fat body (FB) is a highly specialized fat storing tissue physiologically analogous to mammalian adipocytes and the liver. Here, we show that *Drosophila* adipocytes contain functionally distinct LD sub-populations that occupy distinct regions of the cell interior: small peripheral LDs (pLD) just below the cell surface that make contact with the plasma membrane (PM), and larger perinuclear LDs. The two LD sub-populations have distinct roles in fasting and development, and are differentially decorated with the perilipins Lsd2 and Lsd1, respectively. The pLD pool shrinks during fasting and expands upon over-nutrition, while perinuclear LDs remain comparatively unchanged. Furthermore, we identify the sorting nexin protein Snazarus (Snz) as a key regulator of pLD homeostasis. Snz forms distinct foci on the endoplasmic reticulum (ER) and enriches at contact sites between pLDs and the PM, the latter association mediated via a non-canonical phospholipid-binding interface on the Snz PX domain. Loss of Snz perturbs pLD morphology and TAG mobilization during fasting. In contrast, *Drosophila* over-expressing Snz exhibit increased pLD size, elevated TAG, and enhanced starvation resistance. Consistent with this, Snz foci expand in response to high nutrient diet. Snz functionally interacts with fatty acid desaturase Desat1, which is required for Snz-driven TAG up-regulation. We propose that *Drosophila* FB cells spatially organize their LD stores to optimize fat storage and mobilization, with Snz acting as a coordinator of TAG synthesis at ER-pLD-PM tri-junctions.

547 Ribonucleoprotein Clueless play important role in mitochondrial protein import and function. *A. Sen*, R. Cox Biochemistry and Molecular Biology, Uniformed Services Univ., Bethesda, MD, Uniformed Services Univ., Bethesda, MD.

Mitochondria are the major contributor of cellular ATP and functional mitochondria are key to maintain proper cellular function. While the mitochondrial genome encodes for only 13 polypeptides in humans, almost 1800 proteins are encoded by the nuclear genome and must be imported into the organelle. The bulk of these proteins are translated in the cytosol and subsequently transported to the mitochondria using canonical molecular chaperons. Recently, there is increasing evidence suggest that co-translational import is also contributes to mitochondrial protein import. However, the molecular machinery and mechanism are not well defined. We are characterizing the role of Clueless (Clu) in this process. Clu is essential for proper mitochondrial function. Mutant flies are sick, uncoordinated, sterile and have damaged nonfunctional mitochondria. These defects are a direct effect of the lack of Clu because Clu also interacts with outer mitochondrial membrane proteins such as Tom20, Porin and PINK1. Recently our lab and others have shown that Drosophila Clu and the mammalian counterpart, Cluh, are ribonucleoproteins that preferentially bind nucleus-encoded mitochondrial mRNAs. Moreover, we have also found that Clu binds ribosomal proteins on mitochondrial membrane and genetically and physically interact with the major mitophagy pathway complex Pink1-Parkin. Based on our findings we propose a model where Clu binds mRNAs destined for co-translational import on to mitochondrial membrane and may contribute to mitochondrial quality control through sensing and engaging to the mitophagy machinery. Currently we are exploring Clu's role as a ribonucleoprotein in further detail and identifying potential candidate mRNAs in order to explore the contribution of Clu in mitochondrial co-translational protein import.

548 Structural analysis of the flightin-myosin interaction in insect flight muscle thick filaments: Insight into the molecular basis of muscle mechanical properties. *L. Menard*, N. Wood, J. Vigoreaux Biology, University of Vermont, Burlington, VT.

Structural changes in the myosin II light meromyosin (LMM) that influence thick filament mechanical properties and muscle function are modulated by LMM-binding proteins. Flightin (FLN) is an LMM-binding protein indispensable for the function of *Drosophila* indirect flight muscle (IFM); it impacts thick filament viscoelasticity and plays an essential role in sarcomere organization and stability. FLN has a three domain structure that includes WYR, a novel 52 amino acid domain conserved throughout Pancrustacea. FLN mutants lacking either the N-terminal or C-terminal domains flanking WYR partially rescue the IFM phenotype of FLN null flies and implicate the WYR domain as essential for FLN function. A recent cryo-EM study of *Lethocerus* IFM thick filaments (Hu et al. Sci. Adv. 2016) showed a non-myosin density identified as FLN associated with a locally unwound region of the LMM. In this study, we analyzed image files from Hu et al. to uncover a shift in LMM coiled-coil rotation in a region of FLN interaction and revealed that FLN bridges myosin dimers within and between layers. We used Circular Dichroism (CD) to: (1) Test the hypothesis that WYR binds the LMM, (2) characterize the secondary structure of WYR, and (3) examine the structural impact WYR has on the LMM. Resultant ellipticity reveals a structural profile for WYR and supports an interaction with the LMM that coordinates a conformational shift in both binding partners. We find that the secondary structure of WYR is concentration and ionic strength dependent and displays predominant negative ellipticity at ~190-192nm with a 222/208 ratio of ~0.38, trademark of a 3_{10} helix. Deconvolution of the CD spectra show that 40-50% of WYR is unstructured and contains a portion of beta structure and a small degree of helical content. WYR in the context of the LMM shows substantial conformation shifts including an increase in the 222/208 ratio that is maximal at a WYR to LMM ratio of 5:2-5:1. While the LMM alone exhibits a 222/208 ratio of 1.1-1.2, in line with previous reports, the LMM experiences a greater 222/208 ratio with increasing WYR concentration due predominantly to decreased intensity of the 208 minima This indicates decreased alpha helical content and a relative increase in 3_{10} content, supporting increased coiled-coil content with an accompanying unwinding of a portion of the LMM. Our results support the hypothesis that WYR binds the LMM and brings about structural changes in the coiled-coil, consistent with the observation of Hu et al of local coiled-coil unwinding near the FLN density on native thick filaments. These studies implicate FLN,

via the conserved WYR domain, for distinct shifts in LMM secondary structure. They also provide a mechanism by which FLN influences the viscoelastic properties of the thick filament, scaling to modulation of whole muscle function, via its interaction with multiple myosin dimers.

549 Tubulin polymerization promoting protein, Ringmaker, and microtubule associated protein 1B homolog, Futsch, coordinate microtubule organization and synaptic growth. Q. Shi, A. Saliba, *S. Banerjee* Cellular and Integrative Physiology, UT Health San Antonio, San Antonio, TX.

Drosophila Ringmaker (Ringer) is homologous to the human Tubulin Polymerization Promoting Proteins (TPPPs) that are implicated in the stabilization and bundling of microtubules (MTs). No *in vivo* functional data exist that have addressed the role of TPPP in synapse organization in any system. Phenotypic characterization of *Drosophila ringer* mutants during larval neuromuscular junction (NMJ) development showed reduced synaptic bouton growth. *ringer* mutants displayed phenotypic similarities and genetic interactions with the *Drosophila* homolog of vertebrate MAP1B, *futsch.* Immunohistochemical and biochemical analyses showed that individual and combined loss of Ringer and Futsch cause a significant reduction in synaptic MT loops at the NMJs and reduced acetylated-tubulin levels. Presynaptic over-expression of Ringer and Futsch caused elevated levels of acetylated-tubulin and significant increase in NMJ MT loops. These results indicate that Ringer and Futsch regulate synaptic MT organization and synaptic growth during NMJ development. Together our results highlight the role of *Drosophila* Ringer in coordination with MAP1B/Futsch in MT dynamics during synapse growth and organization at the larval NMJs. Our data also provide insights that vertebrate TPPPs may also be required in synapse organization and function.

550 Function of Nat9 acetyltransferase in microtubule stability and JNK signaling in Drosophila. *Jung Wan Mok*, Kwang Wook Choi Biological Science Dept, KAIST, Daejeon, KR.

Regulation of microtubule stability is crucial for the maintenance of cell structure and function. Recent studies suggest that microtubules are also involved in cell signaling, but the underlying mechanisms are largely unknown. Here we show that a Drosophila homolog of human N-terminal acetyltransferase 9 (Nat9) is essential for organ development by stabilizing microtubules. Reduced Nat9 causes severe developmental defects in different organs by inducing apoptotic cell death. Depletion of Nat9 in imaginal discs increases the level of pJNK, as a cause of cell death. Reduced JNK signaling components suppresses cell death and restore Nat9 RNAi phenotypes. We found that Nat9 co-localizes with microtubules in tissues and is physically associated with microtubules in vitro and in vivo. Overexpression of Nat9 enhances the stability of microtubule while its loss causes mitotic spindle defects. We show that Nat9 can acetylate the N-termini of both alpha and beta tubulin in vitro. Function of human Nat9 has not been characterized. We demonstrate that human Nat9 can rescue Nat9 RNAi phenotypes in flies, indicating the functional conservation. This study suggests that Nat9 is crucial for stabilizing microtubules by N-acetylation of tubulins and that the stabilized microtubules inhibits JNK signaling to promote cell survival in developing organs.

551 Exploring C(2)M's Ability to Facilitate the Assembly of the Synaptonemal Complex. *J.E. Fellmeth*, H. Nguyen, K.S. Mckim Genetics, Waksman Institute- Rutgers University, Piscataway, NJ.

Meiosis is a process in which an exchange of genetic information between homologous chromosomes creates genetic variation. The synaptonemal complex (SC), a protein complex which holds homologous chromosomes together, regulates genetic recombination during meiosis through interactions with the chromosome axis. We have shown previously that loading of the SC is dependent on cohesins such as C(2)M. A proposed model depicts a cohesin ring on the chromosome axis, made of two cohesin subunits SMC1 and SMC3, and a kleisin (C(2)M) and Stromalin. This study aims to shed light on the relationship between cohesins and the SC. We have analyzed both N- and C- terminal mutants of C(2)M (UASP-inducible constructs) in an effort to disrupt ring formation. We hypothesize that these mutations will disrupt ring formation without affecting other functions of C(2)M and expect that SC will still be able to assemble. If this hypothesis proves true, it indicates that C(2)M functions independently of the cohesion ring to facilitate SC assembly. While these mutants fail to localize (except in discrete foci associated with centromeres), they also fail to promote SC assembly (except at the same foci). When we quantified nondisjunction (NDJ, as a measure of synapsis defects), we also observed an increased rate of NDJ in these mutants. To determine if this phenotype is due to loss of the cohesion ring, we are creating a double mutant containing both an N- and C-terminal mutation to remove C(2)M from the ring structure completely. We expect these mutants to have a complete loss of cohesion localization. We have also created a hybrid construct with C(2)M N- and C-termini with the central domain from Rad21 (mitotic cohesion). This construct localizes but fails to recruit C(3)G indicating the importance of this meiosis specific function for SC assembly. This research furthers the current understanding of meiotic cohesin complexes. Understanding more about the components of this complex will allow us to understand more about the regulation of meiosis and the functions of these meiosis-specific cohesin proteins. In a larger world view, the result of this research should bring us one step closer to understanding chromosomal abnormalities in humans.

552 Segregation dynamics of the supernumerary B chromosomes of *D. melanogaster*. *S.L. Hanlon*[1], S. Eche[1], R.S. Hawley[1,2] 1) Stowers Institute for Medical Research, Kansas City, MO; 2) Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS.

B chromosomes are small, supernumerary chromosomes that are nonessential and are carried in addition to the essential set of chromosomes. Hundreds of different species from a diverse range of taxa have been found to carry B chromosomes, and recently B chromosomes were discovered in a laboratory stock of *Drosophila melanogaster*. Here we investigate the segregation dynamics of the B chromosomes of *D. melanogaster* to understand the broader mechanism of their transmission during female meiosis. We measured the transmission frequency of B chromosomes passed by individual parents from the original stock and found that B chromosomes show an elevated transmission frequency when transmitted through the female parent, but not when transmitted through the male parent. We are currently testing which genetic element(s) contribute to this biased transmission of the B chromosomes, as well as examining their spatial arrangement during the meiotic divisions in females. Together, a complete picture is beginning to emerge of how B chromosomes can be preferentially transmitted during female meiosis, which may provide insight into how newly-arisen chromosomes may propagate rapidly within a population.

553 The influence of essential chromosomes on B chromosome transmission during female meiosis. *S. Eche*[1], S. L. Hanlon[1], R.S. Hawley[1,2] 1) Stowers Institute for Medical Research, Kansas City, MO; 2) Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS.

Recently, B chromosomes were discovered in a laboratory stock of *Drosophila melanogaster*. Similar to B chromosomes in other taxa, the *D. melanogaster* B chromosomes are small, supernumerary chromosomes that are nonessential and are predominantly comprised of heterochromatin. The presence of B chromosomes during female meiosis can disrupt the normal segregation of chromosome *4*, which is the chromosome that likely gave rise to the B chromosomes. Though the B chromosomes and chromosome *4* share several satellite repeat sequences and may use similar mechanisms for their segregation during female meiosis, it is unclear what relationship, if any, exists between B and 4^{th} chromosome segregation. Here we show that 4^{th} chromosome segregation and B chromosome transmission frequency is inversely proportional. When an extra copy of chromosome *4* is passed to progeny, the overall B chromosome transmission frequency is reduced. Conversely, when no copies of chromosome *4* are transmitted, the B chromosomes are inherited at a higher frequency. Since the B chromosomes carry the AAGAT satellite repeat, which is also found on chromosome *4*, we are currently investigating what role this repeat may play on how B chromosomes are transmitted to progeny. We have placed the B chromosomes in a genetic background that lacks the AAGAT repeat on chromosome *4* and are actively monitoring the maintenance of B chromosomes over several generations. Together these results will begin to reveal how the composition and segregation of essential chromosomes influence B chromosome transmission during female meiosis.

554 Dissecting the role of SPC105R in meiotic kinetochore function of oocytes. Jay Joshi, Lin-Ing Wang, *Kim McKim* Waksman Inst, Rutgers Univ, Piscataway, NJ.

The kinetochore plays a key role in proper meiotic function, errors in which can result in aneuploidies, leading to miscarriages, infertility and developmental disorders. SPC105R is an outer-kinetochore protein that orchestrates several activities critical to the two meiotic divisions, including kinetochore assembly, lateral kinetochore-microtubule (KT-MT) attachments, cohesion protection (co-orientation), bi-orientation, and chromosome segregation. Lateral KT-MT attachment depends on SPC105R and involve an interaction between the kinetochore and the lateral surfaces of microtubules. End-on kinetochore-microtubule attachments depend on NDC80, another kinetochore protein, and form through a direct connection between the kinetochore and the ends of the microtubules. We are conducting a structure-function analysis of SPC105R to understand the mechanisms and regulation of these interactions. To identify the key functional domains of SPC105R, its amino acid sequence was compared to its orthologues in other *Drosophila* species as well as other vertebrate species. The N-terminus and C-terminal domains show areas of homology, while the middle region contains a poorly conserved repeat ("PEED") domain. Transgenic constructs have been constructed to make deletions in each of these conserved domains. These transgenes were constructed with neutral missense mutations to express a form of *Spc105R* that is resistant to RNAi. We have found that the C-terminal domain is sufficient to recruit NDC80 and establish end-on KT-MT attachments, but lacks lateral attachments and likely other activities regulating MT attachments. The result is that when the C-terminal domain was expressed in an RNAi background, the resulting oocytes assembled kinetochores but had severely disorganized spindles, with a split karyosome, defects in co-orientation and an absence of lateral KT-MT attachments. These findings suggest that the C-terminus is sufficient to recruit NDC80 to the centromere. Once there, NDC80 can facilitate end-on KT-MT attachments. Deletion of the central repetitive domain has relatively mild defects, consistent with studies in mitotic cells. Deletion of the first 10 amino acids of the N-terminal domain, which is proposed to interact with microtubules, results in bi-orientation defects. This domain may be important for regulating lateral KT-MT attachments, consistent with previous studies suggesting these interactions facilitate bi-orientation of homologs during meiosis I. We are currently generating and analyzing additional mutants that delete the other conserved regions of the N- and C-terminal domains and conducting cytological experiments to determine which mutants have defects in cohesion maintenance, lateral KT-MT attachments, and bi-orientation.

555 PP2A regulates spindle asssembly and cohesion maintenance in *Drosophila* oocytes. Janet Jang, Amy Gladstein, *Kim McKim* Waksman Inst, Rutgers Univ, Piscataway, NJ.

The dynamics of meiosis are regulated by antagonism between kinases and phosphatases. In *Drosophila* oocytes, the chromosome passenger complex (CPC) is required for formation of the acentrosomal spindle and kinetochore assembly. Aurora B, the kinase of the CPC, regulates two sets of targets, the kinetochores and central spindle, either of which can assemble in the absence of the other. Applying an inhibitor to Aurora B, Binuclein 2 (BN2), resulted in loss of the spindle, implying at least one phosphatase antagonizes Aurora B in spindle assembly, and that sustained Aurora B activity is required to maintain a bipolar spindle. Using tissue-specific RNAi, we determined which phosphatase, PP1 or PP2A, antagonizes Aurora B in oocytes. The spindle microtubules were stabilized when Aurora B was inhibited in *mts* RNAi oocytes. *Mts* encodes the catalytic subunit of PP2A, demonstrating that PP2A antagonizes the Aurora B spindle maintenance function. Surprisingly, knockdown of either *tws* or *wdb,* which encode the B55 and B56 subunits of PP2A, stabilized the spindle in BN2-treated oocytes. These results suggest that the two major PP2A complexes may function in the same pathway to antagonize Aurora B spindle assembly activity. We have also found that kinesin KLP10A, a microtubule depolymerizing enzyme, is the principle target mediating spindle loss in BN2-treated oocytes. Analysis of *wdb* RNAi oocytes also showed a loss of sister chromatid cohesion. This phenotype was enhanced when a second B55 subunit, *wrd,* was simultaneously depleted. These results suggest that PP2A is required for maintaining sister chromatid cohesion during meiosis I. A comparison of the moderate cohesion loss phenotype observed in *wrd* RNAi oocytes to the more severe phenotype observed in *wrd wdb* oocytes, suggests arm cohesion is lost more easily than centromere cohesion. We are currently testing the hypothesis that PP2A recruitment and cohesion protection depends on different proteins during the two divisions. Consistent with the observation that sister centromere cohesion during meiosis I depends on the kinetochore protein SPC10R, we have found that WDB localization to the centromeres depends on SPC105R. We are currently examining how PP2A is recruited to the chromosome arms. Either meiosis I cohesion maintenance on the arms depends on Dalmatian, the *Drosophila* homolog of cohesin regulator Soronin, or it is redundant with MEI-S332/SGO. In meiosis II, pericentromeric cohesion protection depends only on MEI-S332/SGO. Our results also confirm a role for Cyclin B in maintaining sister chromatid cohesion, but also have the surprising implication that, that since loss of PP2A during MI causes precocious loss of cohesion, separase may not be inhibited prior to anaphase I.

556 Requirement for the Rcd4:Ana3 sub-complex for centriole duplication and centriole to centrosome conversion. *P. Panda*[1], L. Kovacs[1], N. Dzhindzhev[1], M. Geymonat[1], A. Fatalska[1], M. Richter[1], V. Persico[2], M. Riparbelli[2], G. Callaini[2], D. Glover[1] 1) University of Cambridge, Department of Genetics, Cambridge, UK; 2) University of Siena, Department of Life Sciences, Siena, Italy.

Proteins essential for centriole duplication appear to exist in sub-complexes; Sas6 must bind to Ana2 phosphorylated by Plk4 to initiate pro-centriole formation; Sas4 interacts with centriolar microtubules; the Cep135:Ana1:Asterless network promotes centriole to centrosome conversion enabling recruitment of Plk4 and peri-centriolar material. Here, we describe a new physical complex formed in the *Drosophila* centriole between Rcd4 and Ana3. We describe null and hypomorphic *rcd4* mutant flies that lack centrioles and show structural defects in the basal bodies of sensory organs leading to loss of coordination. The C-terminal part of Rcd4, corresponding to a conserved domain present in the mammalian counterpart protein, shows residual function in such flies. Rcd4 protein loads onto zone I of the centriole during interphase after procentriole formation and prior to centriole to centrosome conversion. Depleting Rcd4 by RNAi in cultured cells has no effect upon the loading of its partner Ana3 but completely prevents the loading of Ana1 and so, centriole to centrosome conversion. Ana3 depletion allows Rcd4 to be recruited but at approximately 50% of levels seen in control cells while also preventing the loading of Ana1. Thus, it appears that Rcd4 and Ana3 can load onto the centriole independently of each other, but both are required for the loading of Ana1. Our findings identify a conserved functional sub-complex essential for centriole assembly.

557 Investigating the Biochemical and Structural Basis of Centrosome Activation. *S. Smith*[1], K. Plevock[1], B. Galletta[1], R. Varadarajan[1], S. Speed[1], T. Lian[2], N. Billington[1], J. Sellers[1], J. Jiang[2], N. Rusan[1] 1) Cell and Developmental Biology, National Heart, Lung and Blood Institute, NIH, Bethesda, MD; 2) Biochemistry and Biophysics Center, National Heart Lung and Blood Institute, NIH, Bethesda, MD.

As the main microtubule (MT) organizing center of most cells, the centrosome's form and function are tightly regulated to coordinate cytoskeleton organization throughout the cell cycle. In interphase, the centrosome nucleates few microtubules. As the cell prepares for mitosis, centrosomes become activated by accumulating additional pericentriolar material (PCM) which is necessary for nucleating and anchoring a robust MT array needed for mitotic spindle assembly. Structured Illumination Microscopy (SIM) shows that the centriole maintains a ring-like structure of the same size throughout the cell cycle, while the PCM forms a more ambiguous, cloud-like shape around the centrioles that expands during mitosis. A key goal in the centrosome field is to understand how PCM, a dense conglomerate of proteins, interacts with the structured centriole wall, and how PCM expansion is triggered during centrosome activation. While several studies show direct protein-protein interactions amongst centrosome proteins, there is minimal information about these protein interactions at the structural level. The goal of this project is to examine how key centrosome components and protein-protein interactions change as the cell transitions between an inactive interphase centrosome and an active mitotic centrosome. In *Drosophila*, Pericentrin-like-protein (Plp) is positioned between the centriole wall and the PCM. Based on this localization and additional genetic data, we hypothesize that Plp can help catalyzes centrosome activation during

mitotic entry. We are taking a structural biology approach to test this hypothesis by leveraging the direct protein-protein interaction data we generated through yeast two hybrid analysis. We will present our latest negative stain EM data of Plp and its interaction partners, including its known PCM partner Centrosomin.

558 The role of CENP-C in kinetochore building and chromosome segregation. *J.E. Fellmeth*, H. Sturm, K.S. Mckim Genetics, Waksman Institute of Microbiology- Rutgers, the State University of New Jersey, Piscataway, NJ.

Meiosis, the process of cell division resulting in haploid gametes, is innately error-prone resulting in aneuploidy, the leading genetic cause of infertility. This comes from the unique complexity of meiosis relative to mitosis; notably the genetic exchange between homologs and biorientation of chromosomes on the spindle. The centromere and kinetochore are critical parts of this process. The centromere is a chromatin region defined epigenetically by a centromere specific histone (CENP-A). The kinetochore is a large proteinaceous complex that loads onto the centromere to act as the interface between the chromosome and the spindle. The kinetochore is the organizing center for spindle attachments, proper orientation of centromeres, and checkpoint signaling. CENP-C is at the interface between the centromere and the kinetochore and proposed to be the first to load and act as a scaffold for other kinetochore proteins. This study probes the role CENP-C plays in kinetochore structure and function. Using UASP-inducible GFP-tagged constructs, we studied the loading dynamics of CENP-C compared to centromere proteins CID (CENP-A) and CAL1. When expressed prior to S-phase, we observed centromere loading of all 3 proteins. When expression was initiated during meiotic prophase however, we observed centromeric loading of CENP-C but not CAL1 or CID). These results suggest that CENP-C has unique dynamic loading during meiotic prophase, unlike the other centromere proteins or the kinetochores. We will use a heat shock inducible Gal4 line and a version of a pulse-chase experiment to investigate whether the unloading of CENP-C also occurs during prophase. To assess the implications of this dynamic localization pattern on chromosome segregation, we have used *Cenp-C* RNAi and *Cenp-C* mutants to measure chromosome nondisjunction as a readout of chromosome segregation errors. We observed a rate of 7-16% nondisjunction in these oocytes relative to the control (<1%) indicating an increased rate of chromosome segregation errors. We are currently using genetic and cytology methods to determine if chromosome segregation errors when CENP-C is depleted are caused by defects in crossing over, cohesion or kinetochore function. Accurate chromosome segregation is a critical component of reproduction and understanding the mechanisms for this process will further the basic science understanding of infertility and cancer.

559 Spindle Orientation: Pinning Down the Role of Pins. *N. Lowe*[1], N. Weeks[1], D. Na[2], D. Bergsrtalh[1,3] 1) Department of Biology, University of Rochester, Rochester, NY; 2) Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY; 3) Department of Physics & Astronomy, University of Rochester, Rochester, NY.

Oriented cell divisions are essential for the development of higher organisms; they participate in tissue morphogenesis and the generation of cell diversity. A key component of mitotic spindle alignment is the conserved protein Mud/NuMA/Lin-5 (flies, vertebrates, worms) which acts with Dynein to produce a pulling force that reels astral microtubules, and therefore the metaphase spindle, into alignment. A long-standing model holds that another conserved factor, Pins/LGN/GPR1-2 (flies, vertebrates, worms), provides a cortical anchor for Mud/Dynein, through binding to the N-terminal tetratricopeptide repeats (TPRs) of Pins. However the TPRs are also reported to bind factors that guide Pins localization: Inscuteable, Canoe/Afadin, and E-Cadherin, and binding appears to be mutually exclusive, suggesting spatiotemporal separation and/or different populations of Pins. How then does Pins work? Through a combination of CRISPR-based tagging and expression of tissue-specific tagged proteins we address this question through both biochemical and imaging approaches. Using the self-labelling HaloTag and SNAP-tag enzymes allows the use of an increasing variety of fluorescent probes for imaging including super resolution techniques as well as affinity purification. Generation of proteins tagged in a tissue specific manner can also be achieved either through the use of direct tissue specific drivers or conditional flipouts to remove intervening stops between coding sequence and affinity tags.

560 Dunk regulates myosin recruitment during *Drosophila* cleavage through its interaction with anillin. *J. Chen*, M. Wang, H. Bing Department of Biological Sciences, Dartmouth College, HANOVER, NH.

Drosophila cellularization, as a special form of cytokinesis, is initiated by recruiting non-muscle myosin II ("myosin") to the cleavage furrows that partition the peripheral syncytial nuclei into individual cells. Similar to typical animal cytokinesis, the initial recruitment of myosin in cellularization involves a cortical flow of myosin towards the leading edge of the cleavage furrows. It remains elusive how cells regulate the physical characteristics of myosin flow, such as velocity, duration and directionality. We have previously identified a novel gene *dunk* that is required for producing stable, directional cortical myosin flow during cellularization. Dunk functions by promoting myosin retention at the cell cortex, but the underlying molecular mechanism is unknown. FRAP analysis and mathematical modeling suggest that Dunk stabilizes myosin flow by maintaining an unknown cortical myosin "anchor" that recruits and stabilizes activated myosin at the cortex, rather than directly regulating myosin activation-inactivation cycles. In order to identify the cortical myosin "anchor", we performed a genome-wide yeast two-hybrid screen to look for Dunk-interacting proteins. We identified anillin (encoded by *scrap*sin *Drosophila*), a conserved myosin-binding protein critical for cytokinesis, as the primary binding partner of Dunk. Dunk binds to the highly conserved C-terminal domain of anillin, which also contains binding sites for several important regulators for anillin, including Rho1, $PI(4,5)P_2$, Peanut (a *Drosophila*septin protein) and importin. In *dunk* mutant embryos, the localization of anillin and septin to the cleavage furrows is severely disrupted. Furthermore, *anillin* mutants and *dunk* mutants show similar cortical myosin loss phenotype during early cellularization. These data suggest that Dunk facilitates cortical myosin retention by interacting with and regulating the activity of anillin. Cortical myosin flow provides a universal mechanism for delivering cortical components, including myosin itself. Our work will help elucidate how cells regulate myosin dynamics at the cell cortex to generate stable myosin flow.

561 An integrated analysis of the protein-protein interaction network of the conserved mitotic kinase, Polo. *K. Sierzputowska*[1,2], B. Housden[1,3], J. Wakefield[1,2] 1) Living Systems Institute, University of Exeter, Exeter, United Kingdom; 2) College of Life and Environmental Sciences, University of Exeter, Exeter, United Kingdom; 3) University of Exeter Medical School, Exeter, United Kingdom.

Polo kinase, first identified in *Drosophila* over 20 years ago, is a highly conserved enzyme that functions pleiotropically during multiple stages of cell division. Members of this protein family have crucial roles in cell cycle progression, centriole duplication, mitosis, cytokinesis and the DNA damage response. Although Polo substrates have been identified and some of the regulatory mechanisms uncovered, we are very far from a complete understanding of the cellular and molecular roles of this kinase. Previous work in the Wakefield lab identified 40 proteins that physically interact with Polo in *Drosophila* embryos but the functional significance of these components remains unknown. I performed a highly sensitive assay called Variable Dose Analysis (VDA) in S2R+ cells to determine which of the physical interactors also have functional interactions with Polo by screening for genetic interactions in the context of Polo inhibition. Known Polo genetic interactors, *Map205* and *mtrm*, were identified by the VDA screen, validating its robustness and utility in identifying novel interactors. In addition, components of the ubiquitination system were enriched among the hits. Current efforts are aimed at validating and further characterizing the interactions between polo, SkpA, Cul1, slmb and Pli in cells and *in vivo* to gain deeper insight into the complex functions of Polo kinase.

562 Chromosome Preference during Homologous Recombination Repair of DNA Double-Strand Breaks. *J. Fernandez*, H. Bloomer, J. R. LaRocque School of Nursing and Health Studies, Georgetown University, Washington, DC.

DNA double-strand breaks (DSBs) occur frequently and require efficient repair mechanisms to ensure genomic stability and cell viability. While numerous mechanisms for repair are available, only homologous recombination (HR) is thought to allow error-free repair. By using homologous sequences as a template

for repair, cells are able to minimize inadvertent mutations during the repair process. However, how a cell differentiates between a number of possible homologous sequences available for repair remains largely unanswered. Previous reports have suggested that intrachromosomal or intersister homologous sequences are highly preferred as a template. Yet, few studies have investigated this phenomenon in multicellular systems. Using modified versions of the DR-*white* assay in *Drosophila melanogaster,* we can distinguish between HR repair from intrachromosomal donors and HR repair from the homologous chromosome. In short, a DSB is created at a specific locus via the heat shock-induced I-SceI endonuclease. The DSB can be repaired via HR either from a donor template located downstream of the break or from a distinguishable allele of the donor located on the homologous chromosome. We find that, in the premeiotic male germline of *Drosophila*, repair from the homologous chromosome is frequent when it is the only template available for repair. However, given a choice between intrachromosomal and homologous donors, the former is highly preferred. Our findings in *Drosophila* support previous studies in yeast, suggesting specific mechanisms responsible for either repressing interhomolog HR repair, biasing towards intrachromosomal HR repair, or both. Our novel HR assay may help illuminate the proteins and processes necessary to promote this preference in *Drosophila*.

563 Locating Mutagen-sensitivity Gene *mus109* in the *Drosophila melanogaster* Genome Using Deficiency Mapping. *C. Mitchell*, K. Kohl Winthrop University, Rock Hill, SC.

DNA can be damaged by a variety of exogenous and endogenous sources. Normally, this damage is corrected by DNA repair pathways – pathways which continue to be molecularly characterized. In an effort to further study these pathways, several forward genetic screens have been conducted to identify *Drosophila* mutagen-sensitivity (*mus*) genes. However, the precise genomic location of some of these genes is still unknown, including *mus109*. It is known that mutations in *mus109* cause chromosomal aberrations resulting in larval death, and previous research has mapped *mus109* to the 8F10-9B1 region of the *Drosophila melanogaster X* chromosome – a region that consists of over 520,000 nucleotides and 41 genes. Therefore, this study aimed to molecularly identify the mus109 gene within the *D. melanogaster* genome. First, deficiency mapping was used in conjunction with a mutagen-sensitivity assay to narrow the probable genomic location of *mus109* to 12% of the original region. Computational analysis of genes in the narrowed region was used to identify a candidate gene, and Sanger sequencing was used to identify point mutations within this gene in *mus109* mutants.

564 Using deletion mapping to locate mutagen-sensitivity gene *mus305* in the *Drosophila melanogaster* genome. *J. DeLoach*, K. Kohl Winthrop University, Rock Hill, SC.

All organisms experience DNA damage, and a variety of DNA repair mechanisms exist to combat this damage. The goal of this research was to localize the DNA repair gene *mus305* in the *Drosophila melanogaster* genome. To localize *mus305*, complementation analysis was conducted using three deficiencies while assaying for mutagen-sensitivity. Specifically, two broods were created by crossing virgin female flies containing a known allele of *mus305* to male flies containing one of three deficiencies. Brood 1 offspring were treated with water, which acted as the control, while Brood 2 offspring were treated with MMS. Progeny were scored, and then percent relative survival was calculated as the ratio of mutant to control flies in Brood 2, normalized to the same ratio in Brood 1. This data was used to narrow the potential genomic location of *mus305*. A candidate gene was identified in this refined region, and current research is focused on sequencing this gene in *mus305* mutants.

565 The deubiquitinase *Usp5* is required for cell cycle exit. Z. Stephens, J. Lopez, *J. Bandura* Biological Sciences, Lock Haven University, Lock Haven, PA.

The coordination of cell proliferation and differentiation is crucial for proper development. We have identified *Usp5* as a gene required for cell cycle exit after terminal differentiation in *Drosophila* eye cells. Cells lacking *Usp5* experience ectopic E2F activity, based on the expression of an E2F-responsive reporter gene. In addition, cells undergo ectopic cell divisions in the absence of *Usp5*. *Usp5* encodes a deubiquitinase (DUB) in the ubiquitin-specific protease (USP) subfamily. This is particularly interesting, as proteolysis and reversible ubiquitination are known to play important roles in cell cycle regulation. Our efforts to determine which target proteins of *Usp5* are important for cell cycle exit will be discussed.

566 Modeling Meier-Gorlin syndrome mutations. *A.M. Branstad*, S.L. McDaniel, C.A. Fox, M.M. Harrison Department of Biomolecular Chemistry, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI.

Meier-Gorlin syndrome (MGS) is a form of primordial dwarfism caused by mutations in genes (*CDC6, CDT1, ORC1, ORC4*, and *ORC6*) whose products are essential for licensing origins of replication, a step required for proper cell division. In addition to their small stature, these MGS individuals also present with several tissue-specific phenotypes like small ears, small or missing patella, and microcephaly. These tissue-specific phenotypes are unexpected as the genes involved in MGS are ubiquitously expressed, and their products are necessary for genome replication. To better understand the molecular mechanisms by which these mutations cause MGS, we used Cas9-mediated genome engineering to generate a fly model. Specifically, we mutated tyrosine 162 of Orc4, a member of the origin replication complex (ORC), to a cysteine, mimicking a mutation identified in MGS patients. We find that $orc4^{Y162C}$ homozygous animals are viable, but reach adulthood at slightly reduced levels as compared to their wild-type counterparts. Notably, $orc4^{Y162C}$ homozygous females are sterile, a phenotype shared with hypomorphs in proteins similarly required for DNA replication. We demonstrated that, like mutants in other replication components, $orc4^{Y162C}$ homozygous females fail to amplify the chorion genes in the follicle cells of the ovaries, which are responsible for forming the egg shell. Additionally, upon the introduction of a global replication stressor through the administration of hydroxyurea, $orc4^{Y162C}$ homozygous animals fail to reach adulthood at Mendelian ratios. We are currently investigating whether this MGS mutation affects ORC assembly or function. Additionally, because $orc4^{Y162C}$ homozygous animals display replication defects, we are testing for abnormal levels of DNA breakage, which would indicate replication stress-induced breakage that might lead to cell death. Our data suggest that MGS-associated phenotypes result from defects in DNA replication and that the tissue-specific defects are caused by tissue-specific requirements for robust DNA replication.

567 Cell cycle control by growth sensors: Drosophila E2F1 is controlled post-transcriptionally by mRNA UTR elements. *J.I Ovrebo*[1], N. Zielke[2], M. Straaten[3], M. Lewis[1], B.A. Edgar[1] 1) Departement of Oncological sciences, Huntsman Cancer institute, Salt Lake City, UT; 2) University of Helsinki, Helsinki, Finland; 3) German Cancer Research Center (DKFZ), Heidelberg, Germany.

Cell proliferation is largely controlled by environmental conditions, such as growth signaling molecules and nutrition, that stimulate cell cycle progression. However, the exact mechanism linking multiple growth signals to the decision to enter G1/S remains obscure. Also, current animal cell cycle models do not explain how cells tend to enter the cell cycle only once a certain cell volume is reached.

We want to explore a cell cycle model where cell proliferation is decided by the accumulation of growth promoting cell cycle regulators, or "growth

sensors", translating multiple growth signals to cell cycle control. We believe such growth sensors accumulate in parallel with increased cellular protein translation, which correlates with cell volume. Such a model has previously been described for yeast (Polymenis et. al. 1997), but has so far not been demonstrated in multicellular organisms. A promising growth sensor candidate is the cell cycle transcription factor E2F1, which controls expression of cyclin E and S-Phase entry in a rate-limiting manner (Zielke et. al. 2011). The predominantly transcribed isoform of Drosophila E2F1, E2F1-RA, possesses an unusually long 5' UTR containing features associated with decreased translational efficiency. Translation of such mRNAs is often super-sensitive to the activity of translation initiation factors, many of which are controlled by the Target of Rapamycin (TOR) kinase, which provides increased translational efficiency. We are exploring how E2F1 expression is regulated downstream of the mTOR and EGFR pathways. We are also exploring how E2F1 UTRs affect E2F1 translation and, importantly, whether certain UTR elements contribute significantly to cell cycle regulation. We aim to uncover novel mechanisms that regulate accumulation rate of proliferation promoting growth sensors.

568 Analysis of the*mps-1* T Mps1-PP1 interaction *in vivo*. *S.J. Almazan-Herrera*, A.N. Valle, K.N. Weiler, K. Hughes Kean University, Union, NJ.

monopolar spindle 1 (*mps-1*) . (Moura 2017) *mps1* is is a serine/threonine protein kinase member of the spindle assembly checkpoint (SAC) mechanism, responsible for ensuring kinetochore attachment during prophase for proper chromosome segregation in the mitotic and meiotic divisions (Althoff 2012). *Mps1*-null human and *Drosophila* cells enter anaphase upon completing spindle formation, without allowing enough time for the chromosomes to orient correctly and segregate to their respective poles. (Moura 2017). Moura et al. (2017) found that altering the Protein Phosphatase 1- (PP1-) binding domain of MPSps-1 (KVLF to AVLA) prevents timely metaphase exit in human and *Drosophila* mitotic cells *in vitro,* indicating the importance of this domain to the function of Mps-1. Our project is the investigation into whether mutating the KVLF domain of *Drosophila* MPSps-1 will interfere with the MPSps1-PP1 interaction *in vivo.* We have mapped and balanced 38 wild-type and KVLF mutant transgenic lines. This poster will present our genetic (nondisjunction) and cytological analysis of the KVLF mutant to determine if it is unable to bind PP1 and maintain the SAC *in vivo* as demonstrated *in vitro*.

Althoff F, Karess RE, Lehner CF. Spindle checkpoint-independent inhibition of mitotic chromosome segregation by Drosophila Mps1. *Mol Biol Cell*. 2012;23(12):2275-91.
Moura M, Osswald M, Leça N, et al. Protein Phosphatase 1 inactivates Mps1 to ensure efficient Spindle Assembly Checkpoint silencing. *Elife*. 2017;6:e25366. Published 2017 May 2. doi:10.7554/eLife.25366

569 The roles of *jim lovell* (*lov*) in endopolyploid and mitotic tissues of Drosophila. *K.M. Beckingham*, F. Zhou, R. Dibbs, M. Karki, S. Green, S. Hsu, I. Chu, O. Casmiri, M. Tsay Dept BioSciences, Rice Univ, Houston, TX.

jim lovell (*lov*) encodes a putative transcription factor of the BTB/POZ domain family. Using the Gal4-UAS system we initially established that *lov* plays a role in the endopolyploid growth of the larval tracheae and subsequently demonstrated that it has a similar role in all of the larval tissues investigated. The Drosophila homolog of the oncogene Myc is the best characterized promoter of larval endopolyploid growth and epistasis studies indicate that Lov acts downstream of dMyc in this role. dMyc promotes endopolyploidy by enhancing the essential nucleolar function of ribosome synthesis. Much of the Lov protein in the nucleus localizes to the nucleolus and it seems likely that upstream actions of dMyc affect this pool of Lov protein. However, immunolocalization of Lov on the salivary gland polytene chromosomes has demonstrated that Lov also binds to sites on the chromosome arms that are distinct from the nucleolus. Thus Lov may have roles that are independent of dMyc action. In our initial work in the tracheae, we investigated the effects of loss of *lov* function on key tracheal genes and determined that *uninflatable*, a protein expressed on the apical surface of the tracheal cuticle cells, is downregulated in the absence of *lov*. This finding suggests that in the tracheae, Lov may act to promote both endopolyploid growth and formation of the cuticle.
To determine whether Lov has a role in the growth of mitotic tissues we used the Gal4-UAS system to suppress *lov* in the developing eye and wing. Loss of *lov* function has no effect in the eye but produces a very dramatic phenotype in the wings: the wings fails to inflate and have the appearance of black ropes, often stuck to one another on the dorsal thorax. *uninflatable* is also expressed strongly in the developing wing cuticle and the "black rope" phenotype upon *lov* knockdown can be rescued by Gal4-UAS induced expression of *uninflatable* in the wing, We propose that this wing phenotype is not a consequence of inhibition of mitotic wing cell growth, but rather results from loss of positive regulation by *lov* of *uninflatable* expression in the wing cuticle.

570 Specificity of E2F-dependent transcription in coordinating endoreplication. *M. Kim*[1], N.-S. Moon[2] 1) Department of Biology, Developmental Biology Research Initiative, McGill University, Montreal, Quebec, CA; 2) Department of Biology, Developmental Biology Research Initiative, McGill University, Montreal, Quebec, CA.

Endoreplication is a modified cell cycle characterized by successive alternation of synthesis (S) and gap (G) phases without intervening mitoses. In endoreplicating tissues, dE2F1-dependent activation of Cyclin E (CycE) and S phase-coupled degradation of dE2F1 ensures cell cycle-dependent oscillation of dE2F1 and CycE activity. We previously reported that an alternatively spliced isoform of *de2f1*, dE2F1b, is necessary for proper cell cycle progression, particularly in endoreplicating tissues. dE2F1b differs from the widely studied dE2F1a isoform by the presence of a microexon located in the conserved Marked Box domain, implicated in target specificity of E2F transcription factors. Using actively cycling salivary glands and nurse cells in developing egg chambers, we

further examined the role of dE2F1b during endoreplication. Characterization of target gene expression revealed that only a specific subset of canonical E2F target genes are regulated by dE2F1b. Notably, genes that directly contribute to the processivity of DNA synthesis, such as *PCNA* and *rnrS*, are negatively affected by the loss of *de2f1b* while *cycE,* a crucial regulator of endoreplication*,* remains unaffected. In addition, there is a sustained expression of dE2F1 during S phase. Consequently, dE2F1 and CycE no longer oscillate in endoreplicating tissues. This result indicates that one of the key functions of dE2F1b during endoreplication is to ensure proper expression of dE2F1 and CycE in G1 and S phase, respectively. Overall, dE2F1b-dependent transcriptional program is required to properly drive DNA-synthesis in endoreplicating tissues and to coordinate periodic expression of key cell cycle regulators.

571 Variant cell cycles in the *Drosophila* accessory gland. *A. Box*, S. Church, D. Hayes, P. Hakim, L. Buttitta Dept. of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI.

The *Drosophila melanogaster* accessory gland (AG) is a secretory epithelium functionally analogous to the mammalian prostate. The AG consists of two epithelial cell types: main cells and secondary cells that secrete distinct proteins into the seminal fluid. During metamorphosis, the cells of the AG undergo a failed cytokinesis followed by a round of endocycling resulting in binucleate, octoploid (8N) cells, each containing two tetraploid (4N) nuclei. Although the cells of the adult AG have been thought to be quiescent, we and others have observed changes in gland size with age. Here we show that under normal physiological conditions, portions of the adult AG undergo continued endocycling. These endocycles are locally synchronized, consistent with the presence of ring canals in this tissue. Activation of growth pathways downstream of BMP and dMyc generate excessive endocycling leading to severe nuclear anaplasia, suggesting the adult AG is poised to endocycle and may respond to a variety to signals. We are now investigating the potential influence of steroid hormone signalling on the endocycling and tissue growth we see in the adult AG.

572 Transcriptional Response to Errors in Spindle Orientation and Effects on Tissue Growth. *A. Parra*, C. Johnston University of New Mexico, Albuquerque, NM.

Coordination of cell polarity and spindle orientation with cell growth and proliferation ensures mitotic fidelity and thus proper animal development. Mitotic errors have been associated with aberrant tissue growth in epithelial cells. For instance, activating mutations in cell cycle-promoting genes in *Drosophila* imaginal wing discs (IWD) lead to massive tissue overgrowth. We found that loss of mushroom body defect (mud), a key spindle orientation gene, results in suppression of tissue overgrowth. These results point to an overgrowth-inhibitory mechanism in epithelial cells stemming from errors in spindle orientation caused by mud loss. To identify a potential mechanism for this effect, we performed differential gene expression analysis between overgrowth and growth-suppressed IWD tissue. Further analysis will provide a better understanding of the cell signaling pathways and transcriptional changes that govern tissue level responses to defective cell division, which will be important to improving our understanding of the underlying molecular bases for numerous human diseases.

573 Cortical polarization and emergence of the subapical domain in blastoderm embryos is controlled by Dia and Kinesin-1. *L. Li*, Anja Schmidt, Joerg Grosshans Institute for Developmental Biochemistry Medical School, Georg-August-Universität Göttingen Germany.

The cortex undergoes a stereotypic pattern of polarization during the transition from syncytial to cellular development. Two cortical domains, caps and intercaps are observed during syncytial interphases. In contrast, the typical four domains, apical subapical, lateral and basal, emerge within a few minutes in cellularization. We investigate the molecular and genetic mechanism underlying this specific process of cortical patterning as an example of epithelial polarization.

Here we focus on the emergence of the subapical domain. We and others have previously reported that a signaling cascade involving the unconventional guanyl nucleotide exchange factor ELMO/Sponge, Rap1-GTPase and Canoe controls subapical restriction of Bazooka/Par3 and E-Cadherin complex during cellularization. Linked to the transition from syncytial to cellular development is the relocalization of ELMO/Sponge from a disc-like pattern in the caps to a ring-like pattern with the onset of cellularization. We proposed a model that the novel subapical domain emerges at the interface between cap and intercap region. Mechanism underlying the relocalization of ELMO/Sponge have been unknown.

Searching for factors required for cortical patterning we found that the formin Dia is required for restriction of subapical markers. Furthermore we found that dia controls a polarization of actin caps with (+)-ends of F-actin towards the rim, thus providing a molecular template for ELMO/Sponge relocalization. Furthermore, we found that Kinesin-1 is specifically required for cortical patterning during cellularization. Kinesin-1 may provide a link between the cortex and the centrosomes, which are the initial trigger for cortical patterning. We will present data supporting our hypotheses and discuss their implications.

574 Interaction of key inflammatory pathways in tumor microenvironment in *Drosophila* cancer models control tumor progression. *K. Snigdha*[1], A. Singh[1,2,3,4], M. Kango-Singh[1,2,3,4] 1) BIOLOGY, UNIVERSITY OF DAYTON, DAYTON, OH; 2) Center for Tissue Regeneration and Engineering at Dayton (TREND); 3) Premedical Programs, University of Dayton; 4) Integrative Science and Engineering Center (ISE), University of Dayton.

The interaction between the tumor cells and the surrounding normal cells constitutes the Tumor microenvironment (TME). The Toll-like Receptor (TLR), Jun N-terminal Kinase (JNK), and Tumor Necrosis Factor (TNF) produce inflammatory components in the TME, and are thought to play a critical role in tumor survival and progression. However, the exact nature and mechanism of interactions within the TME remain poorly understood. These core inflammatory pathways are conserved in *Drosophila*. As 90% of tumors are epithelial in origin, we used an epithelial tumor model in the wing imaginal discs of *Drosophila melanogaster* to study the interaction of these key inflammatory pathways in the TME. We established a new TME model by creating FLP-out clones of oncogenic forms of *Yki* or Ras^{V12} in polarity deficient (*scribble* mutant) cells marked by GFP surrounded by normal cells. These mosaic clones allow us to test changes in intercellular and signaling interactions within the tumor, surrounding its microenvironment and in distant normal cells. We studied the activity of TLR, TNF and JNK pathway using immunohistochemistry. We found that *Drosophila* IκB Cactus (TLR component) and activated form of JNK (p-JNK) were induced in the tumor cells whereas levels of *Drosophila* TNF ligand, Eiger were unaffected in both the tumor and the surrounding normal cells. We hypothesized that crosstalk between these key pathways in the TME promotes tumor survival and progression. The genetic epistasis experiments between JNK and TNF revealed that downregulation of the TNF receptors in the tumor does not affect the metastatic abilities of the tumor cells. However, similar experiments between JNK and TLR showed decrease in invasiveness of tumor cells likely due to downregulation of Cactus in the tumor cells. We are currently testing if TLR, TNF and JNK pathways genetically regulate each other or independently affect the TME to control tumor growth. Our research will help to unravel the correlation between inflammatory pathways and tumor progression in an *in vivo* model.

575 Oncogenic properties of Peppled/Hindsight in *Drosophila* imaginal tissue. *G. Xie*, W-M. Deng Biological Science, Florida State University, Tallahassee, FL.

Ras proteins are critical regulators of normal cell proliferation and differentiation. Activated forms of Ras are involved in a wide range of human malignancies. RREB-1 (Ras-responsive element-binding protein 1) is a zinc finger transcription factor that binds to RAS-responsive elements of gene promoters. While it is directly involved in Ras/Raf-mediated cell differentiation, unlike *Ras*, *RREB1* is not considered as an oncogene. To investigate if RREB1 has an oncogenic property, here we studied gain-of-function of *pebbled/hindsight* (*peb/hnt*) in *Drosophila* imaginal discs. We show that ectopic expression of *peb/hnt* induces tissue overgrowth and drives *lgl*-depleted pre-tumor cells to be neoplastic. Transformation of *lgl*-knockdown pre-tumor cells by

overexpression of *peb/hnt* is probably induced by decreased cell adhesion. We further show that *peb/hnt* overexpression increases extracellular Wg stability and spreading. Coexpression of *lgl*-RNAi and *RREB1* can cause dramatic overgrowth in the wing disc. Similar to that of overexpression of *peb/hnt*, overexpression of *RREB1* decreases the protein levels of Arm and DE-Cad. Our study suggests that *peb/hnt*/*RREB1* plays an oncogenic role by modulating cell adhesion in *Drosophila* imaginal epithelia.

576 Modeling primary prostate cancer using the D. melanogaster accessory gland. *S.J. Church*, A Box, D Hayes, A Hakim, L Buttitta Cellular Molecular Developmental Biology, University of Michigan, Ann Arbor, MI.

Prostate cancer is a form of adenocarcinoma that affects 1 out of 7 men and is the third leading cause of cancer deaths in the United States. The accessory gland of Drosophila is the fly functional analogue of the human prostate and could be a powerful model to probe the genetic and cellular changes that occur in prostate cancer. To validate this model, we examined fly versions of oncogenic mutations known to occur in prostate cancer such as hyperactivation of Myc or activation of Yki, the fly homolog of YAP in the accessory gland epithelium. These oncogenic mutations in the fly lead to classical prostate tumor phenotypes such as cellular hyperplasia, hypertrophy, nuclear anaplasia and loss of proper epithelial polarity. In addition, we observe non-autonomous effects of oncogene activation in neighboring cells, demonstrating the power of this model for examining complex tumor vs. non-tumor heterogeneity in cancer development.

577 Role of Hippo and Ecdysone Receptor Signaling in regulation of *dronc*. *Karishma Gangwani*[1], Amit Singh[1,2,3,4], Madhuri Kango-Singh[1,2,3,4] 1) Department of Biology, University of Dayton, Dayton OH; 2) Center for Tissue Regeneration and Engineering at Dayton (TREND); 3) Pre-medical program, University of Dayton, Dayton OH; 4) Integrative Science and Engineering, University of Dayton OH.

The Hippo pathway is an evolutionarily conserved pathway that regulates organ size and tissue homeostasis in *Drosophila* and mammals. The pathway functions by regulating the nuclear availability of transcriptional cofactor Yorkie (Yki), mammalian YAP, which is regulated by the activity of a core kinase cascade comprising the serine threonine kinases Hippo (Hpo) and Warts (Wts) and their accessory proteins. Yki binds with transcription factors like Scalloped (Sd) or Homothorax (Hth) to regulate target genes involved in cell proliferation and survival. Downregulation of the Hippo pathway causes increased cell proliferation and overgrowth, whereas hyperactivation of this pathway leads to cell death due to activation of caspases. Caspases are cysteine aspartic proteases which play essential roles in cellular signaling and development via apoptotic signaling. We showed that the initiator caspase *dronc* (mammalian Caspase 9) is a transcriptional target of Yki. We found that loss of Hippo signaling leads to downregulation of *dronc* expression, whereas downregulation of Sd resulted in derepression of *dronc* expression. We found that known binding partners of Sd like E2F and Tgi are also involved in regulating *dronc* expression. Earlier studies have shown that *dronc* expression is regulated by the Ecdysone receptor (EcR) signaling pathway and mapped a EcR regulatory element on *dronc* promoter. We found that depletion of EcR or its corepressors like Smrter caused derepression of *dronc* expression. Overexpression of Taiman (Tai) a binding partner of EcR and Yki also derepressed *dronc* expression. We hypothesize that *dronc* expression is regulated by the Hippo and EcR signaling pathways. Here, we present our work on the regulation of *dronc* by the Hippo and EcR signaling pathways, and its implications on development.

578 Diet induced adaptation to heat and drought stress due to plastic changes in energy metabolites, hardening capacity and life history traits among fig fruit reared *Zaprionus indianus*. *T N Girish*[1], B E Pradeep[1], Ravi Parkash[2] 1) Department of Biosciences, Sri Sathya Sai Institute of Higher Learning, Prasanthi Nilayam, India; 2) Department of Genetics, Maharshi Dayanand University, Rohtak, India.

Zaprionus indianus, a pest on fig fruits (a rich source of carbohydrates and proline) provides a model for assessing diet-induced plastic changes in stress resistance and life history traits of drosophilids located in the tropical and subtropical regions. The larvae and adult flies reared on ripe fig fruits exhibited a significant increase in the levels of energy metabolites (carbohydrates, proline and body lipids) and resistance to heat (heat knockdown and heat shock survival) and drought when compared to larvae and adults cultured on standard laboratory drosophila diet. Plastic effects of heat or drought hardening were significantly higher in fig reared flies among both the sexes albeit only in young flies (6d) and not in the old flies (30d) compared to those reared on laboratory medium. Heat hardening (HH) was found to induce proline, body lipids and protein levels with a concomitant decrease in the carbohydrate levels. In contrast, drought hardening (DH) induced an increase in the levels of carbohydrates and proline with significant decrease in the levels of proteins and body lipids. In vitro analyses revealed that fig fruit-based diet influenced life history and mating related traits i.e., a shorter duration of development, increased fecundity even after heat hardening, early sexual maturity; faster walking speed and two-fold higher dispersal ability. We speculate that higher levels of fig derived energy metabolites support increased level of resistance to heat and drought in *Z. indianus.* The observed fig diet induced plastic changes in stress resistance, energy metabolites and life history traits are likely to support the invasive potential of *Z. indianus.*

579 Octopamine and its receptors are involved in the modulation of the immune response in *Drosophila melanogaster*. *S. Papenmeier*[1], K. Uliczka[1], T. Roeder[2], C. Wagner[1] 1) Division of Invertebrate Models, Priority Area Asthma and Allergy, Research Center Borstel - Leibniz Lung Center, Borstel, DE; 2) Molecular Physiology, Institute of Zoology, Christian-Albrechts-University of Kiel, Kiel, DE.

Stress is known to be a risk factor for the exacerbation of asthma and other chronic respiratory diseases. It induces the release of biogenic amines (BAs) such as norepinephrine, serotonin and dopamine, which modulate the innate and adaptive immune response. Effects of BAs on innate immunity can hardly be elucidated in complex model organisms such as the mouse due to close interactions between the innate and adaptive systems. In this light, the fruit fly *Drosophila melanogaster* represents a promising model system. Devoid of an adaptive immune system, the fly shares several signaling pathways with high similarities to human counterparts (e. g. Toll receptors, NF-κB signaling). It also uses analogues to human BAs (e. g. octopamine (OA), a chemical relative of norepinephrine) which activate the same signaling pathways. The fly's immune response comprises macrophage-like cells, called hemocytes, which phagocytize apoptotic cells and microorganisms, and the fat body, which secrets antimicrobial peptides (AMPs) to fight microbial infections.
The aim of this work is to elucidate the impact of OA on the immune response of *Drosophila*.
qRT-PCR experiments showed that two (Octβ1R, Octβ2R) out of four OA receptors are most prominently expressed in the fly. By using an infection model for *Pectobacterium carotovorum*, which only causes a moderate infection in wild-type flies, we were able to show that the survival rate of infected females flies deficient for the receptor Octβ1R or Octβ2R was significantly decreased compared to non-infected ones. Simultaneously, in both knockouts the bacterial load was highly increased 24 hours after bacterial injection. Moreover, qRT-PCR experiments revealed a strong upregulation of almost all antimicrobial peptide genes in infected Octβ1R-deficient female flies and even stronger in Octβ2R ones. However, the phagocytic activity of adult as well as larval Octβ1R or Octβ2R-deficient hemocytes was diminished after being challenged either *in-vivo* or *in-vitro* by fluorescent pHrodo *E. coli*bioparticles.
These findings suggest that OA modulates *Drosophila's* innate immune response and may point to a similar immune-modulatory role of norepinephrine in vertebrates.

580 Mir-969 regulates body fat mass through Gr47b. *J. Seo*, W. Redmond, M. Youngblood, L. Redmond, D. Allen, M. Elledge, R. Arellanes, J. Yeahquo, S. Zhang, A. Reiner Department of Biology, Rogers State University, Claremore, OK.

Obesity is closely linked to cardiovascular disease and diabetes; moreover, it is often associated with negative social and emotional consequences. Thus,

both early identification of risk factors and effective therapeutics are crucial to prevent and fight the worldwide epidemic of obesity. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression of various developmental and metabolic processes at the post-transcriptional level. Multiple miRNAs have been demonstrated to be linked to obesity, diabetes, and cardiovascular disease from worms to mammals; miRNAs have also been used as biomarkers and therapeutics for obesity-related disease. However, our understanding of miRNA roles on obesity is far from complete.

We ubiquitously expressed miRNAs using the Actin5C-Gal4 and UAS-miRNA binary transgene expression system in *Drosophila melanogaster* to identify miRNAs controlling body fat mass. We screened 161 miRNA lines and identified 26 miRNA lines whose adult fat contents were one standard deviation away from all screened lines' mean in both sexes. We named the set as **micro**RNAs **C**ontrolling **A**dipose **T**issue (miCATs). Comparing the miCATs to our miRNA loss of function data, we identified mir-969 as an essential regulator controlling body fat mass. Overexpression of mir-969 significantly reduced fat contents; inversely, reduction of mir-969 expression increased fat mass. Further, we analyzed gene expression of possible mir-969 targets in the mir-969 overexpression mutant flies. The qPCR analysis identified Gr47b, a G-protein coupled receptor, as a *bona fide* mir-969 target.

Gr47b function is predicted to work as a gustatory receptor. Thus, we knocked-down Gr47b neuron-specifically to determine whether Gr47b mutant phenocopies mir-969 overexpression mutant. The neuron-specific knocking-down of the gene did not show any obvious mutant phenotypes. However, when Gr47b was knocked-down adipose tissue-specifically, it significantly reduced fat mass demonstrating Gr47b plays essential roles in adipose tissue and works beyond sensing peripheral taste.

581 *Prominin-like* regulates longevity and glucose metabolism via insulin signaling in *Drosophila*. *T. Ryu*[1,2], E. Yeom[2], K. Lee[1,2], K. Yu[1,2,3] 1) Biological science, University of Science and Technology, Daejeon, KR; 2) Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, KR; 3) Korea Institute of Science and Technology (KIST), Seoul, KR.

CD133, also called Prominin-1, is a biomarker for mammalian stem cells. It is involved in cell growth, development, and tumor biology. However, the function of CD133 at the organismal level has not been investigated. In this study, we found that *prominin-like* (*promL*) loss-of-function mutant flies show an extended lifespan and metabolic defects such as increased circulating carbohydrates, lipid storage, and starvation resistance. The mRNA expression levels of *Drosophila insulin-like peptides* (*Dilps*) were reduced in loss-of-function *promL* mutants. Furthermore, the level of phosphorylated AKT, a downstream component of insulin signaling, was lower in *promL* loss-of-function mutants than in the w^- control flies. Importantly, the PromL protein is predominantly expressed in the pars intercerebralis region with insulin producing cells (IPCs) of the adult brain. When we inhibited *promL* in IPCs, these flies showed an extended lifespan, metabolic defects, and reduced insulin signaling. These results indicate that the *promL* gene regulates longevity and glucose metabolism by controlling insulin signaling in *Drosophila*.

582 A mitochondrial rescue of a nuclear defect in starvation resistance and lipid levels in Drosophila. *S. Williams*, Brian Franklin, David Rand Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, RI.

Genes required for proper mitochondrial function are jointly encoded by the mitochondria's and the nuclear genome. Mutations in either genome or incompatibilities between the genomes can cause, or modify, various metabolic disorders. Our group studies how genetic interactions between the mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) affect complex traits. To do this we introgressed several divergent Drosophila mitochondrial haplotypes into multiple different nuclear backgrounds to disrupt mtDNA-nDNA genetic interactions. We identified a specific mitochondrial haplotype, the siI mtDNA, rescues a nuclear defect in starvation resistance in the Drosophila Genetics Reference Panel line 765. This effect was unique to that particular mtDNA-nDNA pairing (siI;765), as all the other mtDNA;nDNA combinations were no different from the nuclear DNA controls (flies with the co-evolved mitochondria). We found that this mitochondrial haplotype also drastically increases triglyceride content and the major nuclear loci responsible for both phenotypes is on chromosome 3. This finding highlights a novel genetic interaction between mtDNA encoded genes and lipid metabolism.

583 Regulation of adult lipid homeostasis by *Drosophila Estrogen-Related Receptor*. *K. Beebe*, M.A. Horner, M. Robins, C.S. Thummel Human Genetics, University of Utah, Salt Lake City, UT.

Nuclear receptors are a large family of evolutionarily conserved transcription factors that play central roles in development, growth and metabolism. Three paralogs make up the Estrogen-Related Receptor (ERR) family in vertebrates: ERRα, ERRβ, and ERRγ. Although *ERRα* is necessary for lipid homeostasis in mammals, neither the tissue-specific nor mechanistic basis of this phenotype is well understood. Previous work from the Thummel lab demonstrated that the *Drosophila* member of this family, dERR, establishes a glycolytic metabolic state that supports larval growth. In contrast, adult *Drosophila* physiology does not involve growth or biomass accumulation, but rather requires efficient oxidative metabolism and ATP production to support the demands of flight and reproduction. We thus engineered a conditional allele of *dERR* to eliminate its function selectively during the adult stage, with the goal of identifying possible new functions for this receptor.

dERR mutant adults display reduced fertility and motility, increased glucose, decreased glycogen, and an almost complete lack of stored triglycerides. Consistent with this effect on lipid metabolism, *dERR* mutants are resistant to diet-induced obesity. RNA-seq and ChIP-seq analyses reveal a central role for *dERR* in regulating metabolism, including promoting glycolysis and lipid metabolism. Consistent with a role for *dERR* in supporting glycolytic flux, GC-MS analysis showed that *dERR* mutants accumulate glycolytic intermediates and display decreased levels of TCA cycle intermediates. Interestingly, despite a conserved role in promoting glycolysis in both larval and adult stages, the majority of genes that change expression in *dERR* mutants do so in a stage-specific manner. Our current studies are focused on determining the molecular mechanisms and tissue-specific functions by which *dERR*maintains proper lipid stores for adult metabolic homeostasis.

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584 The role of RNA-binding protein alan shepard in whole organism metabolism regulation. *C. Gillette*, T. Reis, K. Hazegh Division of Endocrinology, Metabolism, and Diabetes, University of Colorado Anschutz Medical Campus, Aurora, CO.

Metabolism is an integrated, multi-organ process, and is best studied within the context of the whole organism. Mounting evidence points to an important yet poorly understood role for genetic background in the control of organismal fat levels. We previously used an unbiased genetic screen to identify 66 genes that when mutated increase body fat in *Drosophila* larvae. One such gene, the RNA-binding protein alan shepard (*shep*), has no characterized role in metabolism. We used tissue-specific RNAi to determine in which organs *shep* is required for regulation of organismal fat. We find that knockdown of *shep* in the brain phenocopies the high-fat phenotype of the mutant and drives changes in the complex metabolic behaviors of feeding and activity. Additionally, knockdown of *shep* in the fat body results in a lean phenotype accompanied by a modest increase in locomotor activity. Shep's genomic locus codes for 8 mRNA and 6 protein isoforms. Thus far, we have shown that fat body-specific overexpression of the Shep-RE isoform increases organismal fat levels, suggesting a positive associate between Shep-RE expression and the fat body's propensity to store fat. Neuron-specific overexpression of Shep-RE has no phenotype, suggesting that Shep isoforms have tissue-specific functions in metabolism. We further find that Shep transcript and protein levels are regulated in

a nutrient-dependent manner. As the nutritional content of the diet increases, *shep* mRNA and protein expression decreases in the brain. This suggests that *shep* is regulated by a nutrient sensing pathway. Our work is now focused on determining the role of shep isoforms in different organs in regulating overall organismal energy metabolism.

585 Regulation of lipogenesis by *Kr-h1*-mediated acetylation of fatty acid synthase during *Drosophila* larval development. *T. Miao*, H. Bai Iowa State University, Ames, IA.

Lipogenesis, a tightly regulated process, is crucial for animal growth and development. Although it has been widely acknowledged that hormone signaling pathway and transcriptional regulation of lipogenic genes play important roles in lipid metabolism, it still remains unclear how post-translational modifications (PTMs) regulate lipogenesis and lipolysis. Using a FLAG-tag knock-in fly line, we are able to directly monitor the PTMs of endogenous fatty acid synthase (FASN1), the key enzyme for de novo lipogenesis. Interestingly, we find that the acetylation level of FASN1 proteins varies with developmental stages and peaks at 96h after egg laying (AEL), which is correlated to high fatty acid synthase activity and fast larval growth. We also noticed that FASN1 is a short-lived protein with a half-life around 6 hours. The FASN1 protein is ubiquitinated throughout larval development and was mainly modified by K48-linked polyubiquitination, especially at wandering and pupal stages. We recently identified transcription *factor Kruppel-like homolog 1* (*Kr-h1*) as the key regulator linking lipid metabolism to larval development in Drosophila. Here we find that loss-of-function Kr-h1 mutants exhibit decreased FASN1 enzyme activity and FASN1 acetylation, and increased K48-linked polyubiquitination during larval development. In addition, the mRNA expression of two lysine acetyltransferases (KATs) genes *Nej* and *Tip60* are significantly down-regulated in *Kr-h1* mutants, which may contribute to the reduction of FASN1 acetylation in *Kr-h1* mutants. Intriguingly, knockdown of *Nej* accelerates the degradation of FASN1 protein, suggesting that the acetylation of FASN1 may stabilize the protein by inhibiting ubiquitin-mediated proteasome degradation. Taken together, our results suggest that the acetylation of FASN1 may play an important role in fatty acid synthesis and larval development, and *Kr-h1* is one of the key regulators that promote fatty acid synthesis and the acetylation of FASN1 through the transcriptional activation of lysine acetyltransferases.

586 The Regulation of Lipid Metabolism by Heterogeneous Nuclear Ribonucleoproteins (hnRNPs) in *Drosophila*. *J.K. Bhogal*[1], A. Nagengast[2], J.R. DiAngelo[1] 1) Penn State Berks, Reading, PA; 2) Widener University, Chester, PA.

The storage of excess nutrients as triglycerides is essential for all organisms to survive when food is scarce; however, metabolic diseases may arise when triglyceride storage is altered. Yet, the mechanisms by which triglycerides are stored are not completely understood. Genome-wide RNAi screens in cultured cells have identified genes that are important in the regulation of triglyceride storage. One group of genes identified in these screens that our lab is interested in is those involved in mRNA splicing. Our lab has identified a number of splicing factors important for regulating triglyceride metabolism; however, the full complement of splicing proteins involved in achieving metabolic homeostasis is unknown. Heterogeneous nuclear ribonucleoproteins (hnRNPs), RNA binding proteins that inhibit the splicing of introns by preventing the assembly of splicing complexes, have no established metabolic functions. To assess any metabolic functions of hnRNPs, we used the GAL4/UAS system to induce RNA interference (RNAi) to six hnRNP's: hnRNP-K, rumpelstiltskin (rump), smooth (sm), Hrb27C, Hrb98DE, and Hrb87F specifically in the *Drosophila* fat body. Decreasing the levels of *hnRNP-K* and *rump* resulted in a decrease in the amount of triglyceride stored per cell as compared to their controls, whereas decreasing the levels of *sm*, *Hrb27C*, and *Hrb98DE* resulted in an increase in the amount of triglyceride stored per cell. Hrb27C-RNAi flies also had an increase in the number of cells per fat body contributing to the excess triglyceride storage phenotype. To further understand the mechanisms by which Hrb27C controls fat storage, qPCR was performed to determine whether the expression of three metabolic enzymes, CPT1, brummer lipase (bmm), and fatty acid synthase (dFAS), was altered. Hrb27C-RNAi fat bodies had a decrease in bmm levels, suggesting that the triglyceride accumulation phenotype observed in these flies is due to reduced lipid breakdown. Together, these results suggest that the hnRNP family of splicing factors have varying metabolic functions and may act on specific metabolic genes to control their expression and processing.

587 The Role of SR Protein Kinases in Regulating Lipid Metabolism in *Drosophila*. *J.P. Mercier*[1], A. Nagengast[2], J.R. DiAngelo[1] 1) Penn State Berks, Reading, PA; 2) Widener University, Chester, PA.

The survival of animals during periods of limited nutrients is dependent on the organism's ability to store lipids during times of nutrient abundance. However, the increased availability of food in modern western society has led to an excess storage of lipids resulting in a number of metabolic diseases. In order to better understand the genes involved in regulating lipid storage, a genome-wide RNAi screen was performed in cultured *Drosophila* cells and identified several groups of genes involved in controlling lipid droplet formation and storage. One group of genes of interest to our lab includes those involved in mRNA splicing. Our lab has previously shown that a group of splicing factors important for intron/exon border recognition known as SR proteins are involved in controlling lipid storage in *Drosophila*; however, how these SR proteins are regulated to control lipid storage is not fully understood. In *Drosophila*, three SR protein kinases (SRPKs) have been characterized: SRPK, darkener of apricot (doA), and SRPK79D. We used the GAL4/UAS system to decrease the expression of these genes specifically in the adult fat body and then we measured the storage of triglycerides. SRPK and SRPK79D-RNAi flies have lower levels of triglyceride stores and this is due to a decrease in the amount of fat stored per cell, despite having more fat cells when compared to control flies. In contrast, doA-RNAi flies store more triglycerides compared to control animals and this is due to more fat storage per cell. In order to determine if the triglyceride storage phenotypes in the SRPK, SRPK79D and doA-RNAi flies were due to changes in feeding behavior, food consumption was measured using capillary feeding (CAFÉ) assays. Flies with decreased SRPK and SRPK79D levels in their fat bodies eat less, which may be the cause of the decreased triglyceride phenotype. However, doA-RNAi flies also eat less than controls, suggesting that increased food consumption is not the cause of the triglyceride accumulation phenotype in these flies. Together, these findings provide evidence to support the hypothesis that lipid storage is controlled by the phosphorylation of factors involved in mRNA splicing.

588 Phosphorylation controls functions of Lipin in fat and energy metabolism. *S.E. Hood*[1,2], A. Morgan[1,3], E. Nesiama[1], H. Davis[1], H. O'Dell[1], M. Lehmann[1] 1) Department of Biological Sciences, University of Arkansas, Fayetteville, AR; 2) Cell and Molecular Biology Program, University of Arkansas, Fayetteville, AR; 3) University of Arkansas for Medical Sciences, Little Rock, AR.

Lipins are dual-function proteins that act as enzymes in the cytoplasm and as transcriptional co-regulators in the cell nucleus. Cytoplasmic phosphatidate phosphatase activity provided by lipins produces diacylglycerol that serves as a precursor for neutral fats and membrane phospholipids. Nuclear lipins regulate genes involved in energy homeostasis. Both the mammalian lipin 1 paralog and the single *Drosophila* Lipin ortholog are highly phosphorylated proteins. Target of rapamycin (TOR) has been identified as one kinase that contributes to the phosphorylation of both lipin 1 and *Drosophila* Lipin, thereby controlling nuclear translocation. However, other kinases are predicted to phosphorylate serine and threonine residues of unknown functional relevance. We use CRISPR/Cas9 mutagenesis to systematically mutate individual residues or groups of phosphorylation sites of *Drosophila* Lipin to determine their functional importance. First results support our prediction that these sites are of relevance not only for nuclear translocation, but also for the role of the protein in fat storage. Extensive phenotypic characterization of the phosphosite mutants includes analysis of fat body histology and fat content, starvation resistance, potential developmental defects, and longevity. Staining with a Lipin antibody is employed to reveal intracellular distribution of the mutant protein. Mammalian lipins are potential targets of therapeutics in the treatment of obesity. Our data will aid in understanding how activities of these proteins could be specifically modulated.

589 Metabolic characterization of the *Drosophila* E78 nuclear receptor. *S.A. Praggastis*, C.S. Thummel Human Genetics, University of Utah, Salt Lake City, UT.

The alarming prevalence of diabetes and obesity emphasizes the importance of characterizing how metabolic processes are regulated and how their misregulation can lead to disease. Our studies of these pathways focuses on nuclear receptors (NRs), which play a central role in maintaining metabolic homeostasis and normal systemic physiology. Here we describe our functional studies of the E78 nuclear receptor in *Drosophila*. E78 has sequence similarity with both the Rev-erb and Peroxisome Proliferator Activated Receptor (PPAR) subfamilies of mammalian NRs. Our studies suggest that E78 shares regulatory functions with the PPARs and may act as a nutrient sensor to maintain systemic lipid homeostasis in the adult animal.

We used the CRISPR/Cas9 system to generate predicted null mutations in *E78*. Transheterozygous combinations of these mutant alleles have no apparent effect on development and result in reduced female fecundity, as reported earlier in the literature. Metabolic analysis of *E78* mutants revealed that these animals have normal levels of stored triglycerides and glycogen at the entry into metamorphosis. Upon adult eclosion, however, *E78* mutants display moderate decreases in their glycogen stores and mild sensitivity to starvation. Interestingly, this glycogen deficit normalizes in mature *E78*mutant adults, which display severe hypolipidemia. *Drosophila* have two main tissues in which lipids are processed and stored, the intestine and the fat body. By using BODIPY to stain for neutral lipids and examining tissues using confocal microscopy, we found that *E78* mutants have a reduction in the number and size of lipid droplets in the upper region of the intestine, as well as a more mild reduction of lipid stores in the fat body. Interestingly, our earlier studies have shown that the E78 ligand binding domain is active within tissues that are high in lipids including the embryonic yolk, oenocytes, and fat body. Taken together, these observations support the model that *E78* senses dietary lipids and regulates lipid uptake, processing, and/or storage within the adult intestine. My current studies are focused on defining the molecular mechanisms by which E78 maintains adult physiology and metabolic homeostasis in *Drosophila*.

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590 Investigating the regulating metabolic kinases and phosphorylation of the SR protein 9G8 in *Drosophila*. *Y. Patel*[1], J.R. DiAngelo[2], A. Nagengast[3] 1) Biology, Widener University, Chester, PA; 2) Science, Penn State Berks, Reading, PA; 3) Biochemistry & Chemistry, Widener University, Chester, PA.

Obesity is a growing epidemic affecting over a third of the United States population yet the underlying causes are not very well understood. Obesity is associated with excess storage of triglycerides in lipid droplets inside of adipose cells. Studies have shown that knockdown of splicing factors such as SR proteins in cell culture resulted in altered lipid droplet formation. Specifically in *Drosophila*, the knockdown of the SR protein 9G8 in the larval fat body resulted in a significant decrease in triglyceride levels, but increased levels when knocked down in adult female fat body. Previous work indicated that the knockdown of the TRN-SR shuttle protein resulted in significantly elevated triglyceride levels as well. SR proteins are activated for nuclear shuttling by phosphorylation of Serines found in the SR domain. We hypothesize that phosphorylation activates 9G8 for its function related to splicing and subsequent role in triglyceride storage. Potential kinases that would activate 9G8 include Doa, mTORC, and SRPK2. Previous studies of the regulation of lipid metabolism by mTORC and SRPK2 signaling investigated the effects of knockdown of several SR proteins on lipogenic gene expression in human cell culture, however, they did not test 9G8. In this project, we will determine whether 9G8 is activated by SRPK2 signaling, and the effect of phosphorylation on triglyceride storage in *Drosophila melanogaster*. To investigate this, commercially available antibodies will be used to determine the phosphorylation status of 9G8 in different RNAi knockdown backgrounds.

591 Determining whether the SR protein 9G8 binds directly to CPT1 mRNA, affecting alternative splicing in lipid metabolism. *T. Kash*[1], J.R. DiAngelo[2], A. Nagengast[1] 1) Biochemistry, Widener University, Chester, PA; 2) Science, Penn State Berks, Reading, PA.

Obesity, a condition that is projected to increase over the years, currently affects over a third of adults in the United States. Excess energy intake through diet is the main cause of obesity. However, there are genetic, physiological, and environmental factors that also make a person more prone to obesity. There is evidence that alternative splicing occurs in the genes that are involved with triglyceride storage. Alternative Splicing is a post-transcriptional process where introns, non-coding sections of RNA, are removed and exons are ligated together. The role of alternative splicing in the regulation of genes related to obesity is mostly unknown. The beta-oxidation gene Carnitine palmitoyltransferase I (CPT1) is known to be alternatively spliced by the SR protein 9G8 in *Drosophila melanogaster*. Previous research shows that when 9G8 is knocked down in adult female fat bodies of *Drosophila*, triglyceride levels increase. It was discovered that there are 9G8 binding sites on CPT1 mRNA, but it is unknown whether 9G8 actually binds directly to CPT1 mRNA. Therefore, the goal of this project is to determine if 9G8 directly binds to CPT1. To determine if there is direct binding, full length and truncated versions of 9G8 will be tagged using a Gateway entry clone and GST destination vector. Finally, through an RNA-binding immunoprecipitation analysis, it will be determined if 9G8 directly binds to CPT1 mRNA.

592 Probing a *Drosophila* model of diet-induced obesity and type 2 diabetes. *L. P. Musselman* Biological Sciences, Binghamton University, Binghamton, NY.

We use overnutrition to study the pathophysiology and biochemistry of obesity. High-sugar-fed larvae and flies exhibit a number of complications associated with obesity including hyperglycemia, hyperlipidemia, cardiovascular disease, increased infection susceptibility, gut barrier dysfunction, and reduced lifespan. To better understand the mechanisms underlying these phenotypes, we use a combination of genetics, genomics, and metabolomics. These studies have identified a number of genes that are required in the fat body for the tolerance of high sugar feeding. Taken together, these genes tell a complex story of how the fat body helps protect animals from the adverse effects of chronic high-sugar feeding.

593 Screen for central regulators of metabolism. *S. Wyler*[1], C. Yip[1], C. Limboy[1], S. Lee[1], A. Rothenfluh[2], J. Elmquist[1] 1) Division of Hypothalamic Research, University of Texas Southwestern Medical Center, Dallas, TX; 2) Department of Psychiatry, The University of Utah, Salt Lake City, UT.

The increasing prevalence of obesity and adult-onset diabetes represents a major health crisis in the United States. These disorders result from a complex interaction of multiple genetic, environmental, and behavioral factors. Human genetic studies and animal models have identified numerous metabolically important genes. We are using the power of Drosophila genetics to identify new neuronal regulators of metabolism.

The mammalian hypothalamus consists of multiple nuclei (clusters of neurons) that are required for the regulation of metabolic homeostasis. One nucleus, termed the ventromedial hypothalamus (VMH), facilitates glucose homeostasis and adaptations to metabolic challenges, such as high fat diet and exercise. A majority of VMH neurons express the orphan nuclear receptor, steroidogenic factor (SF-1) (fly ortholog *ftz-f1*), which is required for both the development and metabolic function of this nucleus. Identification of genes regulated by SF-1 will facilitate our understanding of the VMH.

We have developed a strategy to identify potential SF-1 transcriptional targets, and to test if their Drosophila orthologs are important regulators of metabolism. We crossed *UAS-RNAi* lines (Bloomington Stock Center, Bloomington, Indiana; Vienna Drosophila Resource Center, Vienna) to *nsyb-Gal4* to generate neuroendocrine specific knockdown of these putative target genes. As an initial assessment of metabolic disruption, mated male and female flies were subjected to a starvation assay (n=48/group). Approximately 15% of genes showed at least a 20% reduction in survival time. Additionally, 15% percent showed a 20% or more increase in survival time. Positive hits were subjected to a second and third round of screening. Targets include genes involved in FGF and BMP signaling, GPCR activity, and protein phosphatases. Interestingly several genes showed a sex-specific effect on starvation resistance. A few targets were selected to further assess their role in lipid and trehalose stores. To identify the specific neuronal circuits that regulate these metabolic effects, we used a

panel of Gal4 drivers to target the expression of the candidate genes in subsets of Drosophila neurons. We have shown that this combined approach, of both Drosophila and mouse genetics, can be used to uncover novel genes involved in the central regulation of metabolism.

594 Characterization of possible phosphoglycolate phosphatase orthologs in *Drosophila melanogaster*. *J.A. Kennell*, M.A. Melo, D. Moskop Biology, Vassar College, Poughkeepsie, NY.

Even though metabolic enzymes can be quite specific in their substrate recognition, they are also able to bind to other substrates, sometimes producing toxic side products that must be degraded to allow normal biological functions to continue. Recently a highly conserved enzyme, Phosphoglycolate phosphatase (PGP), has been identified as a "guardian angel" of sorts in mice, human cells, and yeast, by converting a few toxic side products produced during glycolysis and the repair of oxidative induced DNA damage into non-toxic forms. Without PGP these cells die. Yet little work has been done on the function of PGP in animals outside of mice and humans, specifically in the fruit fly *Drosophila melanogaster*. Based on amino acid sequence similarity, we have identified two previously uncharacterized genes, *CG5567* and *CG5577,* as potential *PGP* orthologs in flies. Interestingly only one *PGP* gene has been identified and studied in mammals; we are interested in determining the specific role of each of the two possible orthologs in fly metabolism and whether they demonstrate an example of redundancy (i.e., both genes play similar roles) or whether over evolutionary time they have become specialized and carry out different functions. Using RNA interference, we have found that partial loss of *CG5567* and *CG5577* throughout the entire body increases the sensitivity of adult flies to ethylene glycol, which when metabolized by the flies produces one of the toxic by products we would predict accumulates if PGP were not cleaning them up. We have generated null mutants for *CG5567* and these flies die during early to late pupation. We are currently characterizing these mutants and will present data from rescue experiments to determine if re-expression of *CG5567* or *CG5577* in certain tissues can rescue the lethality in these mutants.

595 When an oncometabolite isn't an oncometabolite: endogenous L-2-hydroxyglutarate production is common among Dipteran larvae. *N. Mahmoudzadeh*, H. Li, J. Tennessen Department of Biology, Indiana University Bloomington, Bloomington, IN.

The oncometabolite L-2-hydroxyglutarate (L-2HG) has emerged as a potent regulator of metabolism, chromatin modifications, and cell differentiation. While this compound is associated with severe neurometabolic disorders, renal cell carcinoma, and glioblastoma, L-2HG is also produced by healthy cells under standard physiological condition. The endogenous functions of this compound, however, remain poorly understood. Previous work from our lab demonstrated that *Drosophila* larvae accumulate high concentrations of L-2HG (>2mM) under normal growth conditions, thereby suggesting that this compound serves an endogenous function during larval development. To further explore this possibility, we used an evo-devo approach combined with GC-MS-based metabolomics to determine if L-2HG accumulation is a common feature of insect development. Our analysis revealed that L-2HG accumulation is common among Dipteran larvae, such as blow flies and mosquitos. Considering that Dipteran larvae have evolved to grow in moist, hypoxic conditions, we are now examining the possibility that flies, like mammalian cells, accumulate L-2HG in response to oxidative stress.

596 Modeling Metabolic Disregulation in Disease: a causative agent and therapeutic target. *M. Tipping*, E. Arcand, K. Neville, K. O'Connell, A. Piatelli, M. Spinney Biology, Providence College, Providence, RI.

Metabolism is altered in many forms of disease, especially cancer. Our lab focuses on three forms of cancer that have shown promise in metabolic disregulation: glioblastoma, Acute Myeloid Leukemia (AML), and ovarian cancer. To study metabolism in disease we have focused on Isocitrate dehydrogenase (IDH), a metabolic enzyme that is often mutated in two aggressive forms of human cancer, glioblastoma and AML. We have modeled this in *Drosophila melanogaster*by overexpressing the homologous mutation in hemocytes, and observe an increase in the number of hemocytes present in *IDH*mutant larvae. Currently, we are performing a methylation inhibition screen to investigate if epigenetic fluctuations due to *IDH*mutant expression is the cause of this phenotype. Ovarian cancer cells have been shown to exhibit Warburg effect phenotypes as well as preferred-Oxidative respiration phenotypes. To study metabolic changes in ovarian cancer, we are performing a border cell migration suppressor/ enhancer screen in *D. melanogaster*ovarioles. We hypothesize that metabolic phenotypes will be present when key genes are inhibited or overexpressed due to the relation to ovarian cancer in humans. Once key genes are identified, drug response that enhances or fixes metabolic phenotypes can be investigated. We hope to combine all aspects of this project to more fully understand *IDH*'s role in metabolism and epigenetic regulation in disease, as well as exploit metabolic changes for treatment of cancers.

597 Characterization of metabolic defects in *Drosophila melanogaster* due to Insulin-signalling impairment. *J.R. Riesgo-Escovar*, Jéssica Álvarez-Rendón Developmental Neurobiology, Instituto Neurobiologia, UNAM, Queretaro, Queretaro, MX.

Fly homozygous null mutations in insulin signaling are lethal, but milder reductions in insuling signaling delay development, final organ and adult size, and lead to hyperglicemia. We characterized defects in flies consequence of insulin signalling defects throughout adult development and aging using viable heteroallelic mutants. We measured in both sexes and at different ages total levels of lipids and sugars, feeding behavior, activity, circadian rhythm and sleep using*InR, Dp110,*and *dS6K* heteroallelic mutants and controls with the same genetic background.

We consistently observed a decrease in mutant flies' weight. *InR* mutants have higher activity levels and more severe defects, particularly carbohydrate levels; *dS6K* mutants show less activity and higher lipid levels. *Dp110* mutants show no changes in activity levels, but metabolic defects in the first post-eclosion days. No differences were observed in the amount of total food consumed under normal conditions. *InR* mutant females show starvation resistance and changes in food intake at lnger time periods.Longitudinal studies characterizing the defects consequence of low insulin signaling as the flies age are scarce. This study sets the basis of defects stemming and developing from insulin signaling defects.

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598 The Drosophila Estrogen-Related Receptor acts as a nutrient sensor to coordinate larval growth with nutrient availability. M. Sterrett, S. St. Clair, *M. Maniex*, J. Tennessen Department of Biology, Indiana University, Bloomington, IN.

All growth during the Drosophila life cycle is restricted to larval development, when animals increase their body size ~200-fold over the course of four days. To support this exponential growth, larvae rely aerobic glycolysis, a unique metabolic program ideally suited to synthesize biomolecules from carbohydrates. Our previous work demonstrated that aerobic glycolysis is transcriptionally-activated during embryogenesis, when the Drosophila ortholog of the strogen-Related Receptor (ERR) class of nuclear receptors coordinately up-regulates genes involved in glycolysis, the pentose phosphate pathway, and lactate production. We have discovered that dERR activity is not restricted to embryogenesis; rather, dERR also promotes aerobic glycolysis during larval development. Our preliminary analyses demonstrate that dERR protein is expressed in key metabolic tissues, including the fat body, intestine, and muscle. This expression pattern suggests that dERR coordinates glucose-derived biosynthesis with growth conditions. Consistent with this model, we have discovered that the dERR ligand-binding domain (LBD) is activated when larvae are fed a yeast-based diet but not sugar-only or starvation media. Furthermore, the addition of insulin to larval organ cultures fails to activate the dERR LBD, suggesting that dERR represents a novel mechanism for linking dietary conditions with sugar metabolism. We have also determined that dERR is covalently modified under starvation conditions, suggesting that a nutrient-sensitive enzyme controls dERR activity. Finally, we have demonstrated that many dERR target genes are transcriptionally down-regulated upon starvation, indicating that diet-induced changes in dERR activation are

functionally significant. Overall, our studies indicate that dERR promotes aerobic glycolysis in response to dietary compounds and suggest that mammalian ERRs also act as nutrient sensors that coordinate biosynthesis with rapid growth.

599 Drosophila larvae maintain NAD+ redox balance by coordinately regulating lactate and glycerol-3-phophate metabolism. H. Li, M. Sterrett, G. Chawla, A. Luhur, R. Massey, C. Gosney, A. Burton, *M. Rai*, J Tennessen Indiana University, Bloomington, IN.

The dramatic growth that occurs during *Drosophila* larval development requires the rapid conversion of nutrients into biomass. In response to these biosynthetic demands, larval metabolism exhibits the hallmark features of aerobic glycolysis, a metabolic program ideally suited to synthesize macromolecules from carbohydrates. Central to the biosynthetic potential of aerobic glycolysis is lactate dehydrogenase (LDH), which promotes glycolytic flux by regenerating NAD^+. To further explore the role of LDH in this metabolic program, we used a metabolomics approach to determine how *Ldh* mutations influence the larval redox state. Our analysis revealed that although *Ldh* mutants accumulate elevated NADH levels, larvae compensate for this metabolic insult by increasing glycerol-3-phosphate (G3P) production, which serves as a backup mechanism to regenerate NAD^+. Furthermore, we not only demonstrate G3P synthesis serves a previously underappreciated role in maintaining larval NAD^+ levels, but also reveal that the cooperative regulation of lactate and G3P metabolism imparts metabolic robustness on the larval glycolytic program. Lack of G3P dehydrogenase (Gpdh1) and dLDH together, exhibit developmental delays, synthetic lethality and aberrant carbohydrate metabolism. This synthetic lethality indicates that a cooperative relationship between Ldh and Gpdh1 is required for the systemic regulation of larval metabolism. However, it remains to be explored if this relationship is cell non-autonomous. Considering that human cells also activate G3P synthesis upon LDHA inhibition, our findings hint at a conserved mechanism that controls redox balance during animal development.

600 A SGLT1-like protein regulates glucose absorption in *Drosophila* midgut. *Y. Li*[1], H. Bao[1], H.Y. Lim[1], W.D. Wang[2] 1) Department of Physiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 2) Department of Medicine, Section of Endocrinology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

It has been well known that sodium glucose cotransporter SGLT1 is highly expressed in the apical membrane in mammalian enterocytes and plays a pivotal role in active absorption of glucose. However, SGLT1-like glucose transporters have not been reported in *Drosophila*. Therefore, our study is aimed at establishing the *Drosophila* SGLT1-like proteins in order to have a better understanding of intestinal glucose transports in fruit fly.

dSLC5A5 has been previously reported to belong to *Drosophila* sodium/solute cotransporter (SLC5A) family whose members show 24%-30% amino acid identities to human SLC5A. Analysis of amino acid sequences and protein secondary structures reveals high similarity between dSLC5A5 and SGLT1. Therefore, we became very interested in investigating the metabolic functions of dSLC5A5. We found that ubiquitous knockdown (KD) of *dSLC5A5* with RNAi caused significant reduction of whole-body glucose level in flies compared to the controls on normal diet (ND) or high sugar diet (HSD). Interestingly, flies with intestinal specific inhibition of *dSLC5A5* simulated whole-body *dSLC5A5-KD* flies in managing systemic glucose content, indicating the midgut could be an important organ for the gene to maintain glucose homeostasis. We therefor postulated that dSLC5A5 could be involved in glucose absorption in the intestine. To address that, we measured the intestinal glucose content as wel as using a fluorescence glucose analog 2-NBDG to monitor glucose uptake into the enterocytes with RNAi-mediated KD of *dSLC5A5*. Our results show that silencing *dSLC5A5* in the midgut notably impaired the ability of dietary glucose absorbance in the fly relative to the control. In addition to the loss of function study of *dSLC5A5*, we also generated a transgenic fly that carries a fusion protein dSLC5A5-FLAG. Utilizing immunofluorescence staining, we observed that FLAG signal presented in both the cytoplasm and apical membrane of fly enterocyte and treatment of high glucose or proteasome inhibitor could trigger dramatically higher level of the fusion protein in the plasma membrane, suggesting that luminal glucose level affects trafficking or internalization of dSLC5A5 and lysosomal pathway may be involved in the process. Importantly, opposite to the intestinal *dSLC5A5-KD* flies in which uptake of dietary glucose was suppressed, flies that express dSLC5A5-FLAG only in their midguts exhibited enhanced glucose absorption.

To conclude, our study revealed a previously uncharacterized role of dSLC5A5 in regulating glucose uptake in *Drosophila* midgut.

601 Triglyceride lipase *brummer* is required for spermatogenesis. *Chien Chao*, Yanina Y. Pesch, Guy Tanentzapf, Elizabeth J. Rideout Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, BC, CA.

Spermatogenesis is an energetically demanding process. In *Drosophila*, several pathways that are required for the regulation of growth, proliferation, and differentiation during spermatogenesis are known to be key regulators of metabolism. Yet our knowledge of the downstream metabolic processes targeted by these pathways during spermatogenesis remains incomplete. Here, we identify a requirement for triglyceride lipase *brummer* (*bmm*) in spermatogenesis. Normally, *bmm* expression is low in cells near the hub of the testis. This low-level *bmm* expression allows the accumulation of lipid droplets, the storage organelle for triglycerides. As the germline exits the transient amplifying stage, *bmm* expression rapidly increases, and lipid droplets are eliminated. In males lacking *bmm* function, however, lipid droplets accumulate throughout the testis, a defect that is accompanied by a severe reduction in the number of spermatids in the testis, and decreased fertility. Together, our data suggests a model in which the correct regulation of triglyceride metabolism in the testis is essential for spermatogenesis.

602 Impact of bacteria on the nutritional content of fly food. *D.N.A. Lesperance*, N.A. Broderick Molecular and Cell Biology, University of Connecticut, Storrs, CT.

Fermentation is a transformative process in which microbes break down complex substrates into easily digestible components and end products. While humankind has exploited fermentation for food production, the process occurs in nature to the advantage of organisms such as *Drosophila melanogaster*. *Drosophila*, which naturally associates with decomposing fruit, contributes to fermentative processes in the fruit via dispersal of its microbiome, which includes both yeasts and bacteria such as *Lactobacillus* and *Acetobacter*. This in turn results in production of metabolites that are desirable to the fly. While this symbiotic fermentative process is essential to fly health in nature, it has not been explored how the presence of these microbes, namely the bacteria, in typical fly diets impacts fly nutrition or whether the bacteria themselves may serve as a nutritional source. In this study, we examine how bacteria present in multiple fly diets concretely impact macromolecular content of food, including protein, carbohydrates, fat, calories, ash, and moisture. We analyzed a natural fly diet of grapes and three artificial fly diets (including Bloomington Standard and Bloomington CMY diets) for nutritional content, both when sterile and after incubation with typical *Drosophila* microbiota members. We report little nutritional impact of bacteria when provided a diet rich in nutrients, including both in grapes and artificial diets. While the microbes do significantly reduce available carbohydrates in the food due to microbial metabolism, protein levels are surprisingly unchanged, suggesting that bacteria do not contribute significantly to protein nutrition in the fly under nutrient-rich conditions. We also find a significant increase in moisture content of food after incubation with the bacterial cocktail, which may have important implications for fly health. Together, our results fill a basic gap in understanding about the role of the *Drosophila* bacterial microbiome on fly nutrition in a natural setting.

603 Male fecundity is plastically optimized by nutrient conditions. *M. Matsuka*, A. Kubo, H. Nakagoshi Graduate School of Natural Science and Technology, Okayama.Univ, Okayama-shi kita-ku, Okayama-ken, Japan.

Nutrient conditions affect reproductive potential and life span of many organisms through the Insulin signaling pathway. Although this is well-characterized in female oogenesis, nutrient-dependent regulation of fertility/fecundity in males remains unclear. Seminal fluid components synthesized in the accessory gland are required for high fecundity in Drosophila males.

The accessory gland is composed of two types of binucleated cell: ~1000 main cells and ~60 secondary cells (SCs). The transcription factors Defective proventriculus (Dve) and Abdominal-B are strongly expressed in adult SCs, whose functions are essential for male fecundity.
We found both of these expressions were down-regulated in nutrient-poor conditions. In addition, nutrient conditions during the pupal stage mainly affected the size and the number of SCs. These morphological changes clearly correlated with fecundity, suggesting that SCs act as a nutrient sensor.
Furthermore, under normal diet conditions in the adult stage, the reduced size of SCs was recovered to the normal one. Our results show that physiological changes of SCs regulate male fecundity in a highly plastic manner depending on nutrient conditions.

604 Indirect exposure to yeast doubles starvation survival in *Drosophila*. *Y. Luo*, Jacob Johnson, Scott Pletcher Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI.

All organims interact with their environment to gain information that is relevant to their fitness, including food availability, mating opportunities, and indications of danger. Multiple strategies are utilized for this purpose, such as canonical sensory perception, cell-autonomous nutrient sensing pathways, and direct chemical reactions with environmental compounds. Our lab has discovered that the smell of food and the detection of individuals of the opposite sex through gustatory cues can induce profound effects on health and lifespan. The mechanisms underlying these effects are largely unknown. We report here the results of efforts to identify new interactions between *Drosophila* and their environment and to investigate the underlying molecular mechanisms through which they influence physiology.
Using a new high-throughput survival assay, we discovered that starvation resistance is nearly doubled when *Drosophila* are exposed to live yeast without the ability to directly contact it. Surprisingly, we found that canonical olfactory pathways are not required for this effect. Loss of the widely-expressed odorant co-receptor *Orco*, which leaves flies broadly anosmic, or loss of the two ionotropic receptors *Ir25a* and *Ir8a*, which are expressed in olfactory neurons, failed to eliminate the increase in starvation resistance upon indirect yeast exposure. Furthermore, smell blind flies that lack *Orco*, *Ir25a*, *Ir8a*, and *Gr63a* as well as flies with their antenna and maxillary palps removed, also exhibited an increase in starvation comparable to control animals when indirectly exposed to live yeast. Other environmental factors, such as humidity, are not required, but may promote, the effects of live yeast exposure on starvation resistance.
Our results indicate that the increased starvation resistance following indirect exposure to live yeast relies on either sensory perception through an undiscovered mechanism or direct chemical alteration of the animal's internal milieu. Ongoing experiments are designed to elucidate the relevant environment cues that emanate from life yeast and to identify the compounds and biological mechanisms that are required for their effects.

605 Insecticides impair energy homeostasis providing new insights into the worldwide crash of insect populations. *F. Martelli Soares da Silva*, T. Rupasinghe, U. Roessner, T Perry, P. Batterham BioSciences, University of Melbourne, Melbourne, VIC, AU.

Around the world insects are disappearing at an extraordinary rate, posing a threat to ecosystems and our way of life. The widespread use of insecticides has been suggested to be a major factor in this. While most insecticides target the insect brain and impact behavior, the basis of their toxicity is poorly understood. Using *Drosophila,* we examined the profile of lipids in larvae exposed to the neonicotinoid imidacloprid and the macrolactone spinosad. These insecticides created unique lipidomic fingerprints but sharing an overall depletion of lipids involved in energy homeostasis and mitochondrial function. Confocal microscopy showed fat accumulation in fat bodies and reduction in Malpighian tubules and midguts. Measurement of lipid levels in hemolymph indicated lipid mobilization. The double reporter strain, ARE-GFP TRE-red, indicated activation of JNK and Nfr2 signaling in anterior midgut after insecticide exposure. Reduced toxicity and lipid accumulation was observed in flies treated with antioxidant prior to insecticide exposure. Flies mutant for the spinosad target nAChR-Dα6, resistant to spinosad, displayed a disturbed lipid environment after imidacloprid exposure, but not spinosad exposure. Thus, the observed lipid changes depend on signals from Dα6 in the brain. Unexposed mutant flies have increased lipid levels compared with controls, indicating that this might be protective. The observed set of disturbances support the hypothesis that a systemic disruption in energy metabolism could be causing energy loss, contributing to insect death. These findings provide new insights into insecticide toxicity and could help explain why incidentally exposed insects, such as honeybees and butterflies, are disappearing.

606 Sustaining mitochondrial genome integrity and robustness with age. *P. TSAI*, P. O'Farrell Biochemistry and Biophysics, UCSF, San Francisco, CA.

Mitochondrial genomes compete with each other to contribute to future generations and to populate the somatic tissues of each individual. Selective forces determine the successful competitors and the quality of their genomes. We monitored the balance of two mitochondrial genomes, one functional and one not, in various tissues and at different life stages of heteroplasmic flies. Purifying selection favored the functional genome, while the dysfunctional genome enjoyed a replicative advantage. Development to the adult was accompanied by a decline in the relative abundance of the functional genome, a sign that there is a reduction in the impact of purifying selection during aging. Unexpectedly, decreasing the dose of *tamas*, the gene encoding the mitochondrial DNA polymerase (POLG), delayed declines in the functional genome. This benefits the flies by slowing deterioration in behavior and reducing neuronal cell loss. Our observations show that there are changes in the forces of selection acting on mitochondrial genomes at different life stages, and by increasing the persistence of purifying selection we show that weakened selection contributes to organismal decline with age.

607 Nicotinamide riboside rescues the exercise capacity of a *Drosophila* model of Barth syndrome. *D.J. Damschroder*, Robert Wessells Physiology, Wayne State University School of Medicine, Detroit, MI.

Barth syndrome is a mitochondrial disorder characterized by cardiomyopathy, skeletal muscle weakness, and exercise intolerance. This disorder is caused by mutations in the *tafazzin* gene, which codes for a transacylase that remodels the mitochondrial phospholipid cardiolipin. Mutations in *tafazzin*lead to disruptions in cardiolipin remodeling and changes in the stoichiometry of cardiolipin species within the mitochondria, leading to mitochondrial dysfunction, muscle weakness and exercise intolerance.

Endurance training has been shown to help patients with mitochondrial disorders by improving cardiorespiratory health. Due to the severity of their exercise intolerance, most Barth patients gain only minor benefits from endurance exercise when compared to patients with other mitochondrial disorders. If the exercise capacity of Barth patients increases, then the benefits from training, like preserved cardiac function, are likely to as well. Thus, finding methods of improving the exercise tolerance of Barth patients is imperative because of the transformative potential exercise has to improve the lives of Barth patients.

Using a *Drosophila* exercise training protocol developed in our lab, we confirmed that *Drosophila tafazzin* mutants also have significant deficiencies in endurance, and fail to improve speed, endurance or flight capacity after exercise training. Further, we find that *tafazzin* expression is required in both muscle and neuronal tissue for normal endurance and exercise adaptations to occur.

With a view toward uncovering novel therapies, we tested currently available pharmaceutics that we hypothesized could improve the exercise tolerance of *tafazzin* mutants. Nicotinamide riboside (NR) is an orally available precursor to nicotinamide adenine dinucleotide (NAD^+). NAD^+ is an essential co-enzyme needed for glycolysis and oxidative phosphorylation. Increasing NAD^+ with nicotinamide riboside improves mitochondrial function in several model systems, but its effects on exercise intolerance in Barth patients is unknown. Here, we show that NR supplementation can rescue the exercise intolerance of *Drosophila tafazzin* mutants and that chronic nicotinamide riboside supplementation can increase exercise adaptations in mutants without affecting wild type flies. These results suggest that NR supplementation has potential to positively impact mobility, health, and quality of life of Barth patients.

608 Functional studies of the evolutionarily-conserved mitochondrial protein ADCK1 in *Drosophila*. D Wisidagama, *S Thomas*, C Thummel Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT.

The Aarf Domain Containing Kinases (ADCKs) are evolutionary conserved nuclear-encoded proteins that localize to mitochondria. They are predicted to act as mitochondrial kinases involved in lipid homeostasis. Abc1/Coq8 is the best characterized ADCK family member. It is part of a multiprotein complex that is involved in the biosynthesis of Coenzyme Q (CoQ), which contributes to electron transport. ADCK3 and ADCK4 are thought to be involved in phospholipid homeostasis and CoQ stability. In contrast, functions for ADCK1 remain poorly understood. To investigate possible roles for ADCK1 in animal physiology, we generated a null mutant for this locus using CRISPR/Cas9 technology. Null mutants for *Drosophila ADCK1* display larval lethality and double mouthhooks, which are hallmarks of molting defects, often associated with reduced levels of the steroid hormone ecdysone. *ADCK1* does not, however, appear to impact systemic sterol levels since we cannot rescue the mutants with dietary ecdysone. Additionally, these mutants display tracheal breaks and the tissue-specific re-expression of *ADCK1* in trachea is sufficient to rescue their larval lethality. These tracheal rescued adults, however, have a reduced lifespan compared to the ubiquitous expression of wild-type *ADCK1* in *ADCK1* mutants. This suggests that *ADCK1* has important roles in tissues outside the trachea. Taken together, our studies in *Drosophila* establish an important role for *ADCK1* in tracheal development, larval molting, and adult lifespan. Moreover, these phenotypes are consistent with a role for this predicted mitochondrial kinase in sterol modifications and/or intracellular lipid trafficking. Future studies will investigate the specific lipid and sterol species altered in the absence of *ADCK1* function to understand how this predicted mitochondrial kinase contributes to systemic metabolism in *Drosophila*. This work is supported by the NIH (R01CA228346).

609 A germline signaling relay triggers mitochondrial respiration during early oogenesis essential for mitochondrial inheritance. *Z-H. Wang*, Y. Liu, H. Xu Systems Biology Center, National Heart, Lung, and Blood Institute, NIH, Bethesda, MD.

Mitochondria play critical roles for eukaryotes by hosting a number of biosynthetic pathways, including ATP production, to support cellular activities. In most metazoans, as mitochondria are exclusively transmitted through maternal lineage, mothers need to deposit sufficient mitochondria, together with mtDNA, into mature oocytes to power the early embryogenesis. However, it is still unclear how the female produces numerous mitochondria and mtDNA during oocyte growth. In the *Drosophila* ovary, active mtDNA replication initiates upon the completion of germ cell division in the germarium and highly depends on mitochondrial electron transport chain (ETC) activity. Here, we find that ETC activity show the same developmental pattern as mtDNA replication and is regulated through its gene expression to prime the onset of mtDNA replication in the germarium. To investigate the developmental cues involved, we perform a candidate RNAi screen and uncover that reduction in Myc or either insulin or JNK signaling impairs ETC activity in the germarium. Myc up-regulation in the late germarium defines the pattern of ETC biogenesis and mtDNA replication. Furthermore, insulin signaling is also enhanced at the same developmental stage as Myc and forms a feed-forward loop with Myc to maintain ETC activity and mtDNA biogenesis. Unexpectedly, a transient JNK activation in the late germarium controls ETC activity and mtDNA replication by triggering InR expression to support the self-maintained insulin-Myc loop. The female germline was previously shown to preferably replicate functional mtDNA in active mitochondria over lethally mutated mtDNA enriched in defective mitochondria to limit the transmission of lethally mutated mtDNA to the next generation. We find that the JNK-insulin signaling relay ensures selective mtDNA inheritance, mtDNA deposition, and female fertility. Altogether, our studies demonstrate a development regulation of mitochondrial biogenesis that links oocyte development to mitochondrial inheritance.

610 The ribonuclease activity of PPR domain in mitochondrial RNA polymerase is required for priming mitochondrial DNA replication. *Yi Liu*, Hong Xu National Heart, Lung, and Blood Institute, National Institute of Health, Bethesda, MD.

Mitochondrial genome is a compact, circular, double-stranded DNA, which encodes the key components of the oxidative phosphorylation complexes. Thus, DNA replication and transcription of the mitochondrial genome are paramount for energy metabolism and their dis-regulations lead to various disorders. The transcription and DNA replication in the mitochondria are carried out by a set of dedicated proteins encoded by nuclear genome. However, the molecular identity of mitochondrial DNA (mtDNA) replication primase remains elusive. We here present that the *Drosophila*mitochondrial RNA polymerase (mtRNApol) serves not only to generate the mitochondrial transcripts but also the RNA oligos needed for mtDNA replication initiation. *In vitro* mtRNApol synthesizes two populations of transcripts: a group of long transcripts spanning the full length of template DNA, and a group of short transcripts of 20 to 100 nt, which can prime the replication of DNA template in presence of DNA polymerase. MtRNApol is a pentatricopeptide repeat (PPR) domain protein, a large family of poorly-characterized proteins which localize in mitochondria and chloroplast. Our data defines that the PPR domain of mtRNApol possesses 3'-5' exoribonuclease activity. A point mutation in PPR domain abolishing mtRNApol nuclease activity retains its ability to synthesize long transcripts but fails to synthesize short RNA primers. We also find that the PPR domain of human mtRNApol has exoribonuclease activity, suggesting a conserved function of PPR domains in mtDNA maintenance, mitochondrial transcription and RNA metabolism.

611 Altered pheromone biosynthesis is associated with sex-specific changes in life span and behavior in Drosophila melanogaster. *J.H. Bauer*[1], Nithya Joseph[2], Nateshwar Elphick[2], Sasha Mohammad[2] 1) Dept of Chemistry, California State University Sacramento, Sacramento, CA; 2) Dept of Biological Sciences, Southern Methodist University, Dallas, TX.

Many insect behaviors, including foraging, aggression, mating or group behavior, are tightly regulated by pheromones. Recently, it has been shown that pheromones may influence extreme longevity in the honeybee *Apis mellifera*, while changes in pheromone profile have been observed during ageing in *Drosophila melanogaster*. These data suggest a potential link between the pheromone system, behavior and longevity in insects.
Here, we investigate this potential link by examining changes in behavior and longevity in fruit flies with altered pheromone profiles. We demonstrate that oenocyte-specific reduction of desaturase activity is sufficient to dramatically alter the composition of the hydrocarbon mix displayed by the flies. In addition, flies with altered desaturase activity display changes in fecundity and stereotypical mating behavior, and, importantly, extended longevity. These data provide evidence for a potential link between hydrocarbon synthesis and life span, and suggest that longevity may be influenced by behavior.

612 The use of the Drosophila Genetic Reference Panel to map Genes and Gene Networks underlying High Fat Diet-induced Mortality. *Bridget Konadu*, Sumitkumar Patel, Matthew Talbert University of Louisiana at Monroe, LA.

Specific treatments aimed at preventing the onset of obesity-related disease are few. There is interest in identifying molecular mechanisms influencing the transition from "healthy obese" to "diseased obese," which could be identified by examining lifespan on a high fat diet (HFD) in *Drosophila melanogaster.* The Drosophila Genetic Reference Panel (DGRP) was employed to conduct a genome-wide association study of lifespan upon a HFD. Twenty mated female flies of each line from the DGRP (193 lines) were obtained by synchronously crossing virgin female of the lines with Bloomington stock 1 male flies over 2 days on normal media. Flies were kept on HFD at equal density and humidity at 23°C on a 12 hour light/dark cycle. Deaths were recorded daily to determine average lifespan. Over 2 million SNPs with MAF >5% were used to perform a genome-wide association study via the DGRP pipeline. Top ranking genes were tagged by SNPs associated with P-values of magnitude 10^{-5} or less. Two of these candidate genes were further assayed for energy homeostasis parameters using the GAL4-UAS system and knockout mutants. For *RdgA*, we found that *RdgA* knockout mutants increased lifespan on normal media but knockout was highly lethal for flies exposed to a HFD. RdgA whole-body knockdowns did not show a change in lifespan on HFD, though they did exhibit increased triglyceride storage. However neuron specific knockdown of RdgA resulted in a significant increase in lifespan on HFD. *Pvf3* whole-body knockdowns showed increased starvation

resistance on HFD and a highly increased triglyceride storage on normal media. Thus far, our attempts to validate GWAS candidates indicate a complex and context-specific involvement for these genes in longevity during HFD.

613 Conserved role of uric acid and purine metabolites in longevity and healthspan. *T.A. Hilsabeck*[1,2], K.A. Wilson[1,2], J.N. Beck[1,3], C.S. Nelson[1], R.B. Brem[1,2,4], P Kapahi[1,2,3] 1) The Buck Institute, Novato, CA; 2) University of Southern California, Los Angeles, CA; 3) Department of Urology, University of California San Francisco, CA, USA; 4) Department of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA, USA.

High uric acid levels, or hyperuricemia, has been correlated with aging-related frailties and diseases such as hypertension, dyslipidemia, type-2 diabetes, cardiovascular disease, and kidney stones in clinical cohort studies. Uric acid, the end product of purine degradation, builds up with both age and a nutrient-rich diet and has also been linked with premature death and higher all-cause mortality risk. This is a particular problem in the west where there is an overabundance of food, with hyperuricemia afflicting over 20% of the US population. Despite being a risk factor for increased morbidity, the genetic and mechanistic causes of increasing uric acid levels are not well known. Tests of unknown genetic associations have previously been performed utilizing several human cohorts identifying SNPs in human FOXO3, IGFR1, and PTEN as associated with altered uric acid levels or gout incidence, but *in vivo* tests have not been performed to validate these genes. This is primarily due to the lack of applicable animal models, which has led to relatively few new therapeutics being developed. To uncover these mechanisms, I performed a *GWAS* of multiple uric acid-related metabolites measured in 174 different fly strains from the *DGRP* collection on two different diets and identified 5 novel genes associated with their accumulation. Further, we identified that inhibition of various genes in the insulin like signaling (ILS) pathway dramatically reduced uric acid concretion formation and uric acid load through inhibition of NADPH Oxidase (NOX) and ROS formation. This work identifies the ILS pathway to be an important modulator of purine metabolism and a potential therapeutic target for hyperuricemia and associated aging-related related pathologies. These novel genes and other genes from human *GWAS* studies were validated using our lab's fly model for uric acid accumulation, showing that knockdown of these genes plays a role in the downstream phenotype of concretion formation due to uric acid elevation. Our lab has developed a powerful approach to identify novel associations between genes and purine metabolite levels in the fly, and then examine their conservation in human studies to provide insight into pathways that regulate human lifespan and healthspan.

614 Genetic screening to identify factors that regulate lifespan and homeostasis of intestinal stem cells during aging in Drosophila. *S. Naito*, H. Nishida, SK. Yoo RIKEN Center for Biosystems Dynamics Research, Kobe, JP.

How lifespan of organisms is regulated is a fundamental and unresolved question. Towards the end of life, homeostasis of tissue stem cells becomes aberrant, occasionally leading to tumorigenesis. In Drosophila, gut dysplasia induced by intestinal stem cell (ISC) proliferation during aging is one of the factors that define lifespan. To identify the components that regulate lifespan and intestinal stem cell homeostasis during aging, we performed an EMS-based genetic screen. First we measured lifespan of 960 EMS stocks and also their susceptibility to oncogenic stress induced by artificial tumors. We found that the flies that have the longest lifespan tend to be sensitive to oncogenic stress: they die readily if artificial cancer is generated. We hypothesized that these long-lived flies may not demonstrate tissue dysplasia that normally occurs during aging and that it is why they live the longest. To test this hypothesis, we further examined lifespan and ISC homeostasis during aging in both male and female animals of the top 10 long-lived stocks. We discuss correlation between lifespan and ISC homeostasis during aging in these long-lived flies. Our final goal is to identify the genes that are responsible for long lifespan and ISC homeostasis during aging. This works provides a fresh insight into how lifespan and tissue homeostasis are regulated during aging.

615 Regulation of Lifespan by dSirt6 in *Drosophila melanogaster*. *Jackson Taylor*, Jason Wood, Chengyi Chang, Matthew Finn, Julianna Liu, Stephen Helfand MCB, Brown University, Providence, RI.

Sirtuins (SIRT1-7 in mammals) are a family of NAD+ dependent deacylases which regulate multiple cellular pathways involved in the aging process. SIRT6, in particular, has emerged as a key regulator of longevity, with roles in DNA repair, metabolism, and chromatin modification. Sirt6 knockout mice are short lived, while overexpression (OE) of Sirt6 extends lifespan in male mice. Despite its involvement in many age-related pathways, the precise molecular mechanisms by which SIRT6 extends lifespan are not well understood. Here, we use the powerful genetic tools of Drosophila melanogaster to investigate the role of SIRT6 (dSirt6 in Drosophila) in regulating lifespan. We find whole-body OE of dSirt6 extends lifespan in flies, up to 38% in males and 39% in females, while RNAi knockdown of dSirt6 shortens lifespan. This lifespan-extending effect is repeatable in multiple OE systems, including both constitutive and inducible driver lines. Interestingly, OE in the fat body alone is sufficient to extend lifespan. dSirt6 OE also improves survival of both young and old flies treated with paraquat, an inducer of DNA damage. dSirt6 OE alters chromatin *in vivo* in the form of reduced of H3K9 and H3K56 acetylation, at both young and old ages. This finding suggested altered transcriptomic state, which led us to perform RNA-sequencing. RNA-seq experiments demonstrate that dSirt6 OE leads to differential expression of several hundred genes in the brain and fat body. Most notably, dSirt6 OE attenuates age-related increases in immune genes, while also reducing ribosomal gene expression and maintaining proteasomal gene expression during aging – all processes associated with aging and longevity. Finally, dSirt6 OE counteracts the age-related increase in transposable elements (TEs) while dSirt6 knockout larvae exhibit greatly increased TE expression, supporting the role of dSirt6 in repression of TEs and the importance of this function in the aging process. Together, these data demonstrate that dSirt6 OE extends organismal lifespan through multiple mechanisms, including enhanced DNA repair and diverse regulation of gene expression.

616 Associated microorganisms as methionine sources or sinks that influence *Drosophila melanogaster* longevity. *H. Wilcox*, J. Chaston Plant and Wildlife Sciences, Brigham Young University, Provo, UT.

Drosophila melanogaster is a model for studying animal aging, and experiments in *Drosophila* have helped expand our understanding of lifespan-extension. For example, animals fed on a calorie- or methionine-restricted diet display increased longevity compared to full-fed controls. The basis for microbial influence on lifespan has also been a topic of recent interest, although the molecular mechanisms for these influences are not well established. To better understand how associated microorganisms are related to *D. melanogaster* lifespan we screened the lifespan influence of 41 strains of genome-sequenced bacteria and performed metagenome-wide association to identify bacterial genes that are predicted to influence fruit fly longevity. An analysis of the most significant genes identified cysteine and methionine metabolism as the sole significantly enriched pathway among the top significant hits. To confirm these predictions, we measured the influence on *Drosophila* lifespan of bacteria bearing mutations in methionine metabolism genes. The results supported that numerous bacterial methionine metabolism genes influence *D. melanogaster* longevity. To better understand how these genetic influences are related to nutrient cycling in the fly we measured the metabolomes of aging flies and their diets. In young flies the bacterial mutants influenced the levels of numerous metabolites, including those in glucose and methionine metabolism. In old flies a smaller set of metabolites were influenced, including methionine. We propose a model where bacteria that function as methionine sinks tend to promote lifespan; whereas if the bacteria are sources of methionine fly longevity is restricted. We are currently collecting additional metabolite data on aging flies reared with additional mutants to test this idea.

617 Octopamine Receptors *OAMB* and *Octβ2R a*re Required in Muscle for Exercise Adaptations. *Alyson Sujkowski*, Robert Wessells Physiology, Wayne State University, Detroit, MI.

Endurance exercise is an effective therapeutic intervention with substantial pro-healthspan effects. Male *Drosophila* respond to a ramped daily program of exercise by inducing a conserved suite of physiological responses similar to those seen in mice and humans. The activity of octopaminergic neurons is sufficient to induce the conserved cellular and physiological changes seen following endurance training. All 4 octopamine receptors are required for at least

one aspect of the exercise response, but only one, *Octβ2R,* is required for all of them. Here, we used the gene-switch Gal4 system in order to achieve adult-specific knockdown of alpha- and beta-adrenergic octopamine receptors in several target tissues. We determined that reduced expression of either *OAMB* or *Octβ2R* in adult muscles abolishes exercise-induced improvements in endurance, climbing speed, flight, cardiac performance and fat-body catabolism in male *Drosophila.* Perhaps surprisingly, *OAMB* expression in the heart is also required cell-nonautonomously for adaptations in other tissues, such as adipose tissue and skeletal muscle. These findings indicate that activation of specific adrenergic receptors is required for *Drosophila* endurance exercise, and suggest the possibility that secreted, cell non-autonomous factors from cardiac and/or skeletal muscle play a key role in exercise adaptations.

618 Effects of histamine signaling on the pH gradient in both larval and adult gut of *Drosophila melanogaster*. *S. Plaska*[1], K. Tekiela [2], D. Beachnau[1], M. G. Burg[1,2] 1) Biomedical Sciences, Grand Valley State University, Allendale, MI; 2) Cell & Molecular Biology, Grand Valley State University, Allendale, MI.

The study of histamine and its function in *Drosophila melanogaster* has been primarily focused on its role as a neurotransmitter used by photoreceptors and its effects on a number of other behaviors, such as grooming, thermal preference, and sleep[1]. However, the study of histamine's function elsewhere in the fly has been hampered by lack of information regarding the location of histamine in tissues outside the nervous system. Histamine's location in the gut has been reported to be close to regions where the gut pH becomes more acidic, with four distinct regions of the larval gut containing detectable levels of histamine: one in the foregut, two in the midgut, and one in the hindgut region[2]. We have focused on one of the regions in the larval anterior midgut known as the copper cell region, where copper cells are located that are thought to be functionally analogous to the acid-secreting parietal cells of the vertebrate stomach[3]. By adding 0.8% bromophenol blue to the food that flies are reared on, the pH gradient was examined along the entire excised gut from larvae and adult flies. Wild-type (*Oregon-R*) flies exhibited the previously described acidic regions[4], including regions of the foregut, anterior midgut (copper cell region), as well as hindgut, with the region close to the copper cell region being the most acidic. However, larval and adult gut from either the histamine-deficient Hdc^{JK910} or Hdc^{P211} mutants demonstrated a more basic pH in the copper cell region when compared to wild-type larval or adult gut. The regions whose pH is affected by the lack of histamine normally appear to contain histamine in wild-type flies, determined by histamine immunofluorescent staining and confocal microscopy. To determine whether histamine signaling plays a role in acid regulation, ort^{P306}larval and adult gut was examined in a similar manner using 0.8% bromophenol blue in food and was shown to have a pH gradient alteration similar to that observed in the Hdc^{JK910} and Hdc^{P211} mutants. Since the ort^{P306} mutant has normal levels of histamine but induces a defect in the *hclA* histamine receptor[5], this result implies that histamine signaling may be a requirement for maintaining acidic gut regions in both larvae and adults. These results provide evidence of another physiologic system in *Drosophila melanogaster* that is regulated by histamine signaling, which may now be amenable as a tool to use in examining the genetic regulation of gut pH.

Citations:

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619 Nora virus proliferates in dividing intestinal stem cells and affects *Drosophila* fitness and susceptibility to intestinal bacterial infections and abiotic stress. Samantha Haller[1,2], Adrien Franchet[1,2], Vincent Barbier[1,2], Laurent Daeffler[1,2], Stefanie Becker[1,2], Kwang-Zin Lee[1,2], Igor Orlov[1,3], Danièle Spehner[1,3], Jean-Luc Imler[1,2], *Dominique Ferrandon*[1,2] 1) Université de Strasbourg, Strasbourg, France; 2) IBMC, CNRS UPR 9022, Strasbourg, France; 3) Department of Integrative Structural Biology, IGBMC, UMR 7104 du CNRS, U964 INSERM, Illkirch, France.

In our studies of bacterial intestinal infections, we were intrigued that Oregon-R wild-type stocks maintained by different investigators in the laboratory displayed varying susceptibilities to *Pseudomonas aeruginosa* or *Serratia marcescens*. A key observation was a correlation between the presence of Nora virus, a picorna-like virus, with the more susceptible stock. Bleaching of the infested stock made the stock less sensitive to the intestinal infections, whereas contaminating the clean stock with a pure preparation of the Nora virus re-established the enhanced sensitivity, thus establishing that Nora virus indeed sensitizes flies to intestinal infections. We failed to detect an effect in several models of systemic infections.

Nora virus is present in many laboratory stocks, with a high variability of its burden, and has been reported to be harmless with no effects on fly fitness. We found that in our conditions it does affect lifespan, intestinal barrier function and homeostasis, as well as microbiota load. We shall further provide extensive evidence that the proliferation of the virus depends on the proliferation of intestinal stem cells.

We conclude that Nora virus has important effects on the physiology of the intestinal epithelium that likely lead to altered fitness of the flies. Thus, it is important to control for the absence of Nora when performing studies on *Drosophila* fitness, intestinal infections, and also the microbiota, as it may be a confounding factor potentially accounting for some of the discrepancies reported in the literature between different laboratories.

620 The role of microRNA-33 in adult *Drosophila* phenotypes. *H. Schultz*, J.A. Kennell Biology, Vassar College, Poughkeepsie, NY.

MicroRNA-33 (miR-33) is a well conserved microRNA located within an intron of the *SREBP* gene. While the role of miR-33 in regulating cholesterol homeostasis has been well characterized in mice and humans, less is known about the role of miR-33 in *Drosophila*. We have recently identified miR-33 as a possible negative regulator of adult cuticle pigmentation in *Drosophila.* Loss of miR-33 causes increased abdominal pigmentation while overexpression of miR-33 in the developing cuticle leads to reduced pigmentation. We have previously identified Akt, a key effector of the insulin and TOR signaling pathways, as a positive regulator of abdominal pigmentation. Interestingly, Akt has a miR-33 binding site in its 3' UTR and miR-33 is able to directly target Akt in cell culture based reporter assays. Akt and miR-33 genetically interact as well, suggesting that miR-33 could act as a negative regulator of cuticle pigmentation through its inhibition of Akt and insulin/TOR signaling. We have also shown that nutrition significantly impacts pigmentation levels in miR-33 mutant lines compared to controls, suggesting there is an interaction between miR-33 genotype and nutritional status during development. In addition to its role in pigmentation, loss of miR-33 has been reported to cause loss of stalk cells in the adult ovary, though the phenotype was reported to be incompletely penetrant. We are currently exploring the role of miR-33 in the adult ovary and whether manipulation of miR-33 expression causes cell autonomous changes in the numbers of these stalk cells and whether age and nutritional status impacts this phenotype.

621 Cellular heterogeneity underlying poly-functional fat body tissue in Drosophila melanogaster. *Vanika Gupta*, Brian Lazzaro Cornell university, Ithaca, NY.

The insect fat body is a multifunctional tissue that can serve as a generic model for how poly-functional organs achieve diversified tasks, including management of immune response to infection. Fat body functions span those of at least three vertebrate organs: immune system,adipose tissue and liver. The day body is the primary systemic immune organ in insects, but also serve as the metabolic control organ responsible for storage and release of lipids. This is analogous to vertebrate adipose tissue which is a cellularly heterogeneous tissue. We hypothesize that cellular heterogeneity in the fat body allows subsets of cells to specialize in each function, collectively resulting in tissue with highly varied capabilities. We are using single-cell RNA sequencing on the 10X platform to

test the hypothesis of cellular heterogeneity in drosophila fat body. Using tissue-specific drivers, we have tagged adult fat body cells which will be sorted using FACS. We seek to identify cell subpopulations within the fat body which are tesponsible for specific functions. We are using flies Which remain either challenged or unchallenged with a gram-negative bacteria Providencia rettgeri, both while engaged in egg development and in the absence of reproductive investment. We will use this data to understamd how poly-functional tissues balance competing physiological functions, providing mechanistic understanding for the classical life history tradeoff between immunity and reproduction.

622 Tumor-derived Upd3 mediates muscle dysfunction and lipid loss in Drosophila. Xiaoxiang Xiang[1], Guangming Ding[1], Yanhui Hu[2], Richard Binari[2], Norbert Perrimon[2], Stephanie Mohr[2], *Wei Song*[1] 1) Medical Research Institute, Wuhan University, Wuhan, Hubei 430072, CN; 2) Department of Genetics, Harvard medical School, Boston, MA 02115, USA.

Using yki-tumor-bearing flies that mimic human cancer-associated cachexia and develop severe host wasting, including muscle dysfunction and lipid loss, we have demonstrated tumor-derived ligands (ImpL2 and Pvf1) as conserved pathogenic factors of host wasting. In order to explore novel regulators of tumor-host interaction, we developed PathOn, a new software analyzing canonical pathways and the matching ligands. PatON analysis indicated that Upd3, the ligand for Jak/Stat signaling, is greatly induced in yki-tumor midgut by more than 20 folds, while targets of the JAK/STAT pathway are upregulated in muscles, suggesting that Upd3/Jak/Stat signaling may play a role in organ communication. We uncovered that Upd3 removal or blockade of Jak/stat signaling in the yki tumors significantly terminates tumor growth. To investigate the role of tumor-derived Upd3 in host wasting, we induced an activate Hop (TumL) in yki-gut tumors to maintain Jak/Stat signaling and, meanwhile, knocked down Upd3 expression. Strikingly, muscle dysfunction and lipid loss were remarkably alleviated without affecting yki-tumor growth. Biochemical and genetic measurements indicate that Upd3/Jak/Stat signaling regulates functions of muscle and adipose tissues via, at least, impairment of insulin responses. Thus, our results demonstrate that yki-tumors produce Upd3 to couple self-growth and host wasting.

623 Circadian control of ecdysone biosynthesis and release during *Drosophila* metamorphosis. *J. Cavieres-Lepe*[1,2], J. Ewer[1] 1) Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, CL; 2) Programa de Doctorado en Ciencias, mención Neurociencia, Universidad de Valparaíso, CL.

Circadian clocks impose daily periodicities to many behaviors and physiological processes in many different organisms. In *D. melanogaster,* adult emergence is controlled by the circadian clock, which restricts emergence to a specific window of time. This gating of emergence depends on the activity of the central circadian pacemaker in the brain and of a peripheral clock located in the prothoracic gland (PG), which produces the molting hormone, ecdysone. Recently, we have shown that central brain clock neurons communicate with the PG clock by transmitting time information to PTTH neurons via the neuropeptide sNPF; the PTTH neurons in turn communicate with the PG via the PTTH neuropeptide. However, the exact role of the circadian clock in PTTH signaling and the production and release of ecdysone is currently unknown. We found that the genes involved in ecdysone biosynthesis, *spook, spookier* and *nvd* contain an "E-Box" in their promotor region, suggesting a possible role in the circadian regulation and we currently are evaluating their circadian expression prior to adult emergence. As a separate approach, we are determining if ecdysone is rhythmically released from the PG during metamorphosis.

Establishing the cellular mechanism by which PTTH modulates ecdysone signaling in the PG during circadian adult emergence could serve as a paradigm to understand how daily steroid hormone rhythms are generated in animals.

624 TIMELESS phosphorylation regulates light entrainment and resetting of the *Drosophila* circadian clock. *Y. Cai*, C. Ochoa, C. Truong, K. Murphy, D. Wilson, J. Chiu Entomology and Nematology, University of California Davis, DAVIS, CA.

Circadian clocks interpret time cues from external environment and orchestrate temporal organization of physiology and metabolism in organisms from all kingdoms of life. In the *Drosophila* clock, the protein TIMELESS (TIM) plays a key role in sensing light, which is regarded as perhaps the strongest zeitgeber for circadian entrainment. *tim*^{0} mutants are arrhythmic in circadian output behavioral rhythms and are impaired in light-dependent circadian entrainment and resetting. At the molecular level, light triggers rapid proteasomal degradation of TIM proteins by promoting its interaction with the intracellular blue-light receptor CRYPTOCHROME (CRY). Previous studies reported that light-dependent TIM degradation is dependent on TIM phosphorylation. However, TIM phosphorylation sites that are important for mediating light responses have not been identified to date. We performed affinity purification of TIM proteins from fly tissues followed by mass spectrometry and identified 8 TIM phosphorylation sites. Many of these phosphorylation sites are located in characterized functional domains. To facilitate characterization of TIM phosphorylation events, we generated transgenic flies expressing non-phosphorylatable serine/threonine to alanine *tim*mutants. A number of these *tim*mutants display altered circadian output behavioral rhythms and/or impaired responses to light. In this presentation, we will focus specifically on the functional characterization of TIM(S1404) phosphorylation. Blocking TIM(S1404) phosphorylation results in more stable TIM proteins in flies, suggesting that TIM(S1404) may function to mediate light-dependent circadian entrainment and resetting. In summary, our results provide new insights into the mechanisms by which site-specific TIM phosphorylation regulate light entrainment and resetting of the circadian clock through the control of TIM stability.

625 Effect of Circadian Desynchrony on Body Triglyceride Content in *Drosophila*. H.L. Soeder, *A.M. Yu* Department of Biology, University of Wisconsin - La Crosse, La Crosse, WI.

Shift work is defined as a work schedule at odds with the typical human sleep-wake cycle. Shift work schedules may be irregular and involve substantial exposure to light during subjective night. Long-term shift work is associated with elevated risk of obesity and type 2 diabetes in humans. However, it is not clear whether this increased risk is due to associated behavioral risk factors or to a physiological effect of the shift work schedule itself. The goal of this study was to determine whether long-term exposure to a light:dark cycle similar to that experienced by human shift workers will alter triglyceride levels in fruit flies. If a simulated shift work schedule alters triglyceride levels in flies, that would indicate that flies could potentially be used as a novel model system for shift work associated metabolic disorders in humans.

w^{1118} flies were raised at defined density (60 larvae per food vial) for two generations on standard cornmeal-agar medium without added yeast. Upon eclosion, second generation adult male flies were housed in fresh food vials (10 flies per vial) in temperature-controlled incubators set to either a 12:12 light:dark cycle (control flies) or a 6:6 light:dark cycle (shift work flies). Flies were maintained on these schedules for 9 days. Triglyceride content was measured in aged control and shift work flies via colorimetric assay. To account for individual differences in body size, triglyceride content was normalized to total protein content.

Results showed a trend towards decreased triglyceride content in flies subjected to a simulated shift work schedule, although the difference did not reach statistical significance. We conclude that the simulated shift work schedule tested alone is not sufficient to alter body triglyceride content in fruit flies. Studies are currently underway to determine whether flies lacking an endogenous circadian clock will have altered body triglyceride content under simulated shift work schedules.

626 Sestrin, a novel target in the mTOR pathway that extends healthspan and mobility. *T. Cobb*[1], A. Sujkowski[1], M. Kim[2], B. Kim[2], J. Lee[2], R. Wessells[1] 1) Wayne State University, Detroit, MI; 2) University of Michigan, Ann Arbor, MI.

Modern lifestyle, often characterized by over-nutrition and lack of exercise, causes prolonged activation of mTOR complex 1 (mTORC1)/S6K and chronic

suppression of mTORC2/AKT, which together promote age-related muscle pathologies including insulin resistance, fat accumulation, mitochondrial dysfunction and functional decline. Using *Drosophila* as a model organism, we recently found that Sestrin acts as an important regulator of both mTOR complexes, and that the absence of Sestrin brings about several age-associated pathologies including fat accumulation, mitochondrial dysfunction and skeletal/cardiac muscle degeneration. In diverse tissues including skeletal muscle, Sestrin strongly potentiates the activity of mTORC2/AKT signaling, while slightly downregulating mTORC1/S6K signaling. mTORC2/AKT signaling is essential for proper metabolic regulation, as well as for cell survival and growth, while chronic mTORC1/S6K activation can be detrimental to muscle metabolism and physiology. Sestrin dependent regulation of mTOR complexes is important for maintaining muscle health throughout life. We hypothesize that Sestrin can be a novel molecular target in the mTOR signaling network that can limit chronic mTORC1 activation and preserve mTORC2 activity in muscle, thereby promoting life- and healthspan. Transgenic induction of Sestrin in skeletal and cardiac muscle can prevent age-dependent loss of endurance and mobility. Importantly, endurance exercise induces Sestrin expression, while Sestrin deficiency nullifies the effects of long-term exercise in improving mobility and metabolism. These data suggest that Sestrins may mediate the beneficial effects of exercise in preserving muscle health. We reveal the beneficial effects of Sestrin in protection against aging and age-associated functional and structural degeneration of skeletal and cardiac muscle. We identify the Sestrin protein family as a potential therapeutic target for the prevention of age-associated mobility decline, ultimately enabling the development of innovative methods for preserving mobility and improving quality of life in later ages.

627 Neuropeptide F receptor acts in the *Drosophila* prothoracic gland to regulate body size and developmental timing. *J. Kannangara*, M. Henstridge, L. Parsons, C. Mirth, C. Warr School of Biological Sciences, Monash University, Clayton, Australia.

In *Drosophila*, growth is primarily regulated by the steroid hormone, ecdysone. Ecdysone is produced in and secreted from the prothoracic gland in response to various environmental and genetic cues. The pathways which work to connect input from these external factors to ecdysone biosynthesis involve neuropeptides such as the *Drosophila* insulin-like peptides, which work through the insulin signalling pathway in order to regulate ecdysone biosynthesis. Here we identify Neuropeptide F Receptor as a novel regulator of *Drosophila* growth. Knockdown of *Neuropeptide F Receptor* specifically in the prothoracic gland was found to generate a developmental delay and increased body size. Interestingly, while *Neuropeptide F Receptor* mutants also have a developmental delay, they instead have a smaller body size. Likewise, knocking down the ligand to Neuropeptide F Receptor, *Neuropeptide F*, specifically in *Neuropeptide F*-expressing neurons also results in a significant delay to development and smaller body size. The developmental delay seen when knocking down *Neuropeptide F Receptor* in the prothoracic gland can be rescued by feeding larvae ecdysone-enriched food. Furthermore, there is reduced expression of ecdysone biosynthesis genes in these animals, as well as reduced ecdysone levels. These data suggest that Neuropeptide F Receptor is involved in regulating ecdysone biosynthesis in the *Drosophila* prothoracic gland and presents a novel mechanism by which body size and developmental timing is regulated in *Drosophila.*

628 Juvenile hormone mimics phenocopy parasitoid wasp attacks in *Drosophila melanogaster*. *R.F. Spokony*[1,2], C McGrail[1], R Calero[3], S.M. Lee[1], M Sarder[1], E Migunova[4] 1) Natural Sciences, Baruch College, CUNY, New York, NY; 2) Biology, The Graduate Center, CUNY, New York, NY; 3) Macaulay Honors College, CUNY, New York, NY; 4) Biological Sciences, Fordham University, New York, NY.

The common insecticides methoprene and pyriproxyfen are juvenile hormone mimics (JHM). Exposure to JHMs leads to disruption of adult development. Lamellocytes are specialized hemocytes induced after parasitoid attack to capsulize eggs deposited in *Drosophila melanogaster* larvae. Lamellocytes differentiate in the lymph gland, sessile pockets, and from circulating hemocytes. The JAK/STAT pathway is involved in the immune response in vertebrates and invertebrates. Activation of this pathway in muscles following parasitization is required to induce lamellocytes. We found that larval exposure to sublethal doses of methoprene or pyriproxyfen leads to lamellocyte formation in wild caught Drosophila isolines from the DGRP collection and other wild type genotypes. Following methoprene treatment, larval lymph glands are reduced in size, as in parasitized larvae. Formation of lamellocytes is juvenile hormone receptor dependent; no lamellocytes were formed in JHM exposed Met^{Del} $gce^{2.5k}$ larvae. Additionally, the JAK/STAT pathway is activated following methoprene exposure; the DOME-MESO reporter is turned on in the fat body and the 10XSTAT92E reporter is turned on in the muscles. We are currently testing if DOME (the receptor), STAT92E (the transcription factor), and UPD2 and UPD3 (the cytokines), are required in the lymph glands, muscles or fat body for JHM lamellocyte induction.

629 The role and regulation of age-induced polyploidy in *Drosophila*. *A.S. Dehn*, V.P. Losick Mount Desert Island Biological Laboratory, Bar Harbor, ME.

Polyploid cells frequently arise during normal aging and with age-associated diseases, including cancer. It remains poorly understood whether age-induced polyploidy functions as a beneficial tissue repair strategy or a driver of disease. The adult abdominal epithelium of *Drosophila* is composed of post-mitotic, diploid cells that become polyploid with age. We have characterized multiple *Drosophila melanogaster* strains and discovered that polyploid cells begin to form by 20 days of age with approximately 20% of the epithelial cells becoming multinucleated. By 40 days of age, large multinucleated cells, some with 30+ nuclei, are evident and appear to help to maintain epithelial integrity. In addition, we found that epithelial nuclear DNA content does not change and diploidy is maintained, indicating that age-induced polyploidization is a result of cell fusion, and not endoreplication. Taken together, these results and our on-going studies will provide novel insights into how polyploid cells emerge with age and affect tissue function.

630 Measuring hemolymph osmolarity in the "mini-osmometer". *J. Sosa-Pagan*, A. Rodan Dept of Internal Medicine, University of Utah, Salt Lake City, Utah.

Plasma or hemolymph osmolarity is maintained within a narrow range in both flies and humans. Our lab is interested in understanding the molecular basis for iono- and osmoregulation. However, measuring osmolarity of the fly hemolymph is hampered by the small hemolymph volume of individual flies. Here we present a simple and inexpensive freezing point osmometer for measuring hemolymph osmolarity, adapted from the design of Singleton and Woodruff (Dev Biol 161: 154, 1994). It is composed of a small crystallization dish placed inside a larger crystallization dish. Ethanol chilled to about -80 °C is added to the larger dish and cold temperature is maintained with dry ice pellets place inside the dish. To extract the hemolymph from flies, the thoraxes of 15 flies are punctured with a Minutien pin. The flies are then placed inside a 0.65 mL conical vial with a perforation at the bottom made with a 25G needle. This vial is placed inside a 1.7 mL conical vial and centrifuged for 7 mins at 7,000 rpm. *Drosophila* hemolymph is collected from the 1.7 mL vial and loaded into small, thin capillary tubes (0.15 mm ID x 0.25 mm OD), which are placed into the small crystallization dish. Room temperature ethanol is added to the dish to create a temperature gradient from the cold bath in the large dish to the room temperature bath in the smaller dish. The capillaries are video recorded, and the time it takes the hemolymph to freeze is measured and correlated to osmolarity using standards recorded at the same time. We have measured hemolymph osmolarity of 341 ± 61 mOsm in control flies fed normal food, similar to previously published measurements. To measure the sensitivity of our instrument, we subjected flies to a feeding scheme where they were starved (with access to water) for 18 hrs, then fed for 24 hours on normal fly food or fly food supplemented with 300 mM NaCl, followed by 6 hrs in empty vials (dehydration). After these maneuvers, the hemolymph osmolarity of the flies fed normal food prior to dehydration was 175 ± 72 mOsm, whereas the hemolymph osmolarity of flies fed high-salt diet prior to dehydration was 308 ± 60 mOsm ($p < 0.05$). The accessibility of this instrument for measuring hemolymph osmolarity opens the door for the study of molecular aspects of osmoregulation in *Drosophila*.

631 The non-cell autonomous contribution of RAB21 in Drosophila gut renewal. *S. Nassari*, S. Jean Université de Sherbrooke, Department of anatomy and cell biology (Sherbrooke, Quebec).

RAB21 is a small GTPase regulating autophagy through the endolysosomal sorting of VAMP8, a protein required for full autophagosome-lysosome fusion.

Autophagy is an important process in gut homeostasis. Significantly, decreased autophagic flux and mutations in autophagy-related genes are associated with intestinal pathologies. RAB21 was first identified in human intestinal cells, where it localizes at the apical side of enterocytes (Ec), which define the main intestinal cell population. Importantly, in the context of defective intestinal homeostasis, RAB21 protein level is decreased in Ec. These data suggest a putative role for RAB21 in gut homeostasis.

Our goal was to characterize the involvement of RAB21 in Ec, and its influence on gut homeostasis. To answer this question, we used the drosophila gut as a model system, given that it is an excellent model to investigate intestinal biology. The drosophila intestine is composed of intestinal stem cells (ISC) that differentiate into progenitor cells to yield differentiated enteroendocrines (Ee) and Ec cells. Using cell specific temporal inducible system, we monitored and assessed RAB21 gain and loss of function in adult drosophila Ec.

RAB21 silencing led to multiple defects reminiscent of hyperplastic growth. As such, the depletion of RAB21 led to an increased cell density and was associated with a loss of gut permeability. Furthermore, more Ee cells were present in RAB21-depleted gut, while conversely, the proportion of Ec was decreased. These results correlated with a gain in mitotic cells, associated with increased cell death. Interestingly, we found that the UPD3 cytokine was upregulated at the mRNA and protein levels, as well as the activity of the related JAK/STAT signaling pathway. Importantly similar results were obtained using a dominant negative form of RAB21, demonstrating the specificity of the various phenotypes.

Altogether, our data indicate that RAB21 plays an important role in Ec-mediated intestinal homeostasis, presumably through the regulation of Ec survival. Therefore, upregulating RAB21 in Ec could potentially improve intestinal pathologies associated with Ec loss.

632 Role of glucagon-like endocrine pathway of *Drosophila* in metabolism and stress. *L. Kucerova*[1], T. Konikova[1,2], H. Sehadova[1], M. Zurovec[1,2] 1) Biology Centre of the Czech Academy of Sciences, Institute of Entomology, Ceske Budejovice, CZ; 2) University of South Bohemia, Faculty of Sciences, Ceske Budejovice, CZ.

The regulation of energy homeostasis is a fundamental feature of all living organisms. *Drosophila* Adipokinetic hormone (Akh) is a small peptide (8 amino acid residues long), which is produced by specialized cells in corpora cardiaca, regulates metabolic responses to stress and mobilizes energy stores. The evolutionarily closest peptides found in vertebrates are gonadotropin-releasing hormones, but at the functional level, Akh rather resembles mammalian glucagon. *Drosophila* Akh stimulates single G protein-coupled receptor AkhR (CG11325). Null mutant flies in Akh as well as AkhR are obese, resistant to starvation; they have slow metabolic rate and significantly low levels of hemolymph saccharides.

In this work, we compared phenotypes of mutant flies with abolished Akh signalization (Akh^1, $AkhR^1$) as well as the short time effect of ectopic application of Akh. We performed microarray analysis in order to identify genes regulated by Akh. We also compared resistance of flies with altered Akh signalization against a metabolic toxin 2-deoxy-D-glucose and an oxidative stressor paraquat.

Short time effect of single Akh injection brought mostly downregulation of genes expressed in gut and fat body. Majority of influenced genes had metabolic function and we also detected impact on several hormones and neurotransmitters as well as slight downregulation of a few oxidoreductases and detoxification genes. Consistently, the co-application of Akh with oxidative stress toxin paraquat caused higher mortality in control and Akh^1 flies, but not in $AkhR^1$ flies. Mortality of Akh^1 and $AkhR^1$ mutants after paraquat treatment without ectopic application of Akh was comparable with controls and gene transcription analysis of paraquat response in Akh^1 mutants did not bring many significant differences. Suggesting that Akh signalization pathway is not connected directly to oxidative stress response. However, it can interfere with detoxification and stress pathways through its influence on metabolism and energy reserves.

633 Proteasome β5 subunit overexpression improves proteostasis and extends lifespan. N. Nguyen[1], A. Rana[2], C. Goldman[1], R. Moore[4], J. Shen[5], J. Tai[1], Y. Hong[1], D. Walker[2,3], *J. Hur*[1] 1) Dept of Biology, Harvey Mudd College, Claremont, CA; 2) Department of Integrative Biology and Physiology, University of California Los Angeles, Los Angeles, CA; 3) Molecular Biology Institute, University of California Los Angeles, Los Angeles, CA; 4) Dept of Biology, Pomona College, Claremont, CA; 5) Dept of Biology, Pitzer College, Claremont, CA.

The free radical theory of aging attributes the root causes of aging to damage of cell components by free radicals that are generated as an inevitable byproduct of metabolism. One manifestation of accumulating damage to proteins is the formation of ubiquitinated protein aggregates. These have been shown to be actively harmful to cells by binding to and sequestering protein degradation machineries. Accordingly, multiple reports have shown that increasing protein degradation can decrease the presence of such aggregates and extend lifespan. We extend and focus these findings to the Drosophila proteasome β5 subunit during adult stages. Reports in worms and human tissue cultures have shown that overexpression of the β5 subunit is regulatory, and cells respond to the imbalance created by overexpression of the β5 subunit by transcriptional upregulation of the rest of the proteasome subunits. In contrast, while we find that overexpression of the β5 subunit in flies during adulthood only results in significantly increased chymotrypsin-like enzymatic activity, there is no significant increase in transcript levels of the other proteasome subunits. Despite this, overexpression of β5 during adult stages are sufficient to significantly increase mean lifespan. Moreover, the increased longevity does not overlap with dietary restriction mechanisms, significant improvements to physiology, or resistance to severe stresses. Our work suggests that increased chymotrypsin-like activity of the proteasome is sufficient to increase lifespan independent of dietary restriction, significant implications to physiology, or stress resistance.

634 Epigenetic Inheritance of Alcohol Sensitivity in *Drosophila melanogaster*. *J. Abdalla*, M. Manier George Washington University, Washington, DC.

In natural populations, *Drosophila melanogaster* oviposit in fermenting fruit and encounter alcohol at concentrations up to 14%. My research investigates whether alcohol consumption in *D. melanogaster* affected their offspring's ability to resist intoxication. We used Capillary Feeder Assays (CAFEs), where adult males and females were fed liquid media containing sugar, water, and alcohol (A treatment) or sugar and water (N treatment). After six days of CAFE feeding, we set up breeding vials with five males and five females in all possible combinations (F×M): A×A, A×N, N×A, N×N. Progeny were collected as virgins into single-sex vials and assayed two days later for alcohol resistance. For this assay, we recorded the time required for half the flies to become sedated on their backs after exposure to alcohol vapor (time to 50% sedation or ST50). We found that progeny with two alcohol-fed parents had an increase in resistance over the N×N control that was approximately twice that of progeny of one alcohol-fed parent. We found little difference in maternal and paternal effects, and sons and daughters were both affected. This acquired alcohol resistance (AAR) effect was detectable for at least five generations after the initial parental alcohol feeding. Moreover, as little as a single day of alcohol feeding was sufficient to produce AAR in A×A progeny, and as few as three days of alcohol feeding elicited AAR in A×N and N×A progeny as well. AAR continued to be detectable in progeny for the entire reproductive life of the parents, suggesting that epigenetic changes generating this effect are stable over time. Ongoing experiments are equalizing caloric value of the A and N CAFE treatments to eliminate any potential confounding factors due to caloric restriction, and we are evaluating the alcohol metabolism of progeny using an alcohol absorbance assay. Finally, RNAseq of mothers, fathers, and sons and daughters from different treatments will identify candidate pathways that are differentially regulated in response to parental alcohol consumption.

635 Improving enzyme annotation in FlyBase. Phani Garapati, Jingyao Zhang, Alix Rey, *Steven Marygold* FlyBase, Dept. of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, GB.

Drosophila melanogaster has been used as a model system to study enzyme function for over a century, and a substantial proportion (~30%) of its protein-coding genome encodes enzymes. Nonetheless, many *D. melanogaster* enzymes remain unidentified or poorly classified within biological databases, hampering research progress and inter-species comparisons. In order to address these shortcomings, we are systematically reviewing enzyme data obtained from several key databases and the primary literature. After integrating and evaluating these data, we ensure that all verified activities are annotated using the appropriate Gene Ontology (GO) and Enzyme Commission (EC) terms, and provide feedback to the source databases as necessary. To date, we have reviewed 4 major classes (oxidoreductases, lyases, isomerases and ligases), resulting in the annotation of 135 new enzyme-encoding genes and the removal of annotations for 77 erroneous cases. Validated enzyme sets are presented as easy-to-use lists within FlyBase 'Gene Group' reports, while the edits we make to GO/EC annotations ensure the improvements are incorporated within key third-party sites, including UniProtKB, GenBank/NCBI, QuickGO, AmiGO and the recently formed Alliance of Genome Resources.

636 Natural genetic variation in Drosophila contributes significantly to exercise response. *N.C. Riddle*, L.P. Watanabe Biology, University of Alabama at Birmingham, Birmingham, AL.

During the last decade, significant progress has been made to develop different exercise systems and to establish *Drosophila melanogaster* as a model for exercise research. While exercise is widely recognized as an important aspect of a healthy lifestyle, how exercise interacts with genetic background and various environmental factors to produce a phenotypic response are not well understood. We have taken advantage of the genetics tools available in Drosophila to investigate how genetic differences impact exercise levels and exercise response. Exercise levels and outcome measures such as weight and climbing ability were measured in the strains of the Drosophila Genetics Reference Panel 2 (DGRP2). Carrying out genome-wide association studies, we find that hundreds of genetic variants contribute to exercise-related traits. There are large differences between the genetic variants that control these exercise-related traits between the sexes, suggesting that at least part of the genetic networks are sex-specific. Interestingly, despite the fact that increased activity levels and an active lifestyle generally is associated with better health and longevity, in our data set, we find no correlation between the activity levels measures and lifespan. This lack of correlation is evident in basal animal activity levels, and also in induced exercise activity levels. Our results suggest that sex-specific genetic networks control responses to exercise and that the relationships between exercise, healthspan, and lifespan are complex, requiring further study.

637 Regulation of larval growth by *drd*. *L.M. Hopson*, M. Pasawala, E.M. Blumenthal Biological Sciences, Marquette University, Milwaukee, WI.

The mechanisms involved in the determination of growth and development in insects are not completely understood. The gene *drop-dead* (*drd*) is required for normal development in *Drosophila melanogaster* in that *drd* mutants exhibit adult lethality, decreased body mass, malfunctions in different barrier structures, and brain degeneration. We have previously found that the decreased body mass phenotype results from the absence of *drd* expression in the respiratory tracheae. Thus, we want to understand how *drd* expression affects the developmental mechanisms that govern adult body mass. Decreased adult mass has previously been shown to correlate with prolonged larval development. To investigate this relationship with respect to *drd*, we measured the developmental timing of drd^{1} and drd^{lwf} mutants to determine if the decreased adult body mass is also associated with prolonged larval development. We found that *drd* mutants showed a significant extension in the time from egg laying to pupariation from 134 hours in controls to 158 hours in mutants. One potential cause of prolonged development is hypoxia; given the expression of *drd* in the respiratory system, we tested whether *drd* mutant larvae are hypoxic. Two hypoxia-induced genes, *scylla (scyl)* and *charybde (char)*, were measured using qPCR in 2nd instar larvae. *scyl* and *char* expression levels were not significantly different in *drd* mutant larvae vs controls, suggesting that the growth defect in *drd* mutants is not due to hypoxia. To identify other genes that may function together with *drd* in the tracheae to control growth, expression of members of three gene families, cytochrome p450s, juvenile hormone epoxide hydrolases, and the epsilon subfamily of glutathione-S-transferases, which have been shown to have altered levels of expression in *drd* mutant flies, were knocked down by RNAi in the tracheae. Five genes; *GstE8*, *GstE3*, *Cyp6a22*, *Cyp28c1*, and *Cyp9f2* were found to be required in the tracheae for survival to adulthood. *Supported by NSF IOS-1355087 to EMB and by a Ronald E. McNair scholarship to LMH.*

638 Effects of dietary sugar on fruit fly place learning and memory. *M. Ganesan*, E. King, T. Zars Biological Sciences, University of Missouri-Columbia, Columbia, MO.

Developmental, cognitive and metabolic disorders have been prevalent for decades when the consumption of processed food rich in sugar and fat has increased worldwide. Extensive research is available on food-induced metabolic disorders but not on cognitive disorders and decline despite learning and memory being the two most fundamental neurological processes essential for survival. Studying the biological targets of alteration by sugar resulting in learning and memory defects is, therefore, crucial. The aim of this project is to determine the mechanisms underlying a potential detrimental effect of dietary sugar on place learning and memory in *Drosophila melanogaster*. In preliminary experiments, we observed that fruit flies fed sugar had low place learning and memory. Place learning in flies uses the heat-box, where single flies are trained to avoid one part of a chamber that is associated with high temperature. We examined a subset of fly lines, high learning and memory lines, from the Drosophila Synthetic Population Resource (DSPR). The DSPR is a population resource obtained by crossing 15 founder *D. melanogaster* lines from different parts of the world with each other for 50 generations that produced ~ 800 Recombinant Inbred Lines (RILs). DSPR is, hence, a population with high natural variation that renders them useful to study phenotype-genotype relation. There are two treatment groups (dextrose in solution and food spiked with dextrose - HSD) plus a control group. Young adult flies were exposed to normal fly food (control), dextrose in solution, and HSD for four days. Place learning and memory were then tested. Flies that were fed dextrose in solution and the HSD had a significantly lower learning and memory in most cases. Thus, it appears that ingestion of high levels of sugar has a deleterious effect on nervous system function and complex behavior. Gene expression, cellular and molecular studies should provide insights on the mechanism of dietary sugar induced learning and memory deterioration.

639 Identification of Novel Cell-surface Proteins in Axon and Glia Development in the Drosophila Visual System. *Zhengya Liu*[1,2], Yixu Chen[2], Yong Rao[1,2] 1) Integrated Program in Neuroscience, McGill University, Montreal, Quebec, CA; 2) McGill Centre for Research in Neuroscience, McGill University Health Centre, Montreal, Quebec, CA.

Much of our impression of the world is based on sight. Normal vision relies on the proper formation of visual circuits during development. This process is dependent on a series of coordinated cellular movements, specific cell-cell interactions and recognition. The initial recognition between axon and glia during development is a critical step in establishing the correct pattern in visual circuit development. Our understanding of the molecular mechanisms controlling axon-glia interactions during development, however, is incomplete. Drosophila has proven to be an excellent model for understanding visual circuit development and function. Our previous study shows that the transmembrane protein Borderless (Bdl) is specifically expressed in wrapping glia (WG) during visual circuit development and is required for the extension of glial processes and the ensheathment of photoreceptor (R-cell) axons. Bdl functions by interacting with the Ig transmembrane protein Turtle (Tutl) on R-cell axons. However, it remains unknown how the glia-specific expression of Bdl is controlled. Also, loss of bdl does not completely block glial extension and axon ensheathment indicates the involvements of other unknown cell-surface recognition

systems. To identify novel cell-surface players involved in regulating WG and R-cell development, we performed a systematic RNAi genetic screen and identified several interesting candidate genes.

640 N-cadherin orchestrates self-organization of neurons within the columnar unit in the *Drosophila* medulla. Olena Trush[1], Chuyan Liu[1], Xujun Han[2], *Makoto Sato*[1,2] 1) Graduate School of Medical Sciences, Kanazawa University, Kanazawa, Ishikawa, JP; 2) Institute for Frontier Science Initiative, Kanazawa University, Kanazawa, Ishikawa, JP.

Columnar structure is a basic unit of the brain, but the underlying mechanism of its development remains largely elusive. The medulla, the largest ganglion of the fly visual center, provides a unique opportunity to reveal the mechanisms of three-dimensional organization of the columns. In this study, we reveal the donut-like columnar structures along a two-dimensional layer in the larval medulla that evolves to form three distinct layers in pupal development using N-cadherin (Ncad) as a marker. Column formation is initiated by three core neurons, R8, R7 and Mi1 that establish distinct concentric domains within a column. We demonstrate that Ncad-dependent relative adhesiveness of the core columnar neurons regulates their relative location within a column along a two-dimensional layer in the larval medulla according to the differential adhesion hypothesis. We also propose that there is a mutual interaction between three layers during formation of the three-dimensional structures of the medulla columns.

641 Modeling Parkinson's in *Drosophila* using the Rapid Iterative Negative Geotaxis (RING) assay. *Christopher Roblodowski*[1], Tahir Mumtaz[2], Jason Sajan[2], Cyrano Yee[2], Qi He[2] 1) Queensborough Community College, Bayside, NY; 2) Brooklyn College, Brooklyn, NY.

Our previous work has suggested that the *Drosophila* protein Dunc-115L, a homolog of human AbLIM, is involved in axon projection of the CNS and the visual system through its actin binding domain, VHD. Our results are consistent with a potential role of Dunc-115 in neurodegenerative diseases such as Parkinson's as some Parkinson's causing genes identified in humans belong to the actin and actin- binding protein category. A landmark for human Parkinson's patients is the loss of motoneural activities resulting in resting tremors and balance loss. With this in mind, we selected the rapid iterative negative geotaxis (RING) assay to assess motoneural activities in flies by comparing climbing rates. This approach offers a simple and reliable measurement of neuromuscular functions. Our results show that Dunc-115-VHD-deleted mutants have a lower climbing ability versus wild type and this difference became more dramatic as the flies aged. Since these mutants had a significantly reduced neuromuscular function, this demonstrates that the VHD domain is critical for this function. Moreover, these results support the potential role of Dunc-115L in Parkinson's.

642 Conservation of the Netrin receptor Frazzled in insects. *Ben Wadsworth*[1,2], Tim Evans[1] 1) Biological Sciences, University of Arkansas, Fayetteville, AR; 2) Cell and Molecular Biology graduate program.

Axons in the developing embryo receive and react to signals that direct their growth to reach target tissues at specified locations. The signal pathways that direct midline crossing of axons during embryonic development have been comprehensively examined using the *Drosophila melanogaster* ventral nerve cord or the vertebrate spinal cord as a model. A number of these signaling mechanisms are conserved; however, disparities have been found between species either in the general strategy or the molecular signals controlling axonal response to guidance cues. The Netrin-Frazzled/DCC pathway has been shown to aid in midline crossing of axons in the embryonic ventral nerve cord of *Drosophila*. However, it is uncertain if this function of Frazzled is conserved in other insects. The goals of this research are to gain insight into the evolutionary conservation of axon guidance by the Netrin receptor Frazzled (Fra) and to expand our understanding of how Frazzled affects midline crossing in the flour beetle *Tribolium castaneum*.

Our lab has successfully cloned the *frazzled* ortholog from *Tribolium* and employed it in gain of function studies. In these studies ectopic expression of TcFra induces midline crossing in *Drosophila* embryos. We will apply a CRISPR approach to replace the *Dmfra* gene with *Tcfra* in *Drosophila* embryos to assess whether TcFra is sufficient to rescue the loss of endogenous Fra. We will also examine *Tcfra* in *Tribolium* embryos to elucidate the function of TcFra in vivo. We expect the *Frazzled* ortholog in Tribolim (*Tcfra*) to be sufficient for replacing loss of function in *Drosophila* Fra. Additionally, we expect to see a similar function of Frazzled in beetles to those observed in *Drosophila*. These studies will expand our knowledge of axon guidance of midline crossing in a species that does not share some of *Drosophila's* derived guidance characters, ultimately allowing us to see a more ancestral guidance scheme. Therefore, understanding the fundamental mechanisms of midline crossing for differing evolutionary trajectories will provide a clearer understanding of axon guidance in the developing embryo across organisms.

643 Transcriptional regulation of *robo2* in the *Drosophila* embryonic nervous system. *Muna Abdal-Rhida*[1,2], Gina Hauptman[1,3], Stephanie Hood[1,2], Tim Evans[1] 1) Biological Sciences, University of Arkansas, Fayetteville, AR; 2) Cell and Molecular Biology graduate program; 3) Fulbright College Undergraduate Honors program.

During nervous system development, neuronal axons are guided to their synaptic targets by receptors expressed on the surface of the axon. The *Drosophila* Robo2 axon guidance receptor is a member of the evolutionarily conserved Roundabout (Robo) protein family, and controls a number of axon guidance decisions during embryonic development. The various roles of Robo2 depend both on distinct functional domains within the receptor protein, and on the dynamic transcription of *robo2* in various subsets of cells throughout embryogenesis. Thus, understanding how Robo2 regulates distinct guidance outcomes depends in part on understanding how its expression is regulated during embryogenesis.

To determine how *robo2* transcription is regulated in distinct subsets of cells during embryogenesis, we screened a series of 17 transgenic lines in which GAL4 expression is placed under the control of putative regulatory regions derived from DNA sequences in and around the *robo2* gene. We crossed each line to a *UAS-GFP* reporter and characterized GFP expression in the embryonic ventral nerve cord during stages when axon guidance is occurring. We identified two non-overlapping DNA regulatory regions located within *robo2* that can activate transcription in distinct subsets of *robo2*-expressing lateral longitudinal neurons in the embryonic ventral nerve cord, where Robo2 acts to promote formation of longitudinal axon pathways. We also identified two additional regions that can activate transcription in midline cells including midline glia, where Robo2 acts non-autonomously to promote midline crossing of commissural neurons during early stages of axon guidance.

Using these identified regulatory regions, we have built transgenic constructs which allow us to restore *robo2* expression to defined subsets of neurons to test for rescue of *robo2*-dependent axon guidance phenotypes in the embryonic CNS and to distinguish cell-autonomous vs non-autonomous roles of *robo2* in specifying distinct axon guidance outcomes. Our results suggest that *robo2's* dynamic expression pattern is specified by multiple distinct regulatory regions, and that its expression in specific subsets reflects a combination of genetically separable regulatory sequences.

644 Conserved and divergent aspects of Robo receptor signaling and regulation between *Drosophila* Robo1 and *C. elegans* SAX-3. Trent Daiber, *Tim Evans* Biological Sciences, University of Arkansas, Fayetteville, AR.

The evolutionarily conserved Roundabout (Robo) family of axon guidance receptors control midline crossing of axons in response to the midline repellant ligand Slit in bilaterian animals including insects, nematodes, and vertebrates. Despite this strong evolutionary conservation, it is unclear whether the signaling mechanism(s) downstream of Robo receptors are similarly conserved. To directly compare midline repulsive signaling in Robo family members from different

species, here we use a transgenic approach to express the Robo family receptor SAX-3 from the nematode *Caenorhabditis elegans* in neurons of the fruit fly, *Drosophila melanogaster*. We examine SAX-3's ability to repel *Drosophila* axons from the Slit-expressing midline in gain of function assays, and test SAX-3's ability to substitute for *Drosophila* Robo1 during fly embryonic development in genetic rescue experiments. We show that *C. elegans* SAX-3 is properly translated and localized to neuronal axons when expressed in the *Drosophila* embryonic CNS, and that SAX-3 can signal midline repulsion in *Drosophila* embryonic neurons, although not as efficiently as *Drosophila* Robo1. We show that the SAX-3 cytoplasmic domain can signal midline repulsion to the same extent as Robo1 when combined with the Robo1 ectodomain. We show that SAX-3 is not subject to endosomal sorting by the negative regulator Commissureless (Comm) in *Drosophila* neurons in vivo, and that peri-membrane and ectodomain sequences are both required for Comm sorting of *Drosophila* Robo1.

645 Regulation of Axon Targeting in the *Drosophila* Visual System. *Y. Zhang*, Scott Liu, Xin Li Molecular and cellular biology, UIUC, urbana, IL.

Navigation of axons to the correct target area is critical for the formation of neural circuits. Although many axon guidance molecules/receptors have been identified, how they are coordinated to assemble complex neuropils is not well understood. Here we use the Drosophila visual system as a model to address this question and find that guidance molecules and their receptors, Netrins (NetA and NetB) / Unc-5 and Frazzled have critical role in the organization of three of the neuropils of the optic lobe.

646 The role of Rab11 GTPase in neuronal pruning of *Drosophila* sensory neurons. *H.H. Kao*, C.H. Chou, H.H. Lee Institute of Molecular Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan.

Neuronal pruning is an evolutionarily conserved event observed in the developing nervous systems of many organisms to refine neural circuitry. Pruning is a process to eliminate unnecessary dendrites, axons, or synapses in neurons without causing any cell death. Blocking pruning in fly brain mushroom body neurons could abolish short-term courtship memory in adults. However, the underlying molecular mechanism remains largely unclear. During metamorphosis, the peripheral class IV dendritic arborization (C4da) neurons undergo dendrite pruning before regeneration of adult-specific dendrites. Our previous works have identified that IKK-related kinase Ik2 and its downstream signaling mediator Spindle-F (Spn-F) are essential for dendrite pruning of C4da neurons. To further study the molecular mechanism by which Ik2/Spn-F signaling leads to dendrite pruning, we found the co-localization of Spn-F and a small GTPase Rab11 in larval C4da neurons. Rab11 is known to be involved in exocytic and endocytic recycling pathway. We observed pruning defects in neurons with Rab11-RNA interference (RNAi) or dominantly negative (DN) Rab11 expression, suggesting that Rab11 and its GTPase activity are required for dendrite pruning. We also found increased number of neurons with pruning defects in *Rab11* and *spn-F* double mutants when compared with single mutants, showing that there is a genetic interaction between *Rab11* and *spn-F*. Moreover, we found that Ik2 is activated normally in neurons with Rab11-RNAi, revealing that Rab11 mutant does not affect activation of Ik2 and the downstream signaling. In addition to genetic interaction, we also found physical interaction between Spn-F and Rab11 and identified the Rab11-interacting domain in Spn-F. Reintroducing mutant Spn-F without Rab11-interacting domain in C4da neurons of *spn-F* mutants failed to rescue pruning defects, indicating that interaction between Rab11 and Spn-F is critical for pruning. In conclusion, we identified that Rab11 and its interaction with Spn-F play crucial roles in dendrite pruning of C4da neurons. And we also uncovered that there is a genetic interaction between *Rab11* and *spn-F*.

647 The role of APP-like in the development of the *Drosophila* nervous system. *N. Reger*, F. Vonhoff Department of Biological Sciences, University of Maryland-Baltimore County, Baltimore, MD.

The Amyloid Precursor Protein (APP) is most commonly associated with its role in Alzheimer's disease (AD). In humans, APP undergoes proteolytic cleavage to produce an intracellular fragment, a secreted extracellular fragment, and the extracellular amyloid-beta (Aβ) peptide. While extensive work has been done to understand the function of Aβ in AD patients, relatively little is known about the endogenous role of APP. *Drosophila* provides a useful model to study what these roles may be as the fly homolog, APP-like (APPL), has homologous intracellular and extracellular domains but lacks the region required for Aβ formation. As such, *Drosophila* provides a model in which the developmental roles of APP can be studied independently of the disease pathway. Here we show that *appl-* animals have developmental defects in the flight motor neurons. By using a drop assay, we found that 2 day old (2d) *appl-* animals showed significantly worse flight performance (110±8.9mm, n=91) compared to 2d WT animals (44±4.2mm, n=43, p≤0.0001). Examination of the flight motor neuron dendritic arbors showed that *appl-* animals had reduced dendritic area and an increased localization of dendrites at the midline. These data suggest that it is unlikely that the number of dendrites is altered in *appl-* animals; rather it is the positioning of dendrites that is defective. We also observed sexual dimorphism in the affect of *appl* on the development of the scutellar mechanosensory bristles. While loss of scutellar bristles in *appl-* mutants has previously been reported, the differential effect on males vs females has not. We found that females retain the anterior scutellar bristles 40% of the time (n=210) while males retain these bristles 2% of the time (n=242; Fisher's exact, p≤0.0001). Neither males nor females retained the posterior bristles suggesting a possible interaction with polarity genes. The possibility of sex specific enhancement of *appl* phenotypes is interesting as there is some evidence that suggests women are more susceptible to AD than men. Additional work can be done to investigate what genetic components may contribute to this sexually dimorphic effect.

648 Absence of FoxO-induced autophagy allows preferential dendritic growth of larval sensory neurons under nutrient restriction. Amy Poe, Yineng Xu, Christine Zhang, Kailyn Li, *Chun Han* Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY.

During animal development, the nervous system grows in coordination with the rest of the body under normal nutrient condition. However, under nutrient restriction, the development of the brain is protected while the growth of other organs slow down. This phenomenon of organ sparing ensures proper development of more critical organs under unfavorable environmental conditions at the expense of other organs. While the maintenance and proliferation of neural stem cells in *Drosophila* are known to be protected by Alk tyrosine kinase signaling in response to glia-derived Jelly Belly signal, whether similar mechanisms exist to protect branching morphogenesis of differentiating neurons under nutrient stress is unclear. We try to address this question by investigating the scaling of dendritic arbors of peripheral sensory neurons in coordination with the larval body growth. We found that while the growth of both neurons and epidermal cells is regulated by the insulin/TORC pathway, nutrient restriction slows down the growth more dramatically in epidermal cells than in neurons, resulting in overly branched dendritic arbors starting from early larval stages. This preferential dendritic growth is accompanied by the absence of autophagy induction and tempered increase of lysosome biogenesis in neurons compared to epidermal cells. Underlying the contrasting levels of growth and autophagy induction is the differential expression and function of FoxO in neurons and epidermal cells: FoxO is highly expressed in epidermal cells but not in neurons, and under nutrient stress, FoxO suppresses epidermal cell growth while mildly enhance dendrite growth. Functionally, the nutrient restriction-dependent preferential dendritic growth results in heightened animal responses to sensory stimuli. Together, our results reveal a novel mechanism of nervous system sparing under nutrient stress at the level of dendritic arbor growth. Such protection of neuronal growth may give animals a survival advantage in the face of environmental challenges.

649 Down syndrome kinase Dyrk1A/Mnb phosphorylates Abrupt to control dendrite morphogenesis in *Drosophila*. *Dae-woo Kwon*[1,2], In Jun Cha[3], Kweon Yu[1,2], Sung-Bae Lee[3], Kyu-Sun Lee[1,2] 1) Metabolism and Neurophysiology Research Group, KRIBB, Daejeon, Korea; 2) Department of Functional Genomics, KRIBB school, UST, Daejeon, Korea; 3) Department of Brain & Cognitive Sciences, DGIST, Daegu, Korea.

Dyrk1a, located in human chromosome 21, is a crucial gene implicated in Down syndrome, Autism spectrum disorder and other neuronal defects. Here we

identified that *minbrain* (*Mnb*), a *Drosophila* homologue of *Dyrk1a*, regulates branching patterns in *Drosophila* dendritic arborization (da) neurons. We found that co-expression of *mnb* rescued the dendritic defects caused by overexpressing *abrupt* in da neurons. We also found that Mnb directly phosphorylates Abrupt at serine 629 residue and phosphorylated Abrupt is inactivated via ubiquitin-proteasome dependent protein degradation pathway. In addition, phosphor-mutant form of Abrupt showed no degradation and rescue pattern by Mnb. These results indicate that Mnb kinase is critical for determining the dendritic patterning of da neurons by regulating the protein stability of abrupt.

650 Probing the role of thrombospondin in synaptogenesis and locomotion. A. Wooten[2], E. Lowenstein[3], E.D. Mendoza Ortiz[4], *Norma Velazquez Ulloa*[1] 1) Biology, Lewis & Clark College, Portland, OR; 2) BCMB, Lewis & Clark College, Portland, OR; 3) PMCB, OHSU, Portland, OR; 4) Facultad de Ciencias, UNAM, Mexico.

Thrombospondin (TSP) is an extracellular matrix glycoprotein that has been shown to have a role in synaptogenesis in the mammalian brain. In mammals, TSP is released by astrocytes at glutamatergic synapses. The TSP family of glycoproteins is composed of 5 members. There is a single homologous gene in *Drosophila melanogaster* (D-TSP) and there is conservation in the
protein domains that are involved in TSP's function in synaptogenesis. In *Drosophila*, D-TSP has been studied in the myotendinous junction at embryonic stages and in the chordotonal organ at larval stages. However, whether D-TSP plays a role in synaptogenesis during the larval stage has not been investigated. Hence, we set out to determine if D-TSP functions at glutamatergic synapses in the larval NMJ. We hypothesized that D-TSP would be necessary for normal NMJ formation. To test this hypothesis, we knocked down D-TSP in specific tissues and quantified different features of the NMJ structure in flies with normal expression of D-TSP and flies with decreased expression of D-TSP. Knockdown of D-TSP was achieved by using the GAL4-UAS system, crossing a D-TSP-RNAi fly strain with GAL-4 strains in which GAL4 expression was controlled by tissue-specific drivers: in muscle (C600-DCR2), pan-neuronally (elav-GAL4), or in both, neurons and muscles (elav-GAL4; BG57-GAL4). We used immunohistochemistry to visualize larval NMJ structure at muscle 4 in segments A3 and A4. Visualization of NMJ structure included established NMJ markers (muscles with phalloidin, presynaptic axons with HRP, and the postsynaptic density with DLG). Our preliminary results suggest a change in NMJ morphology in the knockdowns. Most saliently, we see a difference in NMJ complexity, such that the distribution of NMJs by number of branches changes from a majority of NMJs having 1 or 2 branches in NMJs from control larvae to a majority of NMJs having 3 or more branches when D-TSP is knocked down. This change in complexity is reflected in changes in the number of branches and number of branch points in strains with decreased D-TSP. We are also quantifying muscle area, number of boutons, and the number of islands. Some NMJs also seem to have a clumping phenotype that we plan to characterize further. In addition to the effects at the NMJ, preliminary observation of locomotor activity suggests a difference in activity, with increased turning in larvae with knocked down D-TSP. We are in the process of validating the knockdown of D-TSP by RT-qPCR. Our results suggest that D-TSP plays a role in synaptogenesis at the larval NMJ, and that these structural changes in the NMJ have consequences on locomotor behavior.

651 A γ-secretase dependent cleavage event promotes Wnt-mediated synaptic maturation at the NMJ. *Lucas Restrepo*, Alison DePew, Timothy Mosca Department of Neuroscience, Thomas Jefferson University, Philadelphia, PA.

Newly formed synaptic connections are not yet optimally functional. They undergo maturation to transition from structurally simple, functionally unrefined connections to structurally complex connections capable of robust transmission. This is critical, as failures in maturation underlie neurodevelopmental disorders like autism and epilepsy. At the *Drosophila* neuromuscular junction, synaptic maturation is marked by the initial outgrowth of synaptic boutons followed by the acquisition of apposed postsynaptic membrane. Maturation failures are indicated by so-called "ghost boutons" that have presynaptic, but no postsynaptic, staining. A core pathway that promotes this maturation involves the motoneuron-derived Wnt ligand Wingless (Wg) and the muscle-expressed Frizzled2 (Fz2) receptor. In response to Wg binding, muscle Fz2 receptors are endocytosed, trafficked to the nuclear periphery, and their C-termini (Fz2-C) are cleaved and imported into the nucleus. This entry promotes gene transcription associated with synaptic maturation. Though considerable work identified players in the endocytosis, trafficking, and nuclear import of Fz2-C, the identity of the Fz2-C protease involved has remained elusive. To identify proteases involved in maturation, we performed a candidate RNAi screen against proteases that, when mutated, would cause the same maturation defects as failed Fz2 signalling. We identified Presenilin and Nicastrin, two subunits of the γ-secretase complex, as essential for synaptic maturation. Both can localize to the postsynaptic membrane and loss of either increases the incidence of ghost boutons. Synaptic α-spectrin levels are also markedly decreased, consistent with impaired development. Active zone formation, however, is normal. Further, we identify a behavioral correlate of impaired synaptic maturation: that of reduced larval motility and peristaltic motion. Expression of either transgene in the postsynaptic muscles (but not presynaptic motoneurons) of their respective mutants rescues these defects, suggesting that they function in muscle. In the absence of Presenilin or Nicastrin, Fz2 cleavage is blocked, resulting in the absence of nuclear Fz2-C import. This defect occurs specifically at the cleavage step, however, as expression, endocytosis, trafficking, and localization of Fz2 is otherwise normal. Demonstrating that this nuclear entry is the key event in promoting maturation, muscle expression of a pre-cleaved Fz2-C peptide rescues the *presenilin* or *nicastrin* mutant maturation phenotypes. Our data suggests that γ-secretase activity in the muscle is critical for Fz2 cleavage and synaptic maturation. As γ-secretase is associated with Alzheimer's disease, understanding more about its basic function is a significant step forward in grasping how it affects human disease.

652 GTPase regulator associated with FAK (Graf) is Required for Color Photoreceptor Patterning. *Jessica Gosselin*, Lauren Ducoste, Pamela Boodram, Jens Rister Biology, UMass Boston, Boston, MA.

The postmitotic cell-fate distinction between blue- and green-sensitive photoreceptors in *Drosophila melanogaster* is controlled by the Hippo pathway. This highly conserved pathway is primarily known for controlling tissue growth and suppressing tumors in various organisms, as it coordinates both cell proliferation and cell death. In the blue-sensitive Rh5 photoreceptors, the Hippo Pathway is "off", and in the green-sensitive Rh6 photoreceptors, Hippo is "on". This binary cell fate decision results in a wildtype ratio of about 35% Rh5 to 65% Rh6.

Here we show that a mutation named *daltonien* causes a dramatic change of this ratio to approximately 5% Rh5 and 95% Rh6. Therefore, the *daltonien* mutant shows an increase in the activity of the Hippo Pathway, as more photoreceptors are in the Hippo "on" state, suggesting that a repressor of the Hippo pathway is affected. Indeed, expression of the Hippo pathway kinase Warts is dramatically expanded together with Rh6 in the *daltonien* mutant, while expression of its negative regulator Melted is almost completely lost and only detected in the few remaining Rh5 photoreceptors.

However, the gene that is affected by the *daltonien* mutation is currently unknown. Recombination mapping followed by deletion mapping led to five different candidate genes: *Graf*, *CG8260*, *mRpL3*, *CG17209*, and *CG8952*. Only the RNAi-mediated knockdown of *Graf* in photoreceptors strongly resembled the *daltonien* mutation: We expressed two independent RNAi constructs for *Graf* in the Rh5/Rh6 photoreceptor subset with the *Senseless-GAL4* driver and both resulted in lower numbers of Rh5 photoreceptors with medians of approximately 8% and approximately 17% Rh5 expressing photoreceptors. Both RNAi and the GAL4 driver controls had Rh5:Rh6 ratios similar to wildtype, with the median being approximately 42% and approximately 38% Rh5 expressing photoreceptors, respectively. Therefore, Graf appears to act cell autonomously to promote Rh5 fate and to repress Rh6 fate. In addition, we generated a null mutant for the *Graf* gene using CRISPR/Cas9, which phenocopied the *daltonien* and the *Graf* RNAi phenotypes with a median of Rh5 in approximately 2% of the

photoreceptors. Taken together, these results indicate that *Graf* is necessary to promote Rh5 fate and to repress Rh6 fate. As it is a GTPase regulator, we are currently testing how it negatively regulates Hippo signaling in postmitotic photoreceptors.

653 Wnt/PCP signaling regulates morphogenesis and arrangement of the columnar structures in the medulla. *Xujun Han*[1], Miaoxing Wang[1], Makoto Sato[1,2] 1) Institute for Frontier Science Initiative, Kanazawa University, Japan; 2) Graduate School of Medical Sciences, Kanazawa University, Japan.

Columnar structure is a basic unit of the brain, but the mechanisms of column formation remain largely unclear. The medulla in the fly visual center is an excellent model to study column formation, because columns can be easily visualized by immunostaining and powerful fly genetics is also available. Neurites of medulla neurons are organized along three layers, and each layer is regarded as a two-dimensional sheet. To properly organize columnar structures, some neurons should have particular orientations during column formation, which may be under the control of the planar cell polarity (PCP) signaling, as PCP is known to regulate the orientation of cells along a two-dimensional tissue. The PCP signaling is also known to be regulated by the gradient of Wnt ligands. Thus, we hypothesized that Wnt/PCP signaling may play roles in establishing the polarities of the essential columnar neurons along the medulla layers.

To this aim, we first confirmed that PCP core factors are required for medulla column organization. As Wnt4, Wnt10 and Frizzled2 (Fz2) exhibit striking expression patterns which form graded distributions along the dorso-ventral axis in the medulla, we examined the roles of the Wnt4/10, Fz2 in medulla columns. Indeed, we found the abnormal shape and irregular arrangement of the columns in *Wnt4*, *Wnt10*, *fz1*, and *fz2* mutant flies. Additionally, we found genetic interactions between *fz2* and PCP factors. We also found that the core columnar neurons, R8 and Mi1, show planar polarities during the medulla column development. Loss of Wnt or PCP factors caused abnormal orientation of the terminals of R8 and Mi1. The link between column formation and Wnt/PCP found in our study may provide very important insights for the understanding of the mechanisms of column formation.

654 Haploinsufficiency of the Mitochondrial Genes *Scheggia* and *SesB* Disrupts Neurodevelopment and Behavior. *C.L. Hartwig*[1], A. Gokhale[1], A.H. Freeman[1,2], S.A. Zlatic[1], C. Sapp-Savis[3], T.A. Vadlamudi[3], F. Abudulai[3], V. Faundez[1] 1) Department of Cell Biology, Emory University, Atlanta, GA; 2) Center for the Study of Human Health, Emory University, Atlanta, GA; 3) Department of Chemistry, Agnes Scott College, Decatur, GA.

Microdeletions are common mutations associated with neurodevelopmental disorders in humans. The model for a microdeletion disease mechanism is a haploinsufficiency of multiple genes. Among microdeletions, the most frequent in humans involves the 22q11.2 chromosomal segment, which encodes the mitochondrial transporter SLC25A1. To address the contribution of mitochondria to neurodevelopmental phenotypes in microdeletion syndromes, we modeled a hemideletion of *Drosophila* dSLC25A1 (*scheggia)*, and its interactor, the mitochondrial ADP-ATP translocator dSLC25A4 (*sesB)*. We demonstrate that *scheggia* and *sesB* interact genetically to influence neuron development and function. The morphology of the neuromuscular junction is quantitatively different in *scheggia* and *sesB* loss of function alleles and loss of *sesB* increases neurotransmission during high frequency stimulation. Sleep behavior is also altered in a neuron specific and allele differential manner. *Scheggia* expression in catecholaminergic neurons is required for sleep while *sesB* expression is necessary in glutamatergic neurons. Our results demonstrate that haploinsufficiencies of mitochondrial genes contribute to the pathogenesis of neurodevelopmental disorders.

655 Found in neurons (Fne) promotes an invasive state in *Drosophila* sensory neurons. *R. Alizzi*[1], C. Tenenbaum[1], D. Xu[1,2], W. Wang[3], E. Gavis[1] 1) Molecular Biology, Princeton University, Princeton, NJ; 2) University of Oxford, Oxford, UK; 3) Lewis-Sigler Institute, Princeton University, Princeton, NJ.

Dendritic arbor morphology influences how neurons receive and integrate extracellular signals, and altered morphology is associated with neurological disorders such as autism. The *Drosophila* larval class IV dendritic arborization (da) neurons form a highly branched two-dimensional arbor between the epidermis and the extracellular matrix (ECM) that allows them to respond to noxious stimuli. We found that the Hu family RNA-binding protein Found in neurons (Fne) is critical for multiple aspects of class IV da neuron development. Preliminary analysis showed that Fne expression can promote increased levels of integrin and decreased levels of the cell adhesion proteins Coracle and Armadillo, suggesting Fne may function through post-transcriptional regulation of cell-cell and cell-ECM interacting proteins in these neurons. Additionally, *fne* mutant neurons exhibit decreased stable microtubule content and increased branch instability, suggesting that Fne is able to promote stable dendrite growth along the ECM. The identification of transcripts encoding cytoskeletal regulators, cell-cell and cell-ECM interacting proteins as Fne targets using TRIBE (targets of RNA-binding proteins identified by editing) further supports these results. Analysis of one target, *basigin*, a protein known to be involved in both cell adhesion and cytoskeletal organization, showed that *fne* mutants have lower levels of Basigin, and that *basigin* knockdown leads to lower levels of stable microtubules, similar to what is observed in *fne* mutant neurons. These findings, in light of evidence that Hu proteins are ectopically expressed in cancer cells, suggest Fne regulates dendrite morphogenesis by regulating target genes to induce directed, invasive growth. This process is required to give neurons distinct characteristics from the surrounding epidermal cells and enables dendrites to adequately cover the required territory but would be detrimental if homologs of Fne promote a similar function in cancer cells. By elucidating the role of Fne, we will gain a greater understanding of normal developmental processes and how misregulation of these processes can contribute to disease.

656 The Transcription Factor Gooseberry, a pax3/pax7 homolog, interacts with Wingless to control neuronal function. *M. Perez*[1,2], C. Dominicci-Cotto[1], B. Marie[1] 1) Institute of Neurobiology, Anatomy and Neurobiology Department, Medical Sciences Campus, San Juan, PR; 2) Department of Biology, University of Puerto Rico, Rio Piedras Campus.

The transcription factor gooseberry (Gsb) and the signaling molecule wingless (Wg) are critical for nervous system development. At early developmental stages, Gsb controls neuroblast differentiation by antagonizing Wg signaling. We recently discovered that Gsb is important, within late motoneurons, for synaptic growth, plasticity and stability. Interestingly, Wg also controls neuromuscular junction growth and plasticity but presents opposite phenotypes. We therefore hypothesize that Gsb and Wg maintain antagonistic interactions defining neuronal functions.

We manipulated Gsb and Wg expression and analyzed synaptic growth, plasticity and stability. Gsb loss of function increases synaptic growth and plasticity. These phenotypes can be rescued in a Wg mutant background, suggesting that Gsb antagonizes Wg to control synaptic growth and plasticity. Gsb loss of function also affects synapse stability, provoking synaptic retractions. This phenomenon is amplified if Wg expression is reduced, suggesting that Gsb controls synaptic stability independently of Wg, and raising the possibility that Wg has a neuroprotective role at the synapse. In addition, Gsb overexpression reduces synaptic growth and impairs synaptic plasticity, while Wg overexpression leads to overgrown and overplastic synapses. When Gsb and Wg are overexpressed simultaneously, the phenotypes are identical to the Gsb over expressors suggesting that Gsb renders the synapse resistant to Wg. In contrast, when we overexpress Gsb and activate the Wg pathway by expressing a dominant negative form of the kinase shaggy (Sgg), we found that both growth and plasticity phenotypes are restored to control levels. This finding strongly suggests that Gsb inhibits the Wg signaling pathway upstream of Sgg and downstream of Wg. We conclude that Gsb and Wg, two molecules essential to nervous system development, interact to control mature neuronal function.

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657 The conserved microRNA *miR*-34 regulates neuromuscular junction morphogenesis. *M. Misra*, H. Hwang Biology, Washington College, Chestertown, MD.

MicroRNAs are small non-coding RNAs that interfere with messenger RNA translation and protein synthesis. *miR-34*, a conserved microRNA present in both flies and mammals, has been implicated in the regulation of pruning during neuron development and in protection against neurodegeneration and aging. Whereas previous studies have focused on *miR-34*'s role in the central nervous system (CNS), we examined its effects on the development of connections

between motor neurons and muscles in the peripheral nervous system (PNS) of *Drosophila melanogaster*. The GAL4/UAS system was used to drive overexpression of *miR-34* or expression of a *miR-34* sponge that knocked down functional *miR-34* levels in motor neurons. Larvae were dissected at the crawling third instar stage of development. We then used immunofluorescence labeling to detect pre-synaptic and post-synaptic components of the neuromuscular junction (NMJ) on muscle 12 of abdominal segments 2-5. Overall, we found no significant difference in the average number of boutons between the control and the experimental groups. However, NMJs in the *miR-34* knockdown group were significantly longer and more branched than in the control group. In addition, both the knockdown and overexpression groups showed significant reductions in average bouton diameter. Together, our results indicate that *miR-34* influences multiple aspects of NMJ morphogenesis. *miR-34* may be an important target for future studies of motor neuron diseases, given that these diseases are characterized in part by changes in NMJ structure.

658 *Tao* negatively regulates retrograde BMP signaling during neuromuscular junction development in *Drosophila*. S.F Politano[1], R.R. Salemme[1], J. Ashley[2], J.A. Lopez[1], T.A. Bakula[1], K.A. Puhalla[1], J.P. Quinn[1], M.J. Juszczak[1], L.K. Phillip[1], R.A. Carrillo[2], *P.J. Vanderzalm*[1] 1) Department of Biology, John Carroll University, University Heights, OH; 2) Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL.

The growth and development of synapses in both vertebrates and invertebrates is coordinated by several conserved signaling pathways, including the canonical Bone Morphogenic Protein (BMP) pathway. At the *Drosophila* neuromuscular junction (NMJ), retrograde BMP signaling is critical for expansion of synaptic termini, or boutons, concomitant with larval muscle growth. We aimed to determine whether the conserved Hippo signaling pathway, critical for proportional growth in other contexts, also functions in NMJ development. We found that the serine-threonine kinase *Tao,* a component of Hippo pathway, is required for normal NMJ growth; loss of neuronal *Tao* resulted in supernumerary boutons, suggesting that Tao normally inhibits NMJ growth. Previous studies showed that Tao activates the Hippo pathway by initiating a kinase signaling cascade that inhibits Yorkie by phosphorylation and subsequent cytoplasmic retention. In NMJ development, however, Tao functions independent of the Hippo pathway since removal of *yorkie* function did not suppress the loss of *Tao* phenotype. Instead, we found that Tao inhibits the BMP pathway, as motor neuron nuclei display increased pMad levels and BMP target gene expression is elevated in *Tao* mutants. NMJ neurotransmission is also impaired upon loss of *Tao*, including a decrease in evoked excitatory junctional potentials. Taken together, these results support a role for *Tao* as a novel inhibitor of BMP signaling in motor neurons during synaptic development.

659 A molecular control of temperature dependent synaptic growth: Autophagy, Proteasome and Map Kinases. *K.M. De Leon Gonzalez* , B. Marie Institute of Neurobiology, University of Puerto Rico, San Juan , PR.

Little is known about the effects of varying temperatures on the nervous system. Using the *Drosophila* Neuromuscular Junction (NMJ), we asked how rearing temperature (15°C, 20°C, 25°C, 29°C) can affect synaptic growth. We observed an increased in the number of synaptic boutons in animals reared at higher temperatures. Indeed, animals reared at 29°C had a 100% increase in synaptic growth when compared to animals reared at 15°C. Interestingly, we found that the number of boutons from the 1s motor neuron increased with temperature while the boutons from the 1b motor neuron remained constant. This result indicates that motor neurons might be differentially sensitive to the changes in temperature.

Next, we identified highwire (Hiw), a E3 ubiquitin ligase, as a key regulator of temperature dependent synaptic growth. Hiw is a negative regulator of synaptic growth and its mutation induced a temperature independent overgrowth phenotype. We then asked whether autophagy, a known negative regulator of Hiw, was involved in this regulation. Autophagy mutants showed a temperature independent undergrowth suggesting that autophagy may control temperature dependent of synaptic growth. In addition, using lysotracker, we identified that the levels of autophagy changed at different rearing temperatures. We found that animals reared at 29° C had increased autophagic activity when compared to animals reared at 15°C. We hypothesize that, at 29°C, there is little suppression of synaptic growth given by high activity of autophagy and a consequent low activity of Hiw. In contrast, at 15°C, increased Hiw activity suppresses synaptic growth due to reduced autophagic activity. We are now exploring downstream targets of Hiw that could be involved in the temperature dependence of synaptic growth. In particular, we are testing the roles of the MAP Kinases Wallenda, Jun Kinase and P38b.

660 Maintaining Neuronal Function: The Role of the Transcription Factor Gooseberry in Synaptic Growth, Plasticity and Stability. *C.M. Dominicci-Cotto*[1], M. Perez[1,2], B. Marie[1] 1) Anatomy and Neurobiology Department, University of Puerto Rico- Medical Sciences Campus, San Juan, PR; 2) Department of Biology, University of Puerto Rico, Rio Piedras Campus.

During nervous system development, transcription factors promote neuron differentiation, migration and connectivity. Once fully developed, neurons need to function throughout their lifetime while responding to changes in activity. Here, we hypothesize that transcription factors play an important role in mature neurons. To address this, we focused on Gooseberry (Gsb), a transcription factor homologous to the vertebrate pax3/7, which controls neuroblasts development during embryogenesis. It was previously shown that Gsb is present in mature motoneurons (MNs) and regulates the maintenance of homeostatic compensation at the neuromuscular junction (NMJ). Here, we investigate the role of Gsb in growing and fully developed MNs.

To understand Gsb's function, we manipulated its expression at various stages of MNs development and asked whether the growth, plasticity and stability of the NMJ were affected. We altered Gsb expression at early (embryonic, post-mitotic neuron), late (end of larval stage 2), 24 or 2 hours before dissection (larval stage 3). A decrease in Gsb expression promotes synaptic growth early, late and 24 hours before dissection. In contrast, an increase in Gsb represses synaptic growth at all stages, even 2 hours before dissection. This data suggest that Gsb is not just an early developmental regulator, but it also regulates synaptic growth at distinct stages of MNs development. We also studied how Gsb expression could affect activity-dependent synaptic plasticity at the NMJ by quantifying the appearance of *de novo* synaptic structures after repeated stimulation. We found that early or late Gsb overexpression reduces synaptic plasticity, while a decrease promotes it. To study the role of Gsb in synaptic stability, we quantified the frequency of synaptic retractions. We found that a decrease in Gsb promotes an increase in synaptic retraction at the NMJ. These results suggest that Gsb maintains synaptic integrity at early and late stages of MNs development. In conclusion, we found that Gsb regulates synaptic growth, stability and plasticity at the growing and mature NMJ.

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661 Expansion Microscopy(ExM) enables subcellular localization of neurotransmitter receptors to single neurites in the neurons of the *Drosophila*motion vision pathway. *E.M. Rogers*, P.W. Tillberg, D. Alcor, M.B. Reiser Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA.

The construction of mechanistic models of neuronal function has been well served by the detailed study of a neuron's morphology and electrophysiological properties. However, such models tend to treat a neuron as a 'black box' rather than a highly complex molecular machine. To refine these models, it is necessary to probe neurons' molecular architecture, especially the subcellular localization of neurotransmitter receptors, which has been a longstanding challenge. We have developed methods in which neuron-specific Gal4 and LexA drivers are used to express epitope-tagged receptors that are imaged using Expansion Microscopy(ExM) with a lightsheet microscope. In expansion microscopy the immunohistochemically stained fly brains are chemically (and isotropically) expanded[1] .This procedure allows us to visualize subcellular localization of neurotransmitter receptors at a resolution that is substantially improved over traditional light microscopy methods. As a case study, we are using the *Drosophila* motion vision pathways. Our lab has developed a model, based on electrophysiological and morphological measurements, in which directional selectivity is computed by the integration of spatially offset inhibitory and excitatory inputs to T4 and T5 neurons[2]. We applied the aforementioned methods to visualize the localization of inhibitory GABA receptor subunit

Rdl[3], [RM2] along single neurites of T4 and T5 neurons. We find that the T4 and T5 neurites that contain Rdl puncta are in close proximity to the terminals of the GABAergic CT1 neuron[4]. This additional piece of data may enrich our current model by providing the spatial organization of a source of inhibition in the motion vision pathways, and the application of these methods, more generally, will improve models for neuronal function.

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662 Expansion of octopaminergic neurons during chronic exercise. *K. Richardson*, A. Sujkowski, R. Wessells Wayne State University, Detroit, MI.

The activity of octopaminergic neurons has been shown to be critical for the adaptive response to chronic exercise in *Drosophila*. Using flies with GFP-labelled octopaminergic neurons (Tdc2-GFP), we observe that exercised male *Drosophila* have an increased number of GFP labeled neurons following three weeks of exercise that is not seen in controls. We hypothesized that this octopaminergic expansion may be a key part of the adaptive response to exercise. Therefore, we aim to determine the mechanism through which this neuroplasticity may be occurring, providing a basis for how the brain adapts to exercise. There are two possibilities for how the expansion of Tdc2-positive neurons may be occurring: 1) existing octopaminergic neurons are increasing dendritic branching, 2) other types of neurons within the brain are co-expressing octopamine following exercise. To determine if one, or both of these possibilities is correct, we used confocal microscopy to visualize Tdc2-GFP colocalization with neurotransmitters such as dopamine and serotonin. The number of octopaminergic neurons is quantified in exercised brains using neuron tracing software. We hope to establish a time course for this neuronal expansion during exercise training, and determine which populations of neurons, if any, are being recruited during the observed octopaminergic expansion.

663 Identification and functional characterization of neurons controlling systemic body growth by affecting the prothoracic gland in *Drosophila melanogaster*. *E. Imura*[1], S Kondo[2], H Tanimoto[3], R Niwa[4], Y Shimada-Niwa[4,5] 1) Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan; 2) Genetic Strains Research, NIG, Japan; 3) Grad. Sch. of Life. Sci., Tohoku Univ., Japan; 4) Faculty of Life Environ. Sci., Univ. of Tsukuba, Japan; 5) Life Science Center for Survival Dynamis, TARA, Univ. of Tsukuba, Japan.

Steroid hormones play key roles in many aspects of development, growth, and reproduction in multicellular organisms. In insects, the principal steroid hormone ecdysteroid controls systemic growth and developmental transitions, represented by molting and metamorphosis. During the larval stages, ecdysteroid is biosynthesized in the prothoracic gland (PG) and secreted into the hemolymph. The level of ecdysteroids fluctuates in a stage-specific manner; the peaks of ecdysteroids trigger developmental transitions, while basal levels negatively affect body growth. It has been reported that several signals, especially the neuronal signals, affect ecdysteroid biosynthesis in the PG and are essential for controlling the proper timing of growth and developmental transitions; the prothoracicotropic hormone (PTTH)-producing neurons and serotonergic $SE0_{PG}$ neurons innervate the PG, regulating ecdysteroid biosynthesis. However, considering the precise and adaptive regulation of ecdysteroid biosynthesis, we hypothesize that there must be other regulatory neuronal systems in the PG.

Based on this hypothesis, we aimed to identify and characterize novel PG-innervating neurons in this study. Using the FlyLight database, we particularly focused on one type of PG-innervating neurons that have neuronal connections with not only the PG but also PTTH neurons. Inhibiting these neurons increased pupal size, while had little effect on the timing of pupariation, indicating that the pupal overgrowth is not a consequence of an extended larval growth period. This suggests that these neurons inhibited systemic body growth by affecting the PG. Previous research showed Warts/Yorkie/microRNA *bantam* signaling in the PG controls systemic body growth by regulating basal ecdysteroid biosynthesis. Consistent with this report, inhibiting these neurons increased *bantam* expression in the PG. In this presentation, the involvement of these neurons in ecdysteroid biosynthesis will also be discussed.

664 Analysis of Ciliary Trafficking of two TRP channels in *Drosophila* Chordotonal Organ. *Youngtae Kwon* Department of Life Science, University of Seoul, seoul, KR.

Cilia are important eukaryotic cellular organelles required not only for cell motility but also for various cellular processes including detection of external stimuli. Recent studies revealed that regulation of protein trafficking into the proper ciliary sub-compartments is essential for the function of cilia. *Drosophila* chordotonal cilium, which is essential for the auditory and other mechano-electric transduction, is a good model for studying ciliary protein trafficking. Two distinct members of TRP superfamily ion channels, TRPV and TRPN, which have distinct roles in auditory transduction, have been found in the distinct ciliary sub-compartments of chordotonal cilia; TRPV is restricted in the proximal sub-compartment while TRPN is exclusively localized in the distal one. To understand the mechanisms underlying TRP channel localization into distinct ciliary sub-compartments, we analyzed the trafficking of two TRP channels in developing chordotonal cilia. The results showed that the two channels have distinct courses of ciliary trafficking. TRPN was initially targeted to the tip of developing cilia and retained in the distal sub-compartment throughout embryonic development. On the contrary, TRPV was initially dispersed along the elongating cilia except the distal tip where TRPN was located, and later restricted to the proximal sub-compartment of cilia at the final stage of embryonic development. We next analyzed the role of some known proteins required for the ciliogenesis in the localization of TRP channels. Lack of Beethoven (BTV), a cytoplasmic dynein motor protein, differentially affected the localization of the two TRP channels, the pre-ciliary apical transport for TRPV, and the ciliary entry for TRPN; while the intra-ciliary transport seemed to be unaffected for both TRP channels.. REMPA, a component of IFT-A complex, however, was required for the ciliary entry of both TRP channels. Finally, dTULP, an IFT-A-interacting protein, was required for the ciliary entry of TRPV, while the intra-ciliary localization of TRPN was defective in *dTULP* mutants. Taken together, our results suggested that two distinct parallel pathways are involved in the proper localization of the two TRP channels from the pre-ciliary to the intra-ciliary trafficking stages in *Drosophila* chordotonal cilia.

665 Exploring receptor/insecticide interactions of the nicotinic acetylcholine receptor gene family using CRISPR/CAS9. *T. Perry*[1], W. Chen[1], M. Ghazali[1], D. Christesen[1], Y. Yang[1], N. Luong[1,2], P. Batterham[1] 1) School of BioSciences, The University of Melbourne, Parkville, Victoria, AU; 2) Institute Molecular Biology Biotechnology FORTH, Heraklion, Crete.

The nicotinic acetylcholine receptors (nAChRs) are a conserved gene family that facilitates synaptic signalling through the conversion of a chemical to an electrical signal. Toxins have proven to be powerful tools in the discovery and characterization of receptors in the nervous system, including nAChRs. Insect nAChRs have been a regular target of insecticides and the loss of receptor function can lead to high levels of resistance. Another pathway to resistance is the modification of the receptor to remove the binding motif of insecticides.

The Drosophila melanogaster genome encodes 10 nAChR subunit genes and the functional nAChR is a pentamer of subunits that means many different combinations are possible. The various pentameric subtypes can have significantly different binding and signalling properties. The identities and stoichiometry of subunits within these subtypes are not well understood, and area hampered by technical difficulties with expressing functional receptors in

heterologous systems. Thus, we have taken an *in vivo* approach to examining which receptors bind to different insecticides. Taking advantage of available Drosophila resources for efficiently deleting and editing the genome with CRISPR/CAS9, we have systematically deleted or modified nine of these nAChR genes. These nAChR mutants were examined for response to insecticides to identify those that are specific targets. Using compounds from three insecticide classes, the neonicotinoids, sulfoximines and spinosyns, we identified 5 nAChR subunits targeted by at least one of these different insecticide classes and found interesting cross resistance relationships between and within the classes. Interestingly, we also see evidence of compensatory changes in nAChR expression in deletion mutants for individual subunits.

Our data have helped to define the receptor subunits involved in binding of insecticides. We are also beginning to uncover some of the roles these receptors play, with subunit mutations leading to effects on sleep as well as courtship behaviors. There are likely to be many more behaviors that are impacted through loss of these receptors, making the set of deletion mutants a valuable resource for future investigation of nAChR biology. Our results contribute to a greater understanding of the interaction of insecticides and nAChR subunits and the fitness effects that might be associated with target site mediated resistance mechanisms that arise. These findings will help to determine the probability of resistance evolving and aid in design of resistance monitoring and management programs.

666 Characterization of the molecular and physiological role of TET proteins in development. F. Frey[1], J. Ismail[1], F. Hugosson[2], *M. Shirinian*[1] 1) Experimental Pathology, Immunology and Microbiology, American University of Beirut, Beirut, LB; 2) Whitney Laboratory for Marine Bioscience, University of Florida, USA.

Ten-eleven translocation 1-3 (TET 1-3) proteins are members of a family of DNA hydroxylases, a well-characterized epigenetic modification which plays an important role in regulation of gene expression and maintaining cellular identity with an enzymatic activity towards the methyl mark on the 5-position of cytosine (5-methylcytosine 5mC). TET proteins convert 5mC into 5-hydroxylmethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5CaC) through three consecutive oxidation reactions. Drosophila melanogaster has a very well conserved TET homologue including the conserved CXXC and dioxygenase domain and the catalytic domain which harbors the conserved metal binding residues. In this study we focus on understanding the role of dTET in brain development in Drosophila melanogaster. We show that dTET is widely expressed in brains of third instar larvae, especially within the central brain and ventral nerve cord. dTET is found specifically within neurons, neural progenitors (ganglion mother cells). Furthermore, knocking down dTET induces crawling and climbing defects in larvae. We hypothesize that dTET is required for survival as well as proper development of neuronal cells in larval Drosophila brain.

667 Identification of a unique distribution of cells potentially involved in nociception. *Minh-Nguyet Hoang*[1], Justin Patawaran[1], Catherine Hand[1], Andrew Moehlman[2], Helmut Kramer[2,3], Drew Stenesen[1] 1) Dept. of Biology, University of Dallas, Irving, TX; 2) Dept. of Neuroscience, UT Southwestern Medical Center, Dallas, TX; 3) Dept. of Cell Biology, UT Southwestern Medical Center, Dallas, TX.

Nociception is the sensory process tasked with detection and transduction of potentially harmful stimuli subjectively experienced as pain. While Drosophila is an established model system capable of probing the genetics of nociception, a majority of studies focus on neuronal components involved in pain sensation. Here we describe the initial exploration of a genetic tool originally developed to mark a subset of glial cells in the adult brain. Application of this tool in the larval stage uncovered a population of cells bilaterally positioned at each branch point along the dorsal tracheal trunk. Preliminary results suggest genetic manipulation of these cells alters the larval response to noxious heat.

668 Regulation of Wrapping glia differentiation in Drosophila eye disc. *Chia-Kang Tsao*[1,2], Yu Fen Yu Fen [1,2], Y. Henry Sum[1,2] 1) Molecular Biology, Academia Sinica, Taipei, TW; 2) Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan.

The retinal basal glia (RBG) is a group of glia that migrates from the optic stalk into the third instar larval eye disc while the photoreceptor cells (PR) are differentiating. There are three major classes of RBG, namely surface glia (SG), wrapping glia (WG) and carpet glia (CG), based on molecular and morphological characteristics. The SGs migrate and divide. The WGs are postmitotic and wraps around PR axons. There are two CGs per eye disc and they have giant nucleus and extensive membrane extension that each covers half of the eye disc. It has been proposed that the SGs migrate under the CG membrane, which prevented their contact with the PR axons lying above CG membrane. Upon passing the front of the CG membrane, which lags slightly behind the morphogenetic furrow marking the front of PR differentiation, the migratory SG can contact the nascent PR axon and be induced to differentiate into WG. We have developed an ex vivo culture system to follow the migration, division and differentiation of RBGs by live imaging. We found that the increase of RGB cell number is primarily (92%) due to SG divisions and only 8% contributed by migration from optic stalk. The WGs showed no cell divisions and appeared de novo in the anterior region, presumably differentiating from the migratory SG. However, we found that SGs are migrating both above and below the CG membrane, thus the CG membrane cannot be a physical barrier to prevent contact between SG and PR axon. We will present new findings suggesting a novel mechanism for the regulation of the SG-to-WG transition by CG.

669 Indispensable role of *globin1* in development and maintenance of the nervous system in *Drosophila*. *N. Kumari*, S. Sarkar Department of Genetics, University of Delhi South Campus, New Delhi, New Delhi, IN.

(Hemo-) globins (Hbs) are evolutionarily conserved heme containing metallo-proteins which could be characterized by the presence of distinct "globin fold." Although, *globin* genes have been classically associated with the oxygen transport function; however, recent studies have linked *globins* to various other biological and physiological processes. *Drosophila* genome harbours three *globin* genes, namely, *glob1*, *glob2,* and *glob3.* Our lab has successfully established the role of *glob1* in various aspects of development such as tracheal liquid clearance, regulation of cellular level of ROS during oxidative stress and maintenance of cytoskeleton integrity during oogenesis. The present study was undertaken to investigate the cellular expression profile and functional relevance of *glob1* in central nervous system development in both embryonic as well as larval tissues. Our study reveals a robust expression of Glob1 in the neuronal tissues, specifically around physiologically active and dividing cells such as outer proliferation center (OPC) of the optic lobes, in the optic stalk, in the region of eye imaginal discs below the morphogenetic furrow. In addition a robust expression of Glob1 has also been observed in various cells of embryonic ventral nerve cord, and A1-A8 (abdominal) neuromeres of the larval brain ganglia. Ubiquitous or neuronal cell specific reduced expression of *glob1* causes abnormal development of nervous system and also results in significant lethality during embryonic and larval development. Our study, therefore, suggests a novel and critical role of *glob1* in the development of nervous system in *Drosophila*.

670 Cellular diversity in the *Drosophila* 3rd instar larval ventral cord revealed by single-cell transcriptomics. *T. Brody*, S. Choudhury, T. H. Nguyen, M. Serpe Section on Cellular Communication, NICHD/NIH, Bethesda, MD.

To gain insight into the cellular diversity of the third instar larva ventral cord, we have sequenced 9,000 single cells and assigned them to 22 clusters. We have used the expression of *VGlut, Gad1, VAChT*, *Ddc* and *repo* to define glutamatergic, gabaergic, cholinergic, and serotonergic/dopaminergic neurons and glia respectively. We have also used identified enhancers for *shakB* and *futsch* to drive GFP and RFP in a limited number of ventral cord neural populations. Several of the identified clusters exhibit a high degree of uniformity in gene expression, suggesting that they are likely to represent a relatively uniform cell population. These include six glial populations and four motorneuron clusters. One cluster is identified as putative neural precursors, based on expression of neuroblast precursor genes. Another cluster is marked by expression of all four types of neurotransmitters, but in different cells. We will report on the repertoire of

transcription factors and other proteins that show a high degree of specificity to different clusters or subsets of cells within clusters. We will also describe approaches to further define specific cell types represented within the clusters that exhibit a high degree of diversity.

671 Differential small GTPase activities mediate semaphorin-1a-controlled projection neuron dendritic targeting. *K. Ku*, H. Yu Institute of Cellular and Organismic Biology, ACADEMA SINICA, Taipei, TW.

Olfaction is one of crucial sensory modalities for survival and progeny reproduction in animals. In the *Drosophila* olfactory system, olfactory sensory neurons (OSNs) project their axons to the primary olfactory center, the antennal lobe (AL), where dendrites of projection neurons (PNs) make connections with OSN axons to relay the olfactory information in other brain regions for further odorant decoding. Precise PN dendritic targeting in the AL to connect with OSN axons is crucial for generating an accurate olfactory map to construct the functional olfactory system. Previously, we have shown that Semaphorin-1a (Sema-1a) plays an essential role in the olfactory map generation by preventing PN dendrites from mis-targeting into wrong AL regions. Here, we explore intracellular signaling pathways that mediate Sema-1a-controlled PN dendritic targeting. Consistent with the previously reported function of Sema-1a in motor axon guidance, we found that Sema-1a acts as a repulsive receptor to control PN dendritic targeting by promoting the activity of small GTPase Rho1, which is positively and negatively regulated by RhoGEF Pebble and RhoGAPp190, respectively. However, a perplexed question revealed from our study is that the Rho1 activity can only mediate part of the Sema-1a repulsion in the PN dendritic targeting. To resolve this discrepancy, we have investigated whether other small GTPases also participate to mediate the repulsive Sema-1a activity in PN dendritic targeting. Interestingly, we found that knocking down or overexpressing dominant- negative RhoL and Rap2l significantly suppressed PN dendritic mis-targeting caused by loss-of-function of *Sema-1a*. Taken together, these results suggest that differential small GTPase activities mediate Sema-1a-controlled PN dendritic targeting.

672 The DNA damage response gene *nopo* has distinct interphase and mitotic functions in neurogenesis. *R.S. O'Neill*, C.J. Fagerstrom, N.M. Rusan Cell and Developmental Biology Center, National Heart, Lung, and Blood Institute, NIH, Bethesda, MD.

Genes identified in human cortical malformations such as microcephaly reveal key cellular mechanisms controlling brain size and structure. Many microcephaly genes function in DNA damage response (DDR) or at the centrosome and mitotic spindle. The human gene *TRAIP* is mutated in microcephalic primordial dwarfism. *TRAIP* and its *Drosophila* ortholog, *nopo* (*No Poles*), encode E3 ubiquitin ligases with functions in DDR and S-phase progression, and defects in these functions are thought to cause reduced proliferation, increased apoptosis and reduced neuron numbers in *TRAIP* mutants. However, *nopo* was originally named due to its maternal effect lethal phenotype that includes acentrosomal mitotic spindles in the early embryo, suggesting a role in mitosis. We characterized mNeonGreen::Nopo in larval neuroblasts with live imaging. Nopo localizes in the nucleus during interphase as expected for a DDR protein. However, at the onset of mitosis Nopo streams along mitotic spindles and concentrates at the centrosomes. This spindle localization is dependent on the uncharacterized C-terminal domain of Nopo. Yeast two-hybrid analysis reveals extensive interactions between Nopo and centrosome proteins, including Asl, Cep135 and Cnn. Although *nopo* mutant larval neuroblasts progress through mitosis normally, they have subtle defects in centrosome-spindle cohesion. Beginning in pupal stages, *nopo* mutants fail to develop properly sized mushroom bodies (MBs), a pair of central brain structures involved in learning and memory. The MB size defect is rescued by inhibiting caspase-dependent cell death, but not by expression of a RING-domain mutant or an NLS mutant *nopo* transgene, indicating that the E3 ubiquitin ligase function of *nopo* is required in the nucleus to prevent MB cell death. In addition to small MBs, *nopo* mutants have axon guidance defects including MB axons improperly crossing the midline and misguided axon tracts in the central brain. Mutants for *bendless*, which encodes an E2 that interacts with Nopo, have properly sized MBs that improperly cross the midline similar to *nopo* mutants. Thus, our working model is that *nopo* has multiple functions in neurogenesis: a DDR function in the nucleus during interphase, a centrosome-spindle cohesion function during mitosis, and a potential function with *bendless* in axon guidance. Our work reveals a novel mitotic role for a DDR gene in neurogenesis, suggesting a deeper link between the two pathways commonly mutated in microcephaly.

673 Regulation of Ey Expression in *Drosophila* Medulla Neuroblasts. *H. Zhu*, A. Ray, T. Zhang, X. Li University of Illinois Urbana-Champaign, Urbana, IL.

Sequential expression of five transcription factors (TFs), Homothorax (Hth), Eyeless (Ey), Sloppy paired (Slp), Dichaete (D) and Tailless (Tll), is observed in medulla neuroblasts (NBs) in the *Drosophila* larval brain. The progression of this temporal cascade is necessary for generating the full spectrum of neurons. Although Ey, Slp and D are required to turn on the next TF, Ey expression can still be turned on in *hth* mutant NBs. Therefore, the mechanisms used in the transition of NBs to the *ey* stage remains unknown. To understand how Ey expression is regulated, we first identified a putative cis-regulatory sequence for *ey*. We are currently screening through RNAi and mutant lines for the TFs predicted to bind the cis-regulatory sequence to confirm their participation in the regulation of *ey*.

674 *Drosophila* dADAR isoform structure and function in RNA editing. *Fatemeh Kohram*, Max Lebowitz, Cara Horst, Odunayo Ayodele, Jack Vaughn Biology, Miami University, Oxford, OH.

The *Drosophila* ADAR (dADAR) gene encodes a dsRNA-dependent adenosine deaminase. In embryos, two major isoforms exist, which are full-length (FL) and embryo-specific truncated (TR) dADAR isoforms. FL isoforms contain the catalytic domain and TR do not, while both possess the dsRNA-binding domains. dADAR editing activity is nearly absent before CNS development in embryos, while a spike in editing is seen between 16 h to L2 stages when the CNS develops, during which dADAR protein levels also become detectable. The detection of FL isoform in both the CNS and gut in embryos is in contrast with the knowledge that dADAR editing primarily occurs in the CNS. While the function of FL is well defined, nothing is known about the function of the evolutionary conserved TR isoform. Unlike FL, TR isoform is limited to the gut region and since both dADAR isoforms possess dimerization domains, their colocalization in the gut could result in formation of heterodimers, which in turn may reduce dADAR activity due to absence of the catalytic domain in the TR isoform. We are testing a model in which heterodimers between the two major isoform classes negatively regulate dADAR catalytic activity during embryonic development in the gut region. We chose the yeast two-hybrid system to test interactions between dADAR isoforms. Our results show, for the first time, that the TR isoform can bind to FL isoforms. We predict that the FL-TR heterodimers result in virtual absence of RNA editing in the gut during fly embryo development. We have also measured the relative binding strength in our heterodimers and have shown that heterodimers involving the TR isoform have a considerably lower stability than hetero- and homodimers between various FL isoforms. This may be indicative of a role for catalytic domain in stabilizing dimer structure by RNA binding. Here, we have tested deletions in ADAR to find a threshold where FL-TR heterodimers will cease to form, but FL hetero- and homodimers will still exist. In this study, we introduce a dADAR isoform that lacks the first 21 amino acids to provide the above-mentioned requisite. Based on these findings, we propose that in the absence of the first 21 amino acids, mutation of the catalytic domain will disrupt or strongly decrease dimer formation and RNA binding activity in dADARs, and hence reduce RNA editing activity, an idea now being tested. We will use this newly found role of the catalytic domain to study important RNA binding/editing sites in this protein.

675 Differential effects of vitamin A deprivation on different photoreceptor types. *Clara Poupault*[1], Khanh Lam[1], Alexis Perry[1], Mukesh Kumar[2], Andrej Shevchenko[2], Jens Rister[1] 1) UMass Boston, Boston, MA; 2) Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

In *Drosophila melanogaster*, six rod-equivalent 'outer' photoreceptors are responsible for vision at low light intensities and express the light-sensing Rhodopsin Rh1, while the cone-equivalent 'inner' photoreceptors mediate color vision and express the Rhodopsins Rh3, Rh4, Rh5, or Rh6. As in humans, vitamin A is essential for *Drosophila* vision and vitamin A deficiency leads to severe visual defects. Flies also cannot synthesize vitamin A *de novo* and must

obtain it from their diet. Our goal was to analyze the effects of vitamin A deprivation and vitamin A replacement therapy on outer *vs.* inner photoreceptor structure, as well as on the expression of their different Rhodopsins.

Our goal was to compare the retinas of wild type Canton S (CS) flies raised on minimal yeast food supplemented with vitamin A (vitA+) or without vitamin A (vitA-). Using Rhodopsin antibodies and Phalloidin to visualize the rhabdomeres (specialized compartments that contain the Rhodopsins), we found that flies raised on vitA- food had outer photoreceptors that were dramatically reduced in size and severely reduced levels of Rh1 (in line with previous publications). The inner photoreceptors were also reduced in size, but to a lesser extent, and Rh5 was lost. In contrast, Rh3, Rh4, and Rh6 were expressed, but abnormally concentrated outside of the rhabdomeres.

In a complementary approach, we studied mutants that cannot take up or process vitamin A from their diet, but were raised on standard lab food containing vitamin A. *ninaD*^{1} lacks a scavenger receptor responsible for the cellular uptake of dietary vitamin A in the gut. This mutant had substantially reduced rhabdomeres and also displayed a loss of Rh1 and Rh5. Rh6, Rh3, Rh4, were expressed, but the latter were concentrated mostly outside the rhabdomeres of the inner photoreceptors. *ninaD* mutants therefore resemble vitA- wildtype flies.

ninaB^{1} mutants lack the carotenoid oxygenase to process vitamin A downstream of *ninaD* and also had substantially reduced rhabdomeres. As opposed to *ninaD*^{1} or CS on vitA- food, *ninaB*^{1} had faint residual levels of Rh1 and Rh5. Moreover, *ninaB*^{1} also expressed properly localized Rh3, Rh4, and Rh6. Taken together, *ninaB*^{1} mutants showed lesser defects than the *ninaD*^{1} or CS flies raised on vitA- food. This suggests that other factors participate in vitamin A processing that could compensate for the lack of *ninaB* expression.

Taken together, we found that vitamin A is essential for proper development of the rod-equivalent outer photoreceptors and the cone-equivalent inner photoreceptors, but with differential effects on Rhodopsin levels and their intracellular localization.

676 Localization of histamine to the accessory gland and its effects on the female post-mating response in *Drosophila melanogaster*. *M.G. Burg*[1,2], M. Allen[1], L. Gerritsen[1], A. Prince[1] 1) Biomedical Sciences, Grand Valley State Univ, Allendale, MI; 2) Cell & Molecular Biology, Grand Valley State University, Allendale, MI.

The study of histamine and its function in *Drosophila melanogaster* has been focused on its role as a neurotransmitter used by photoreceptors[1] and its effects on a number of other behaviors, such as grooming, thermal preference, and sleep[2]. In these studies, histamine deficiencies in peripheral or central neural tissue were correlated with a specific disruption in function, such as histamine deficiency in photoreceptors disrupting vision[3] or lack of histamine in specific central neurons in the brain affecting thermal preference[4]. To understand what role histamine may play as an important biogenic amine in tissues outside the nervous system, a systematic approach was taken to localize histamine in non-neural tissue in larval and adult stages. A number of anatomical regions of the fly were examined using histamine immunofluorescence, comparing results between wild-type flies and *Hdc*^{JK910}mutants that produce no histamine[1]. From this examination, two previously unreported locations were found that contain histamine: one in the gut[5] and the other in the male accessory gland. Histamine immunofluorescence in the accessory gland appears to be limited to about 40-50 cells which, given their position, are likely to be secondary cells that are known to be involved in mediating the female post-mating response (PMR)[6]. Previous studies have shown that secondary cells of the accessory gland are responsible for enabling increased egg laying and inhibiting female receptivity to male courtship advances. To determine whether histamine could modulate the female PMR, 4-5 day old wild-type (Oregon-R) females were mated with either similarly aged wild-type (Oregon-R) or *Hdc*^{JK910} mutant males (which lack histamine in the accessory gland), after which female behaviors were studied. Results indicate that histamine deficiency in the male accessory gland interferes with egg laying that is typically observed in females after mating[6]. The effect of histamine deficiency on other aspects of the PMR, such as female receptivity, requires further experimental observations that are currently in progress. These results indicate that histamine does function as a biogenic amine in tissues outside of the nervous system, and in this case, appears to be necesssary for normal post-mating response behavior in female flies.

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677 Transcriptional dysregulation of neuroblasts as a putative hallmark linking neuronal morphology to intellectual disability. *H. A. M. Hatch*[1], H. M. Belalcazar[2], S. Zamurrad[2], J. Secombe[1,2] 1) Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY; 2) Department of Genetics, Albert Einstein College of Medicine, Bronx, NY.

Intellectual disability (ID) disorders affect 2% of the population and are characterized by an IQ score lower than 70 with deficits in adaptive functioning. Mutations in over 400 genes contribute to the pathogenesis of ID disorders, with patients presenting with learning and memory impairments and often syndromic features such as epilepsy, anxiety, short stature, and aggressive tendencies. Our research focusses on the KDM5 family of transcriptional regulators, mutations in which account for 1-3% of inherited ID ranging from mild to severe. The molecular mechanisms by which KDM5 proteins impact neuronal function remain largely unknown, leaving patients without effective treatment strategies. Thus, the overarching goal of this project is to understand how KDM5 contributes to neuronal and transcriptional outputs that influence cognition, and how these processes are altered by mutations associated with ID.

Here, we demonstrate that *Drosophila*, which possesses a single *kdm5* ortholog, serves as a suitable and genetically tractable model to investigate the molecular, cellular, and behavioral defects associated with ID disorders. Fly strains harboring missense mutations in evolutionarily conserved residues of KDM5 recapitulate cognitive deficits observed in ID patients with analogous KDM5 mutations. Combining analyses of these ID allele fly strains with tools such as targeted RNAi knockdown and behavioral paradigms, we are able to probe the neuronal requirements of KDM5 *in vivo*. Here, we present a demethylase-independent role for KDM5 within neural stem cells in regulating neuronal morphology. We (1) demonstrate that KDM5 is required, independent of its demethylase activity, within mushroom body neuroblasts, but not in post-mitotic, mature Kenyon Cells, for proper mushroom body growth and guidance, (2) show that a subset of *kdm5* ID mutants display morphological guidance and growth defects of the mushroom body, a structure critical for cognition, and (3) identify, using Targeted DamID (TaDa), differentially expressed KDM5 target genes within neuroblasts of *kdm5*knockdown and ID mutant strains, allowing us to elucidate KDM5 transcriptional regulatory networks critical for neuronal morphology and cognitive function.

678 Dysfunction in neurons and glia reveals that distinct *PIGA* deficiency phenotypes arise from independent cell types. E. Coehlo[1], J. Pleinis[2], K. Chung[1], A. Rodan[2], *C.Y. Chow*[1] 1) Department of Human Genetics, University of Utah, Salt Lake City, UT; 2) Department of Internal Medicine, University of Utah, Salt Lake City, UT.

Mutations in the *Phosphatidylinositol glycan class A* (*PIGA*) gene cause *PIGA* deficiency, a type of X-linked epilepsy and intellectual developmental disorder. *PIGA* deficiency is an ultra-rare disease with fewer than 12 patients reported. *PIGA* deficiency is characterized by neonatal hypotonia, myoclonic seizures, epilepsy, dysmorphic features, and a number of congenital anomalies. PIGA is involved in the first step of glycosylphosphatidylinositol (GPI) anchor biosynthesis by transferring N-acetylglucosamine (GlcNAc) from uridine 5'-diphospho N-acetylglucosamide (UDP-GlcNAc) to PI to form GlcNAc-PI. GPI is a glycoprotein that attaches the C-terminus of a protein to the cell surface. GPI-anchored proteins play a number of roles in cell-cell signaling, cell migration, and immunity. It remains unclear how loss of PIGA function contributes to the large spectrum of phenotypes observed in patients. Because a number of the patient phenotypes include nervous system abnormalities, we used RNAi technology to knockdown *Drosophila PIGA* (*PIG-A*) expression in neurons and in glia. Neuron-specific loss of *PIGA* function results in behavioral and neurological abnormalities, including severe locomotion defects and sleep disturbances, that are reminiscent of those observed in patients. Because epilepsy is present in all identified *PIGA* deficiency patients, it is surprising that neuronal knockdown does not result in a seizure-like or bang sensitive phenotype. Strikingly, glia-specific knockdown does result in a bang sensitive seizure-like phenotype, but no movement disorder. To understand the molecular underpinnings of these cell type-specific phenotypes, RNAseq analysis was performed on heads from flies with neuronal or glia-specific knockdown of *PIGA* and controls. Transcriptome analysis in neuron vs glia knockdown heads reveals likely mechanisms as to why seizures are observed in glia knockdowns and not in neuronal knockdowns. These observations are in line with recent studies that demonstrate that seizures can arise from neuronal dysfunction that is secondary to a glial defect. This study suggests that treatment of the epilepsy and seizure phenotypes observed in all *PIGA* deficiency patients will require therapies that target primary and secondary dysfunction in glia and neurons, respectively.

679 Dscam regulates lineage dependent repulsion during columnar unit formation in the medulla. *C. Liu*, O. Trush, M. Sato Graduate School of Medical Sciences, Kanazawa University, Kanazawa, Ishikawa, JP.

Columnar structures are found in the brains of many animals and are important for information processing. According to the “radial unit hypothesis”, columns are formed by neurons that are produced from a common neural stem cell in the mammalian cerebral cortex, but molecular mechanisms that regulate column formation remain largely unclear. The fly brain also shows columnar structure in a part of the visual center, called the medulla, and its developmental processes also show similarities to the mammalian brains. In our study, we focused on Down syndrome cell adhesion molecule (Dscam), which is known as a responsible factor for Down syndrome and is a member of immunoglobulin cell adhesion molecules. Because Dscam has ~20,000 different splice isoforms that induce adhesion and/or repulsion among identical isoforms, it is possible that Dscam orchestrates columnar organization by regulating axonal and dendritic interactions between multiple neurons in a column.

According to the radial unit hypothesis, sister neurons produced by the same neural stem cell need to recognize each other to organize a column. If Dscam is involved in this process, its expression should be temporally upregulated in neuroblasts (NBs), neural stem cell-like cells in the fly brain. Indeed, we confirmed that Dscam is temporally upregulated in NBs under the control of the temporal transcription factor, Homothorax (Hth), and its expression is inherited to medulla neurons. These results imply that the sister neurons inherit the same Dscam isoform from the same NBs and causes repulsion between sister neurons. Indeed, neurons of the same lineage project to different columns in wild-type fly brain. However, the axons of the same lineage abnormally fasciculated to form a bundle and projected to the same or nearby columns in Dscam mutant MARCM clones. Finally, the column morphology was significantly disorganized in a mutant in which the number of Dscam isoforms is reduced. These data demonstrate critical roles of Dscam in lineage dependent repulsion during columnar unit formation in the fly brain.

680 Contribution of Phosphatidylserine Exposure in Engulfment of Dendrite Debris by Phagocytes. *H. Ji*, M. Sapar, B. Wang, C. Han Weill Institute for Cell and Molecular Biology and Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Phagocytic clearance of degenerative neural tissues is critical for maintaining tissue homeostasis and for preventing neuroinflammation in the nervous system. Phagocytosis of neuronal debris relies on “eat-me” signals specifically exposed on the surface of degenerating neurons or neurites. We previously demonstrated that phosphatidylserine (PS) is an “eat-me” signal allowing recognition of degenerating neurites by phagocytes both during developmental pruning and after neuronal injury. PS exposure is dynamically associated with dendrite degeneration and ectopic PS exposure causes context-dependent neurite degeneration. However, how PS exposure is regulated during neurite degeneration and whether PS exposure is required for neurite clearance remain largely unknown. In this study, we found that elevation of NAD^+ levels by Wallerian degeneration slow (Wld^s) overexpression effectively blocks PS exposure on degenerating dendrites of *Drosophila* dendritic arborization (da) neurons both during developmental pruning and after physical injury. Using long-term time-lapse live imaging, we found that, in the absence of PS exposure, the dendrite debris of Wld^s overexpressing neurons were engulfed by phagocytes in a way distinct from wildtype dendrites, suggesting that PS exposure contributes to but is not absolutely required for the clearance of degenerating dendrites. In addition, the loss of engulfment receptor Draper (Drpr) severely impaired but did not block dendrite clearance during pruning, suggesting the existence of redundant engulfment receptors. Suppressing PS exposure in neurons by overexpressing Wld^s did not enhance *drpr* engulfment defects, indicating that recognition of PS is primarily mediated by Drpr. Together, our results reveal a general role of NAD^+ metabolism in PS exposure and provide evidence for other “eat-me” signals coordinating with PS in mediating engulfment of degenerating dendrites.

681 Plasma membrane depolarization induces toxic overactivation of IP_3-receptors during neurodegeneration. *N. Karagas*[1], C. Wong[1], K. Tan[2], Q. Wang[1], Y. Chao[1], M. Rousseau[1], I. Levental[1], M. Zhu[1], Y. Zhou[1], J. Hancock[1], H. Bellen[2], K. Venkatachalam[1] 1) McGovern Medical School at the University of Texas Health Sciences Center, Houston, TX; 2) Baylor College of Medicine, Houston, TX.

Neuronal bioenergetics and excitability are functionally coupled by Na^+/K^+ ATPases, which utilize copious quantities of ATP to maintain the electrical polarity of the plasma membrane. Concordantly, insufficient ATP levels in degenerating neurons diminish Na^+/K^+ ATPase activity leading to chronic depolarization. Here, we reveal inositol trisphosphate receptors (IP_3Rs) as unexpected intermediaries between chronic depolarization and attendant dysfunction in neurons. We demonstrate that depolarization promotes association of the plasma membrane-localized enzyme, phospholipaseβ (PLCβ), with its phosphoinositide substrate, PIP_2. Thus, stimulation of PLCβ –coupled receptors in depolarized neurons results in augmented hydrolysis of PIP_2 to IP_3, and elevated IP_3R activation. Pointing to the pathophysiological significance of this modality, attenuating PLCβ–IP_3R signaling prevented the abbreviated lifespan and motor dysfunction observed in *Drosophila* expressing dominant-negative Na^+/K^+ ATPase or neurodegeneration-causing transgenes. These findings indicate that sustained depolarization induces neuronal dysfunction via IP_3Rs and identify PLCβ –IP_3R signaling as an actionable target for mitigating neurodegeneration.

682 Male aggression requires octopamine and glutamate in dual neurotransmitting neurons. *E. Catudio Garrett*[1], L. Sherer[1], H. Morgan[2], H. Shearin[3], S. Stowers[3], S. Certel[1,2] 1) CMMB Graduate Program, Division of Biological Sciences, University of Montana, Missoula, MT; 2) Division of Biological Sciences, University of Montana, Missoula, MT; 3) Department of Cell Biology and Neuroscience, Montana State University, Bozeman, MT.

Co-transmission, or release of more than one neurotransmitter from a single neuron, has emerged as an important aspect of neuron communication. Recent studies suggests co-transmission impacts circuit output with both differential and synergistic affects. To examine the role of dual transmission on behavioral circuits, we determined that neurons that express the biogenic amine, octopamine (OA) also are glutamate positive. As previous work has established OA is required to promote male aggression in *Drosophila,* we manipulated levels of glutamate and glutamate and OA together to determine if aggressive behavior requires the function of both neurotransmitters from OA-Glu transmitting neurons.

To test if glutamate is required in OA-Glu neurons to promote aggression, we reduced vesicular glutamate transporter (vGlut) levels in OA neurons through RNAi interference or the B3 recombinase system. We found that males with a loss of vGlut in OA neurons like males lacking OA, exhibited decreased aggression as measured by latency to lunge, lunge number, and wing threats. However, vGlut loss in OA-Glu neurons did not increase levels of male-male courtship, indicating glutamate has a separate role from OA on male behavior.
To determine if OA or glutamate release targets the same or different downstream neurons, we are examining the behavior of males lacking OA and glutamate receptors. Preliminary results indicate GluR1A null males display low lunge numbers, higher latency, low wing threats and wing extensions. Using GRASP, we determined that OA neurons form putative synaptic connections with GluR1A-expressing neurons and thus identify one target of OA-Glu neurons. Together, our results demonstrate that co-transmission has separable effects on behavior and lead to future studies aimed at understanding the mechanism and circuitry of neurotransmitter corelease.

683 Dopamine deficiency: how dopaminergic circuits compensate for loss of dopamine. *R. Sangston*, J. Hirsh Biology, University of Virginia, Charlottesville, VA.

Dopaminergic circuits play critical roles in a wide range of behaviors important for survival with conserved neural functions in flies and vertebrates. Aberrant functioning of these circuits leads to disease states. However, compensatory mechanisms delay onset of symptoms in some diseases, such as in Parksinsons where clinical symptoms present only after a majority of dopamine neurons degenerate. Understanding these compensatory mechanisms can identify potential methods for therapeutic intervention. Our lab is particularly interested in the role of dopamine in locomotor behavior and have created a *Drosophila* model that lacks dopamine in the central nervous system (Cichewicz et al., 2017). These flies exhibit obvious locomotor deficits reflecting the functional conservation of dopamine between flies and mammals. We have since identified a sub-line of these flies in which a second-site suppressor leads to normal levels of locomotor activity, and we are mapping this genetic element.
One possibility to explain compensation for dopamine loss comes from observations that many vertebrate dopamine neurons express additional co-transmitters. Here we use the temperature inducible cation channel TrpA1 in our *Drosophila* model to activate dopaminergic neurons in the presence or absence of dopamine. We achieve rapid changes in temperature using a simple, inexpensive device to change temperature of an airstream (BioRxiv: http://dx.doi.org/10.1101/439414). We find strong modulation of locomotor activity in response to activating dopaminergic neurons in a wild type background, as well as significant and temporally distinct modulation even in a dopamine deficient background. The genetic tools available in flies will enable identification of this co-transmitter, and whether it is involved in compensatory signaling that aids in compensation for dopamine loss.

684 The Phenomenon of Negative Cross-resistance between Insecticides attributed by Mutation in Nicotinic Acetylcholine Receptor (nAChR) Subunits. *R. Ghazali*[1,2], T. Perry[1,2], P. Batterham[1,2] 1) School of Biosciences, The University of Melbourne, Parkville, Victoria, AU; 2) Bio21 Molecular Science and Biotechnology Institute, Parkville, Victoria, AU.

Nicotinic acetylcholine receptors (nAChRs) play important roles in neurotransmission. In insect, the receptors have been targeted by various classes of insecticide due to the integral function in the central nervous system. Extensive usage of these insecticides however has led to evolution of resistance over time, implicating the efficacy of the insecticides in pest management. Negative cross-resistance, a phenomenon when an allele confers resistance to one insecticide and shows hyper-susceptibility to another, has been evidenced when nAChRs are disrupted in *Drosophila melanogaster* (preliminary data). This study initiates to profile the negative cross-resistance phenotype in available resistant strains using toxicology bioassay. Loss-of-function mutants of nAChR subunits, *Dα1*, *Dα2* and *Dα6* which previously known to confer nitenpyram-, imidacloprid- and spinosad-resistance respectively, are all observed with negative cross-resistance to another insecticide. Importantly, when measured, level of nAChR subunits that target these insecticides are altered in the mutant strains.

The identification of these negative cross-resistance toxins that are more effective in eliminating the resistants would give a huge advantage in existing resistant management. Also, the study has shown that mutations in one gene family member change the levels of expression of others. This has significant implications for resistance to insecticides that target these receptors and to understanding their broader biological function. Understanding the mechanism(s) involved behind the phenomenon might uncover possible regulatory link between nAChR family.

685 Genetic and environmental factors that modify seizure susceptibility in the *Drosophila* voltage-gated sodium channel mutant, *para^{Shu}*. *J. Mrkvicka*[1], P. Lansdon[1,2], H-L. Chen[1,3], T. Kitamoto[1,4] 1) Interdisciplinary Graduate Program in Genetics, University of Iowa, Iowa City, IA; 2) Department of Molecular Biosciences, College of Liberal Arts and Sciences, University of Kansas, KS ; 3) Department of Medical Research, Tung's Taichung MetroHarbor Hospital, Taichung, Taiwan, ROC; 4) Anesthesia, University of Iowa, Iowa City, IA.

Epilepsy is a very common neurological condition affecting people of all ages and ethnicities. Unmanaged epilepsy leads to failing health and severely reduces quality of life of both patients and family members. The tangible and intangible effects of epilepsy greatly tax public healthcare resources and, although efficacious anti-epileptic drugs are available, a significant portion of patients (30%) are refractory to available therapies. Development of novel approaches to treat drug-resistant epilepsy is one of the major challenges of current medical practice. This daunting task is made more tractable by recent clinical and experimental studies that demonstrate how epilepsy severity is modifiable by gene-gene and gene-environment interactions. These studies raise the exciting prospect that in the future, currently refractory epilepsy will be manageable by manipulating identified phenotypic modifiers. To this end, our lab is studying epilepsy-modifying gene-gene and gene-environment interactions using a fruit fly voltage-gated sodium (Na_V) channel mutant, *para^{Shu}*. *para^{Shu}* mutants have severe neurological defects that are modifiable by both genetic and environmental manipulations. Our genetic analyses have identified that reduced function of the genes *Glutathione-S-Transferase S1* (*GstS1*) or *Kelch-like ECH-associated protein 1* (*Keap1*) results in significant suppression of *para^{Shu}* mutant phenotypes. *GstS1* is the putative ortholog of the human gene, hematopoietic prostaglandin D2 synthase, a generally pro-inflammatory enzyme, while Keap1 is an evolutionarily conserved inhibitor of the nuclear factor erythroid 2–related factor 2 (Nrf2), the master transcription factor for antioxidant responses. Interestingly, similar phenotype suppression is achieved by manipulating key environmental factors during development. By removing endogenous commensal bacteria or by feeding the mutants a diet supplemented with milk whey, *para^{Shu}* phenotypes are suppressed. The underlying mechanisms of phenotypic suppression by these genetic and environmental modifiers are likely shared, at least in part. An understanding of their functional interactions should provide insights into the biological processes that are critical for the manifestation and severity of seizures in Na_V channel mutants.

686 *Drosophila Neuroligin 3* affects social behaviour. *J. W. Robinson*[1], S. Aletta[1], R. T. Yost[1], R. Hakimjavadi[1], M. Soliman[1], A. M. Scott[2], R. Dukas[2], A. F. Simon[1] 1) Department of Biology, Western University, London, ON, Canada; 2) Animal Behaviour Group, Department of Psychology, McMaster University, Hamilton, ON, Canada.

Drosophila exhibit social behaviours when in proximity to other individuals. Choosing a preferred social space[1] and deciding to group with others (sociality[2]) are two of these behaviours. Determination of social spacing is linked to many factors such as social experience, genetics, or differential signalling of neural circuitry. So far, it has been determined that the mushroom bodies, a sensory integration center in fly brains, are important for social space[3], as well as cholinergic[3] and dopaminergic[4] signalling. However, we do not know how these different parts of the neural circuitry are potentially integrated, nor do we

know how different social behaviours relate to one another. Here, we show that *Drosophila neuroligin 3 (dnlg3),* a gene encoding a post-synaptic protein that regulates transmission at the synapse[5], affects fly social spacing and sociality, with minor effect on climbing ability. Using genetic and molecular techniques, we identify specific brain regions rich in DNLG3. We assess how separate mutations in *dnlg3*, and how different brain regions where the gene is expressed, can differentially affect social behaviour and locomotion. Our results contribute to the understanding the role of neuroligins in a social behaviour response, as these genes have been linked to proper social interactions from flies to human[6,7].

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687 The Role of Vamp7 in Regulating PDF Controled Sleep Patterns. *M.M. Narvaez*[1], R. Jorquera[2], J.L. Agosto[1], A. Ghezzi[1], M.E. Ramirez[1] 1) Department of Biology, University of Puerto Rico, Rio Piedras, San Juan, PR; 2) Department of Neuroscience, Universidad Central del Caribe, Bayamon, PR.

Many molecular functions require synthesized components that are packed and mediated by vesicular transport proteins from one cell to another. There is a vast group of proteins involved in this transport, different coat proteins attract different types of adapter proteins and this itself is associated with a specific set of cargo receptors and cargo components. It is also known that there is specificity between vesicle transport proteins and their cargos, but not all of the associations have been identified. Vesicle-associated membrane protein 7 (VAMP7) is part of the SNARE complex that mediates vesicle fusion. VAMP7 is enriched in the nervous system, it highly promotes neurite development, cell adhesion, and it is believed that is involved with intracellular signaling cargos. For this reason, we consider that Pigment-dispersing factor (Pdf) is a possible cargo candidate. Pdf is a secreted neuropeptide that acts as a ligand of specific G-protein-coupled receptors and participates in circadian sleep/wake cycle process via intracellular signals. Here, we perform RNAi-induced knockdown of VAMP7 in Pdf-expressing neurons of *Drosophila melanogaster*, and explore its effects on specific pdf controlled behaviors, such as the regulation of circadian rhythms and sleep patterns. Our results show that VAMP7 knockdown induces specific alterations in both circadian and sleep patterns, suggesting that VAMP7 is a potential mediator of PDF-release.

688 The neuronal design underlying consolidated Anesthesia-Resistance Memory (ARM). *E. Antwi-Adjei*[1], M. Schwaerzel[2] 1) Freie Universität Berlin, Department of Biology; 2) Washington University in St. Louis, Department of Biology.

Stimulus directed behavior is regulated by communications within various neural circuits existing throughout the animal's brain. Experience-dependent activities can change the dynamics of the neural circuit by altering synaptic morphologies between these neural circuits. However, unravelling the mechanism behind behaviorally-related plasticity has proven to be elusive. The *Drosophila* melanogaster has a numerically small brain and also abreast with robust olfactory network circuit. In relation to this, we performed olfactory neural network circuit analysis by altering the synapses to study the consolidated part of *Drosophila* olfactory memory i.e. Anesthesia-Resistant Memory (ARM). Bruchpilot (Brp/ELKS)- a presynaptic protein which forms part of the cytomatrix active zone (CAZ) functions in the release of neurotransmitters by establishing a close proximity of Ca2+ ion channels with synaptic vesicles and formation of T bars. Nonetheless, reducing Brp levels specifically in Antennal lobe (AL), Kenyon cells (KCs), Dopaminergic neurons (DANs) and Mushroom body Output Neurons (MBONs) via genetically targeted RNA-interference (RNAi) impaired the formation of the aversive ARM. The extensive effect on learning pinpointed the existence of recurrent loop network between the synapses of Kenyon cells (KCs), Dopaminergic neurons (DANs) and Mushroom body output neurons (MBONs). The recurrent activity in the network loop required a concurrent activation of the glutamatergic MBONs and depolarization of the dopaminergic neuron in order to gate the activity of the presynaptic plasticity. In spite of the involvement of these neural circuits in learning and memory, these neural circuits also form part in memory reconsolidation, retrieval and re-evaluation.

689 The role of actin regulating genes in alcohol induced behaviors in *Drosophila*. *A.R. Butts*[1], S.A. Ojelade[2], A.R. Rodan[1,2,3,4], A Rothenfluh[1,2,3,5] 1) Molecular Medicine Program, University of Utah, Salt Lake City, UT; 2) Department of Psychiatry, Program in Neuroscience, UT Southwestern Medical Center, Dallas, TX; 3) Department of Human Genetics, University of Utah, Salt Lake City, UT; 4) Department of Internal Medicine, Division of Nephrology, University of Utah, Salt Lake City, UT; 5) Department of Psychiatry, Department of Neurobiology and Anatomy, University of Utah, Salt Lake City, UT.

Alcohol use is highly prevalent in the United States and across the world, and every year millions of people suffer from alcohol use disorders (AUDs). While the genetic contribution to developing AUDs is 50-60%, many of the underlying molecular mechanisms remain unclear. Previous studies from our lab based on genetic screens of P-element insertions in the *Drosophila* genome revealed that mutations in RhoGAP18B and Ras Suppressor 1 (Rsu1) cause reduced sensitivity to ethanol-induced sedation. Both Rsu1 and RhoGAP18B are negative regulators of the small Rho-family GTPase, Rac1, a modulator of actin dynamics.

Here we investigate the role of Rac1 and the downstream actin-severing protein, cofilin, in alcohol consumption preference. We show that expressing activated Rac1 and dominant-negative cofilin in the mushroom bodies (MB) abolishes experience-dependent alcohol preference. Conversely, dominant-negative Rac1 and activated cofilin MB expression leads to alcohol preference in a 16-hr CAFE (capillary feeder) choice, but not in a 30-min choice assay. This suggests that these flies have normal acute alcohol avoidance, but show fast acquisition of alcohol preference. To test this hypothesis, we have established a CAFE on-line learning assay (COLA), that allows us to monitor feeding behavior and preference with minute resolution. We will present our findings with the aforementioned actin mutants.

690 The neural circuitry of learning dialects in Drosophila species. *B.Z. Kacsoh*, J. Bozler, S. Hodge, G. Bosco Molecular and Systems Biology, Geisel School of Medicine at Dartmouth, Hanover, NH.

Drosophila species are able to communicate the presence of parasitoid wasps to naïve individuals. Communication manifests as oviposition depression in wasp-exposed, teacher flies and naïve student learning from teachers. This suggests a rudimentary Drosophila social structure. Communication between closely related Drosophila species is efficient, while more distantly related species exhibit a dampened, partial communication. Remarkably, partial communication between some species is enhanced following a period of cohabitation. This cohabitation demonstrates the evolution of linguistic variations between species, termed “dialect-learning.” However, it remains unclear as to how the behavioral acquisition and learning dialects can be modulated within distinct or multiple brain regions. Utilizing a deficiency screen style approach, we identify six regions of the Drosophila brain involved in dialect learning. We find these regions by independently turning off synaptic transmission in key areas of the Drosophila brain during dialect learning. We then performed a

deeper examination into three regions identified. One region identified is the antennal lobe, specifically the D glomerulus, where we subsequently identify the odorant receptor Or69a as a mediator of dialect learning. We also identified the optic lobe as a mediator of dialect learning, where we pinpoint subgroups of neurons known as motion detecting neurons in the optic lobe. In particular, we identify L2 and L4 neurons as being necessary for dialect learning, while L4 neurons are also sufficient to drive dialect learning. Finally, we examine the fan-shaped body, where we identify layer 5 as both necessary and sufficient for dialect acquisition. These results demonstrate higher-order complex behaviors are facilitated by multiple brain regions, with unique subsets of neurons. Without dialect learning, information might be lost in translation or muddled, resulting in an inefficient behavioral response that yields significant survival disadvantages. Adult behaviors may emerge only as a result of previous social experiences. Such experiences take place in nature, where relevant ecological pressures are ever present alongside multiple species. This study integrates the vast tool-box and the life history of the Drosophila model in an effort to dissect the neuro-circuitry governing the observation of dialect learning which involves the maximization of inclusive fitness.

691 Modulating dopamine neuron activity transiently suppresses or enhances expression of aversive long-term memories. *J.M. Sabandal*, R.L. Davis Department of Neuroscience, Scripps Research Institute, Jupiter, FL.

The compelling body of work accumulated in *Drosophila* olfactory learning and memory have elucidated the important neural, cellular and molecular networks underlying memory formation, stabilization and retrieval. Additionally, recent findings have identified that forgetting is an active process which include a small cluster of protocerebral posterior lateral 1 dopamine neurons (PPL1 DAns) that vigorously extinguish labile short-term memories. However, information about whether these forgetting cells cause memory failures for consolidated protein-synthesis dependent long-term memories (PSD-LTM) is missing. Here, we interrogated whether active forgetting is involved in PSD-LTM by manipulating PPL1 DAn activity before retrieval and monitored the flies' performance. Blocking synaptic release from PPL1 DAns increased, while ectopic activation decreased the expression of PSD-LTM at 3 days. More specifically, the bidirectional activity of a single PPL1 DAn that innervates the upper stalk region of the mushroom bodies (MB), MBα2/α'2, produced the most robust differential expression of PSD-LTM. Surprisingly, waiting for several days after modulating PPL1 DA-MBα2/α'2 neuron activity allowed the expression of PSD-LTM to resurface, revealing the transient nature of the DAn-induced suppression or enhancement on memory expression up to 14 days. In addition, we discovered that the DA D5 receptor, DAMB, suppressed LTM formation in αβ MBns, and that DAMB function is required for the DAn-induced suppression of PSD-LTM expression. Our findings show how tuning DAn activity can shape memory expression and may translate to memory loss in neurological and psychiatric disorders.

692 Study of female mating decision using an incipient speciation model in *Drosophila melanogaster*. Sheng-Hao Wang, Yu-Chiao Lin, *Tsung-Han Kuo* Institute of systems neuroscience, National Tsing Hua University, Hsinchu, TW.

Decision making is a critical process for both innate and learned behaviors involved in animal survival and reproduction. One of the most important decisions for female animals is mating choice, which plays the central role for the next generation fitness and further influence population through sexual selection. The sexual isolation between the *Drosophila melanogaster* populations in Zimbabwe and those from other populations in the world has been widely studied by evolutionary biologist. In the presence of males from their own population, females from most isofemale lines of Zimbabwe (Z) will not mate with males from elsewhere (M). In this report, we provide new evidences to show that M females also perform preference to mate with their own males, although the discrimination ability is much weaker. While M female preference is largely determined by male courtship activity, pheromones also played an important role to affect female decision. Targeting different pheromone sensing organs further led us to identify the function of forelegs in this sexual preference. Our next step is to compare the expression profile of gustatory receptors in the forelegs between Z and M populations. We are hoping that identifying the receptors responsible for male recognition may provide us new information about the molecular basis involved in sexual selection and potentially help us to further mapping the neural circuits for mate choice.

693 Determining the Role of microRNAs in the Female Drosophila Post-Mating Response. *C. Bennett*[1], G. Carney[1,2] 1) Department of Biology, Texas A&M, College Station, TX; 2) Department of Biological Sciences, University of Idaho, Moscow, ID.

After mating, female *Drosophila melanogaster* exhibit a distinct set of behavioral and physiological changes, collectively referred to as the post-mating response. Hallmarks of this response include increased egg-laying rate and reduced receptivity to mating. Many of these changes have been tied to seminal fluid proteins (SFPs), passed from male to female during mating, via his ejaculate. However, it is possible that a female fly can alter the response with her own endogenous molecules. To test this hypothesis, we screened microRNA (miRNA) deletion mutant strains to identify candidate miRNAs that change the post-mating kinetics of egg laying or receptivity to mating. Three of 29 miRNA deletion lines tested affected remating latency, and 25 of 59 tested affected egg-laying rate. Some of these strains delete multiple miRNAs. We confirmed the effects of 3 of these miRNA deletion mutants (1 remating latency, 2 egg laying) by testing them *in trans* with a deficiency allele. One deletion mutant removes a single, highly conserved microRNA linked to lipid metabolism, which is important for egg production. Mated mutant females increase egg laying compared to control females, and the effect was rescued by ubiquitous miRNA expression in the mutant background. Ubiquitous expression of a competitive inhibitor of the miRNA (via a miRNA-sponge) also increased egg-laying rates post-mating. Current efforts are focused on identifying the molecular pathway(s) in which the miRNA acts.

694 Genomic and neurogenetic approaches reveal a role of *dpr*- and *DIP*- expressing neurons in courtship behaviors. *Hongru Hu*[1], Pam Lovejoy[1], Nicole Newell[1], Colleen Palmateer[1], Pei-Tseng Lee[2], Hugo Bellen[2], Kai Zinn[3], Michelle Arbeitman[1] 1) Department of Biomedical Sciences, College of Medicine, Florida State University, Tallahassee, FL; 2) Departments of Molecular & Human Genetics and Neuroscience, Baylor College of Medicine, Huston, TX; 3) Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA.

Drosophila melanogaster adults display sex differences in reproductive behaviors that are specified by the sex determination hierarchy. This hierarchy consists of an alternative pre-mRNA splicing cascade that culminates in the production of sex-specific transcription factors encoded by *doublesex* (*dsx*) and *fruitless* (*fru*). Both *dsx* and *fru* are required for the potential for reproductive behaviors by directing sex-specific differences in neuronal arborization patterns, connectivity, neuron number, and physiology. Our genomic studies showed that the downstream target genes of male-specific Fru (Fru^M) isoforms are significantly enriched with genes encoding immunoglobulin domain superfamily (IgSF) members, including *defective proboscis extension response* (*dpr*) and *Dpr-interacting protein* (*DIP*) genes. There are 21 *dpr* and 10 *DIP* genes, and other laboratories have shown that the interactions of their protein products mediate synaptic specificity. Additionally, our previous *in vivo* functional analyses demonstrated that *dpr1* is important for the timing of progression through sub-steps of the courtship ritual. Taken together, these results inspired us to investigate the functional role of the *dpr*s and *DIP*s in directing sex differences in connectivity. We are now able to examine the role of different subsets of *fru P1* co-expressing (*fru P1*∩*dpr/DIP*) neurons, to understand the neural circuit basis for sex-specific reproductive behaviors. Using scRNA-seq, we have examined the combinations of *dpr* and *DIP*expression patterns within individual *fru P1*-expressing neurons to gain insight into the combinatorial code of these adhesion molecules. We have visualized *fru P1*∩*dpr/DIP* neurons and found that expression patterns are diverse, change throughout development, and show sexual dimorphism. We also investigated how perturbations of *dpr*s and *DIP*s affect development and morphology of *fru P1*∩*dpr/DIP* neurons, and behavior. Finally, we activated or silenced *fru P1*∩*dpr/DIP* neurons and observed aberrant male courtship, elucidating the roles of neuronal subsets in controlling reproductive behaviors.

695 The dopamine receptor D2R is required in the blood brain barrier for male courtship behavior. *C.R. Love*, B. Dauwalder Biology and Biochemistry, University of Houston, Houston.

We have previously shown that the G-protein Go and sex-specific signaling in the blood-brain barrier (bbb) is required for male innate courtship sustainment towards naïve females. The subperineurial glia (spg) cells form the bbb by providing a contiguous barrier, connected by septate junctions, at the interface between the hemolymph and the brain. Here we show that the glial cells that form the bbb may modulate male courtship behavior through the dopamine receptor D2R. While the neural circuits required to produce scripted actions in the fly, such as the steps for courtship, have been widely investigated, much less is known about how the circuitry and functions underlying the fly behavior is influenced by cell- non-autonomous molecular processes. We have shown that male-specific molecules in the bbb regulate male courtship. Here we identify the *Drosophila* Dopamine 2-like Receptor (D2R) as one of these regulators.

In a screen for sex-specifically expressed genes in the adult bbb, we identified D2R in males and confirmed its presence using qPCR and a *Drosophila* D2R antibody. D2R knockdown with RNAi or over-expression of D2R using Gal4/UAS in the spg of adult males significantly reduces courtship. Knockdown or overexpression of D2R with ubiquitous neuronal Gal4 drivers or spg knockdown specifically during development has no effect on the courtship index. Reduced courtship in D2R mutant males is rescued by expressing an isoform of D2R in the spg specifically after adult maturation. To maintain full male courtship levels D2R likely signals through Go and β-arrestin. Our results indicate an expanded role for dopamine signaling in the glial cells that surround the brain and provide a critical time frame for its action in behavior.

696 Courtship behavior and ovipositor extension neural circuits in *Drosophila Melanogaster*. *H. Cheng*, S. Wei, T. Kuo Institute of system neuroscience, National Tsing Hua University.

The diverse behaviors in *Drosophila Melanogaster* have been studied for many years, the neural circuits behinds these behaviors, however, are still not fully understood. New developed automated laser tracking and optogenetic manipulation system (ALTOMS) provides us a platform to high-throughput screen multiple lines with different behaviors. By activating neurons labeled by different Gal4 driver lines, we identified couples of lines with specific behaviors, including ovipositor extension in females and abdomens bending in males implying their functions in egg laying and mating behaviors. We further inhibited the activity of targeting neurons by temperature-sensitive *Shibire* and showed that thermogenetic inhibition of these specific neurons decrease the copulation rate or the number of laying eggs in corresponding lines. Surprisingly, while both behavior are sexually dimorphic, intersection study suggested that none of these neurons are fruitless positive. Finally, the neural anatomy of these targeting gal4 lines were examined under confocal microscopy. These candidate gal4 lines have provided us new opportunities to further map the neural circuitry which responsible for mating and egg laying behaviors.

697 Alcohol-intoxicated flies become aggressive. *A. Park*[1], T. Tran[1], L. Gutierrez[1], C. Stojanick[1], R. Bohm[2], N. Atkinson[1] 1) University of Texas at Austin, Austin, TX; 2) Texas A&M in Kingsville, Kingsville, TX.

Alcohol-induced aggression is a serious and devastating issue that affects billions of people worldwide. Despite the pervasiveness of this phenomenon, we do not have a clear understanding of the neural and genetic components that drive this behavior. A reason for a gap in our understanding about the molecular basis of alcohol-induced aggression arises from the ethical challenges most vertebrate researchers face when they must consider how to treat their animals. We find that when given a low dose of alcohol, *Drosophila melanogaster* can acquire alcohol induced aggression and may be the ideal model organism to study this disorder. Male flies become more aggressive after consuming a low dose of alcohol and have impaired courtship abilities, whereas female flies become more receptive to courtship and less choosy. We also demonstrate that alcohol-induced aggression is regulated by FruM (Fruitless Male) a transcription factor that specifies maleness and is involved in several other sexually dimorphic behaviors. Finally, we find that low doses of alcohol activate components of the FruM circuit, whereas higher doses of alcohol suppress FruM production.

698 Neural circuits control fly grooming over several timescales. *J.H. Simpson*, N. Zhang, S. Yoshikawa, D. S. Syed, P. Ravbar, J. Mueller, S. Marella, L. Guo MCDB, UCSB, Santa Barbara, CA.

The way animals integrate sensory cues and internal priorities to assemble complex behaviors from simpler movements is not well understood. Fly grooming is a powerful model system to study this problem. Flies groom in response to sensory stimuli with stereotyped leg movements targeted to different parts of the body, and they progress from cleaning anterior to posterior body parts. We use behavioral analysis and optogenetic manipulation to dissect the neural circuit motifs that coordinate the limb movements and subroutine order that result in this flexible motor sequence.

Several different types of mechanosensory neurons can evoke grooming. While mechanosensory bristle neurons play the most critical role, bitter taste and chordotonal organs can contribute to short time-scale alternation between grooming movements. Genetic manipulation of sensory neurons and competitive optogenetic activation of different body parts demonstrates that changing the amount of sensory inputs to a body part alters the probability that it is cleaned. This supports a role for sensory gain in establishing the anterior to posterior hierarchy.

Annotation of grooming movements in many flies using our Automated Behavior Recognition System and subsequent modeling efforts supports our proposal that the probability of selecting a given movement depends on the strength of sensory stimulation, but also on the identity and duration of the previous action. The minor but significant contribution from duration suggests that there is internal control of the alternation between some behaviors. This internal organization is corroborated by identification of some descending neurons that evoke alternating bouts of front leg rubbing and head cleaning.
Using DeepLabCut limb tracking software, we have quantified leg sweep speeds and left-right coordination patterns during different grooming movements in wild-type dusted flies and when different neurons are optogenetically activated. These data imply that central pattern generators may govern the stereotyped movements of individual legs but both sensory feedback and central circuits coordinate the switches between in-phase and out-of-phase modes when the legs work together.

We are mapping the neural circuits that control grooming using screens for descending, dopaminergic, and GABAergic neurons. Some neurons bias grooming toward more posterior movements, while others disrupt normal left-right or front-back limb coordination. These behavioral phenotypes reveal organizing principles and guide the search for neurons that connect sensory inputs to motor outputs.

699 Engineering a *UAS-fried-V5* transgene for phenotypic analysis and rescue of *fried*. *M. Stein*, S. Sabido, J.Z. Morris Natural Sciences, Fordham University, New York, NY.

We previously identified the Drosophila *fried* gene, which is required for oogenesis, as *CG31320*, the gene that encodes the 842 amino acid HEATR2 protein (Diggle, et al., 2014, Morris, et al., 2003, Morris et al., unpublished). *fried* mutants exhibit varied phenotypes such as darkening of the trachea, precocious wandering, larval arrest, and death within 7 days after egg deposition. To correlate rescue of *fried* phenotypes with expression in particular tissues, we engineered a *UAS-fried* transgene with a V5 tag. To generate this contsruct, we first used Gibson cloning to isert *fried* (which we produced via long-range PCR) into a vector containing a *UAS* promoter, a *mini-white* marker, and an *attB* site. We then used site-directed mutagenesis to introduce a V5 tag just before the

stop codon in *fried* and to repair a mutaiton produced by the PCR.

The construct was injected into a strain carrying an *attP* site on chromosome 3L. We are currently generating recombinant flies carrying the *UAS-fried-V5* transgene and a *fried* loss of function mutation on the same chromosome. We will cross those recombinant flies to lines carrying other loss-of-function *fried* alleles and various *Gal4* drivers in order to determine which patterns of *fried* expression rescue which *fried* mutant phenotypes. We will use the V5 tag to follow Fried protein expression and to determine the subcellular localization of Fried protein.

700 Modulation of Gene Expression by Cocaine and Methamphetamine in *Drosophila melanogaster*. *C.A. Highfill*, B.M Baker, R.R.H Anholt, T.F.C Mackay Department of Genetics and Biochemistry, Clemson Center for Human Genetics, Clemson University, Greenwood, SC.

Substance abuse is a global pandemic. In 2015, an estimated 250 million people worldwide used illicit drugs; and in the United States alone, drug use disorders account for monetary losses exceeding 740 billion dollars annually. However, our understanding of the genetic factors underpinning the liability to transition from drug user to drug abuser is incomplete, in part because it is difficult to disentangle variation in social and other environmental influences from genetic variation, and because it is not possible to precisely control drug intake in human populations. *Drosophila melanogaster* is a powerful genetic model system for understanding the genetic basis of individual variation in drug responses. The effects of cocaine and methamphetamine in flies recapitulate those observed in people, and importantly, the genetic background and drug exposure can be controlled precisely. Here, we used RNA sequencing of Canton S (B) flies to evaluate the genome wide effects on gene expression following acute exposure to a defined quantity of cocaine or methamphetamine as young adults and chronic exposure to either drug during development. We obtained triplicate samples of treated and control flies. For the acute exposures we obtained samples of heads and bodies of males and females. For the developmental exposures we obtained samples of first and third instar larvae, and heads and bodies of young male and female adults. For both acute and chronic exposures, we uncover vast amounts of differentially genes that participate in oxidation-reduction and NTRs that have variable developmental/tissue effects. These data suggest other mechanisms, such as Cyp450s metabolism, at work beside neural circuitry that affect drug-seeking behavior. Of interest, no studies examining drug addiction have investigated Cyp450s metabolism effects on drug addiction in the gut or blood brain barrier. Lastly, this study provides more evidence that *Drosophila melanogaster* can be developed as a powerful model system to identify evolutionarily conserved genes that affect drug-seeking behavior.

701 Ethanol exposure affects innate behaviors. *P. Garcia-Trevizo*, P. Sabandal, K. Han Department of Biological Sciences, Neuromodulation Disorders Cluster at Border Biomedical Research Center, University of Texas at El Paso, El Paso, TX.

Alcohol has stimulatory and depressant effects on both humans and flies. Previous research has established that the fruit fly *Drosophila melanogaster* is a good animal model to study alcohol abuse and addiction. However, the neurobiological mechanism by which ethanol modulates innate and reward-seeking behaviors is not well characterized. Our study is directed to narrow this knowledge gap by first characterizing the effects of ethanol on innate and reward-seeking behaviors in *Drosophila*. For the task, we exposed wild type *Canton-S* males to ethanol till sedation for 6 consecutive days, and on the following day we measured the courtship activity of the chronic ethanol-exposed or non-exposed male towards a virgin female. We found that the males with chronic ethanol exposure exhibited shorter latency to court a female compared to control males. This suggests that chronic ethanol exposure enhances courtship drive. We also examined food consumption using the CAFE assay that allows to measure total food consumption over the course of 3 days as well as ethanol preference. We found that both female and male flies previously exposed to ethanol had less food consumption compared to non-exposed flies. This implicates that chronic ethanol exposure has an appetite suppressant effect. We are currently investigating whether chronic ethanol or other factors affect ethanol preference. This study may provide novel insight into the factors and mechanisms by which ethanol alters innate and reward-seeking behaviors relevant to alcohol addiction.

702 Detection of a possible pathogenic phenotype caused by Nora virus infection in *Drosophila melanogaster* . *L. Towery*, A. McCown, A. Benz, D. Crisman, K. Carlson Biology, University of Nebraska at Kearney, Kearney, NE.

Nora virus is a picorna-like virus that contains a positive-sense, single stranded RNA genome. The virus infects *Drosophila melanogaster* with no known pathogenic effects. One hypothesized pathogenic effect of Nora virus is a deficit in locomotor ability of the fly. In this study, geotaxis assays and longevity curves were used to determine if Nora virus infection has an effect on *D. melanogaster's* locomotor ability. Quart sized cages (five Nora virus infected, five Drosophila C virus (DCV) infected, and five uninfected) were established each containing 60 virgin female flies. The cages were marked with a line two thirds from the bottom of the cage. Every third day since cage establishment, the flies were tapped to the bottom, allowed one minute to reach the top, and the number of flies crossing the threshold line were recorded. Also on every third day, the dead flies were removed, the number recorded, longevity curves were created and examined using Kaplan-Meyer survivorship analysis. The data demonstrated a significant decrease in both geotaxis and longevity when the *D. melanogaster* were infected with either Nora virus or DCV, as compared to uninfected controls. This is the first time that a possible phenotype has been associated with Nora virus infection. Overall, the data demonstrate that geotaxis and locomotor dysfunction may be a pathogenic hallmark of Nora virus infection. The project described was supported by grants from the National Center for Research Resources (5P20RR016469) and the National Institute for General Medical Science (8P20GM103427), a component of the National Institutes of Health.

703 Characterizing neuromuscular degeneration *Drosophila mayday* mutants. *J.M. Willis*, D. Weaver, D. Babcock Lehigh University.

Neurodegenerative diseases (ND) are characterized by the degeneration and dysfunction of neurons in the nervous system. Alzheimer's Disease (AD), Parkinson's Disease (PD), and amyotrophic lateral sclerosis (ALS), are a few examples of progressive neurodegenerative diseases. The hallmark that characterizes these diseases is the loss of function in neurons. Most of what we understand about neurodegeneration has come from late stage or postmortem studies. Current treatments seek to target these diseases by alleviating late stage symptoms. Recent evidence has shown that synapses begin to deteriorate long before the neurons die, suggesting that there are earlier events that could be targeted. However, it remains to be elucidated how synapses degenerate. To further understand neurodegeneration in *Drosophila,* we have developed a high throughput behavioral flight assay that has been used to screen mutant flies for an impaired flight phenotype. The impaired phenotype suggests issues with motor neurons or associated muscles. Through a preliminary flight screen, we isolated a mutant in a previously novel uncharacterized gene, CG31475. CG31475 that has a mammalian homolog, Cab45, which is involved with cargo sorting at the Trans-Golgi Network. Thus, we seek to characterize the role of CG31475, now referred to as "*mayday*" and its role in synapse dysfunction in *Drosophila.*

704 Functional Effects of Allelic Variants of Drosophila *Odorant binding protein 56h*. *Sneha Mokashi*[1,2], Joel Johnstun[3,4], Trudy Mackay[1,2], Robert Anholt[1,2] 1) Clemson Center for Human Genetics, Greenwood, SC; 2) Department of Genetics and Biochemistry, Clemson University, SC; 3) Program in Genetics, Department of Biological sciences, NC State University, Raleigh, NC; 4) W M Keck Center for Behavioral Biology, NC State University.

Odorant binding proteins (OBPs) in *Drosophila melanogaster* are thought to play a role in olfaction by transporting hydrophobic odorants through the aqueous lymph to their membrane-bound receptors, thus acting as a liaison between the olfactory receptors and the external environment. However, OBPs can also have pleiotropic roles in mating, development and taste. We found that CRISPR-Cas9 mediated removal of the *Obp56h* gene has sexually dimorphic effects on physiological phenotypes, including recovery from a chill-induced coma and resistance to starvation stress. We introduced transgenes of allelic

variants at the endogenous location of the CRISPR-Cas9 deleted gene via an engineered Phi-C31 genomic integration site. These alleles comprise 11 non-synonymous or potentially regulatory SNPs that segregate in a wild-derived population, the *Drosophila melanogaster* Genetic Reference Panel. This experimental design enables us to delineate for the first time the effects of individual naturally occurring polymorphisms on variation in multiple phenotypes in an otherwise identical genetic background. In addition, we will be able to assess how each allele affects a co-regulated ensemble of transcripts. Results from these studies will provide insights in how molecular variants at a gene that contributes to fitness traits can enable shifts in pleiotropic functions and thus contribute to adaptive evolution. Supported by NIH grant GM128974.

705 Individual differences in innate olfactory behavior and neural coding within isogenic *Drosophila melanogaster* populations. *Matthew Smith*[1], Kyle Honneger[1], Matt Churgin[1], Glenn Turner[2], Benjamin de Bivort[1] 1) Harvard Department of Organismic and Evolutionary Biology, and Center for Brain Science; 2) Janelia Research Campus.

Innate behavioral biases and preferences can vary significantly between individuals of the same species. Although such variability is a frequently observed behavioral phenomenon, it is not known how individual differences in neural structure or physiologyaccount for behavioral variability. Furthermore, it is unclear if such behavioral variability can be attributed to the activity of identifiable cells within defined microcircuits, or the accumulation of innumerable differences of small effect. We have examined how different neural populations can tune innate behavioral variability using *Drosophila*as a model system. We've focused on the olfactory circuitry of the fly due to its extensive characterization and available genetic tools to target specific microcircuits. We show that neural responses to identical olfactory stimuli differ across individuals, and that a population of genetically-addressable inhibitory neurons within an olfactory microcircuit regulates the variability of odor preference behavior. Moreover, we have found the neuromodulator serotonin modifies the extent of inter-animal variability across the population. Taken together, these results indicate that the degree of behavioral individuality across a population of animals may be dynamically tuned (presumably with corresponding changes to individuality of neural responses) and that specific neural circuits mediate this effect. Neuromodulation of individuality may be a conserved and ecologically important mechanism for diversifying behavior in response to environmental stress. Consistently we have found that abrupt changes in diet result in changes in inter-animal variability across the population.

706 Functional variation in the fruit fly antenna lobe predicts individual behavior. *M.A. Churgin*, B.L. de Bivort Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.

Behavioral variation persists even in isogenic animals reared in identical environments. This is especially surprising in animals with nominally stereotyped nervous systems. Importantly, neurodevelopmental disorders such as autism result from pathological idiosyncrasy in neuronal wiring and function. Therefore, it is crucial to gain a fuller view of how nervous systems vary and how this variation affects behavior. Fruit fly olfaction is a powerful model for investigating the neural bases of individuality owing to its well-studied and highly stereotyped nature. Olfactory receptor neurons synapse on projection neurons (PNs) in ~50 glomeruli in the antenna lobe (AL). Recent work has uncovered subtle idiosyncrasies in AL wiring and activation, suggesting this circuit does indeed vary between individuals. Furthermore, individual flies display persistent individual odor preferences (day 1 to day 2, r=0.37, n=50 flies). Which elements of the olfactory circuit explain these varying behavioral preferences in such a highly stereotyped system?

To answer this question, we performed paired behavior and optical physiology experiments in individual flies expressing a calcium reporter only in PNs (GH146>GCaMP6m). We measured odor preference by tracking flies in individual tunnels and filling each half of the tunnel with one of two odors (MCH or OCT). Individual preference was determined as the proportion of time spent in each half of the tunnel. We then performed volumetric two-photon imaging in each individual to record PN responses to an odor panel. We performed principal component analysis on glomerular activations to compare flies in coding space and found that distances are lower within-flies (trial to trial) than across-flies, a sign of individuality in odor coding. Finally, we generated a linear classifier from the first principal component scores to predict individual preference (n=27 flies, r=0.4, p=0.02). Thus, we find that idiosyncratic neural coding in PNs can partially explain behavioral variation. We also describe efforts to link structural variation with individual behavior.

Identifying the sources of neural variability is critical to increasing our understanding of both normal and pathological neurological function. The results described here may shed light on how behavioral individuality can emerge from a nominally stereotyped network through idiosyncrasies in neural coding.

707 Vitamin A deprivation affects motion vision at low pattern contrasts. *D. Dewett*[1], K. Lam[1], C. Poupault[1], M. Kumar[2], A. Shevchenko[2], J. Rister[1] 1) UMASS Boston, Boston, MA; 2) Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

Vitamin A is essential for vision, as it is converted to the retinal chromophore that is required for Rhodopsin function. Similar to humans, flies cannot synthesize vitamin A and have to take it up from their diet. We use *Drosophila melanogaster*as a model to study how vitamin A deprivation affects photoreceptor function and visually guided behaviors. To achieve this, we raised flies on two minimal yeast media: vitamin A rich food and vitamin A deprived food.

Previous studies had shown that the size of the rhabdomeres, the light-sensing compartments of photoreceptors that contain the Rhodopsins, is severely reduced by vitamin A deprivation. To test whether the orientation of the rhabdomeres is also affected by vitamin A deprivation, we measured the deep pseudopupil *in vivo*in wild type flies. The deep pseudopupil is an optical superposition of virtual images of adjacent unit eyes in the center of the eye under antidromic illumination. We found that the deep pseudopupils of flies raised on both food types were composed of seven normally oriented rhabdomeres; vitamin A deprivation therefore does not affect rhabdomere orientation.

To assess the consequences of vitamin A deprivation on visually guided behaviors, we analyzed responses to a strong light stimulus (phototaxis) as well as motion vision (optomotor responses). In the phototaxis assay, there was no significant difference in the responses of flies raised on both food types to a bright light stimulus. This suggests that the deprived flies retained sufficient levels of functional Rhodopsin to detect light. Previous studies showed that the major Rhodopsin Rh1, which is critical for motion vision, is dramatically reduced in vitamin A deprived flies. Therefore, we sought to determine if vitamin A deprivation affected spatial resolution and temporal acuity by measuring the optomotor response to moving stimuli. We displayed a rotating striped drum stimulus around a tethered fly, which elicits optomotor responses. The vitamin A deprived flies showed normal responses to a range of different pattern wavelengths. However, at a pattern wavelength of 24 degrees, already minimal reductions in pattern contrast led to a complete loss of the response. Taken together, wild type flies deprived of vitamin A have a normal orientation of the (reduced) rhabdomeres with normal spatial resolution at high pattern contrast but show dramatic optomotor defects at low pattern contrasts.

708 Investigating the Genetic Control of Nociceptive Neural Circuit Development in *Drosophila* Larvae. *M.R. Chin*[1,2,3], W.D. Tracey[1,2,3] 1) Program in Neuroscience, Indiana University, Bloomington, IN; 2) Department of Biology, Indiana University, Bloomington, IN; 3) Gill Center for Biomolecular Sciences, Indiana University, Bloomington, IN.

Organisms must adapt and respond to changing environments. The sensory nervous system allows for this by first detecting external changes and then transmitting that information to a central system for processing and output. Within the sensory nervous system, sensory neurons are the first step of the circuit and can initiate specific behaviors depending on the type of stimulus detected. As such, a greater understanding of how neural circuits are formed

through out development will lend insight into the basis for complex behaviors. While nervous system development has been well studied, many unanswered questions remain in how neurons within a functional circuit find their appropriate synaptic partners amongst millions of other neurons. This study uses a model circuit, the nociceptive response in *Drosophila* larvae, to further understand how neurons are wired together to generate specific behavioral outputs. Specifically, the focus of this investigation is the gene *defective proboscis extension response 11* (*dpr11*). Dpr11 is an immunoglobulin superfamily protein that is important for appropriate synapse development in the *Drosophila* eye and larval neuromuscular junction, and RNAi knockdown of *dpr11* in nociceptive neurons impairs the thermal nociceptive response in *Drosophila* larvae. However, the molecular role of Dpr11 in nociceptive neurons is still unknown. Initial findings from CRISPR generated *dpr11* mutants suggest that loss of *dpr11* alone does not result in a thermal insensitive phenotype as would be expected from RNAi results. However, these findings lead to interesting questions about how the Dpr protein family functions, as current results suggest it may be in a combinatorial or redundant manner. While still examining the molecular role of Dpr11 in nociceptive neurons, this study will also use nociceptive behavior as a readout to investigate how the Dprome functions *in vivo* to set up efficient neural circuits. Specifically, it is hypothesized that loss of a *dpr* causes upregulation of other related *dpr* genes, leading to a compensatory effect.

709 Multisensory integration into the *Drosophila* mushroom body. *J. Li*, B. Mahoney, S. Caron School of Biological Sciences, University of Utah, Salt Lake City, UT.

Multisensory integration is a fundamental function of all brains, yet its underlying mechanisms of connectivity remain largely unknown. To uncover these mechanisms, we are using the *Drosophila* mushroom body, an associative center in the fly brain that consists of only 2000 principal neurons called Kenyon cells, which are separated into 7 subpopulations. Previous studies suggest that one of these subpopulations, the $\alpha/\beta_{Posterior}$ Kenyon cells, receives multisensory input. From a preliminary anatomical screen, we have identified 6 input neurons connecting the mushroom body to either the visual, gustatory, olfactory, hygrosensory or thermosensory system. In addition, we have identified a group of neurons projecting from the posterior lateral protocerebrum (PLP). Using a genetic technique called GFP Reconstitution Across Synaptic Partners, we have found that all input neurons form synaptic connections with the $\alpha/\beta_{Posterior}$ Kenyon cells. Using a novel neuronal tracing technique, we have found that the $\alpha/\beta_{Posterior}$ Kenyon cells connect to these input neurons at different rates, with the highest connectivity rate made by the visual input neurons and the PLP input neurons. Our results thus far suggest that individual $\alpha/\beta_{Posterior}$ Kenyon cells integrate inputs randomly from this pool of input neurons. Given that many fundamental design principles are conserved from invertebrates to vertebrates, it is likely that the mechanisms of connectivity underlying multisensory integration in the *Drosophila* mushroom body will also apply to vertebrate associative brain centers.

710 Neuromodulatory coordination of action selection and action composition in an elementary decision network. F. Diao, *B.H. White* National Institute of Mental Health, NIH, Bethesda, MD.

Behavioral decisions result in the selection of particular actions. These actions are typically composed of separable motor patterns, which are performed serially and/or in parallel. How the nervous system composes actions from basic motor patterns and whether this process is distinct from action selection is generally not known. To map the parts of the fly brain responsible for selecting a particular action on the one hand, and executing it on the other, we have investigated the circuitry underlying an elementary behavioral decision made by all flies immediately after eclosion. The decision is whether or not to expand their wings, a process that is under the combined control of the hormone Bursicon and environmental variables: In confined environments, flies will delay wing expansion and instead perform an extended environmental search to escape confinement. Our previous work has shown that the behavioral decision to cease environmental search and initiate wing expansion correlates with Bursicon release from a pair of neurons in the subesophageal zone, which when activated trigger wing expansion behaviors, and when suppressed block them. These neurons (B_{SEG}) thus lie at the intersection of the wing expansion decision and its execution.

Using genetic targeting strategies combined with neuronal manipulations of activity, we have now identified the downstream targets of the B_{SEG} command neurons. We find that the B_{SEG} targets divide into functionally distinct populations, which collectively facilitate the wing expansion decision and direct the assembly of the behaviors required to execute it. They do so by participating in either feedforward motor circuits for abdominal contraction and air swallowing—the motor patterns required for wing expansion—or a feedback modulatory circuit that promotes Bursicon release from the B_{SEG} to maintain the execution of those motor patterns. In addition to the B_{SEG}, the positive feedback loop consists of a small set of cholinergic neurons that express the Bursicon receptor, Rickets. When these neurons are silenced wing expansion is suppressed, consistent with an essential role in the wing expansion decision. The feedforward neurons that mediate the motor output are distinct sets of octopaminergic, Rk-expressing neurons. In this system, neuromodulation thus coordinates both composition of the action taken to a behavioral decision and its selection. In addition, our results suggest a mechanism for assembling motor primitives into diverse motor programs, which is a prerequisite for flexibility in generating behavior.

711 Sex, Flies, and DREADD Modulation of Locomotor Response to Methamphetamine. *M. Hibicke*, KJ Sherman, C Nichols Pharmacology and Experimental Therapeutics, LSU Health Sciences Center, New Orleans, LA.

Psychostimulant abuse is a significant health issue with no FDA-approved treatment strategies. While it has been well established that dopamine is the primary mediator of response to stimulant drugs, serotonin is also involved. Serotonergic signaling modulates dopamine levels, and serotonin receptors are highly expressed in brain regions implicated in reward and dependence. However, the underlying molecular mechanisms of serotonergic modulation on psychostimulant response have not been defined. To further develop and refine the fruit fly as a genetic model system to study sympathomimetic drugs of abuse, this study aims to identify brain structures and their interactions underlying the locomotor response to methamphetamine in *Drosophila*, and to elucidate the role played by serotonergic signaling.

Fly strains expressing designer receptors exclusively activated by designer drugs (DREADDs) on discrete neurological circuits were fed medium containing agonist clozapine-N-oxide (CNO, 3.0 mM), methamphetamine (METH, 5.0 mM) or CNO + METH, and monitored next to flies fed vehicle medium in the Drosophila Activity Monitoring System (DAMS) under constant light conditions for at least one week. Neurocircuits were selected by coupling the DREADDs (G_i-coupled UAS-M4D1, G_s-coupled UAS-M3DBar4, and G_q-coupled UAS-M1D3) with Gal-4 drivers selective for the mushroom body (MB247-Gal4), presynaptic serotonin neurons (TRH-Gal4), postsynaptic serotonin neurons (5-HT_7-Gal4 and $5HT_{1A}$-Gal4), insulin secreting neurons of the pars intracerebalis (DILP-Gal4), and a circadian clock protein (PDF-Gal4). Beam breaks recorded by the DAMS were summed into one-hour bins and group means were calculated. Group means were then evaluated individually with repeat measures ANOVA, and expressed as mean beam breaks/hour/day ± standard error of the mean.

METH increased locomotor activity greatly in males and modestly in females, and M3DBar4 flies displayed dose-dependent, sexually dimorphic locomotor responses to CNO alone. These results suggest sex-dependent structural differences in the neurocircuitry, which will be explored in future investigations using UAS-MCD8 to visualize neurons specified by a Gal-4 driver. Additionally, modulation of serotonergic signaling reduced locomotor activity independently and when paired with METH, suggesting that to some extent, serotonergic signaling mediates locomotor activity independently of dopamine. Finally, both activating (M3DBar4-coupling) and inhibiting (M4D1-coupling) DILP greatly reduced METH response, suggesting that regulation of insulin secretion is vital to dopaminergic modulations of locomotor activity.

712 Neuronal Constituents and Putative Interactions within the Drosophila Ellipsoid Body Neuropil. *B. Nguyen*, J. Omoto, P. Kandimalla, J. Lovick, J. Donlea, V. Hartenstein University of California, Los Angeles, Los Angeles, CA.

The central complex (CX), is a structure located in the midline of the insect brain shown to be involved in processing goal oriented visual and motor behaviors. It consists of four distinct neuropil compartments: the Upper (CBU) and Lower (CBL) Halves of the Central Body, the Protocerebral Bridge (PB), and the paired Noduli (NO). In the fruit fly, Drosophila melanogaster, the CBU and CBL are referred to as the Fan-Shaped Body (FB), and the Ellipsoid Body (EB) respectively. Recently, a fifth compartment, the Asymmetrical Body (AB), has been proposed to be considered a part of the CX as well. In this paper, we characterize the neuronal populations which form the EB, provide structural and developmental context for their classification, revise their nomenclature, and show their putative interactions. Based on the intensity and distribution of the DN-cadherin, we define five domains within the EB and three partitions in its associated input neuropil, the bulb (BU). With these distinct divisions, different ring neuron subclasses (EB tangential elements) derived from the DALv2 neuroblast lineage (so-called R-neurons) were characterized based on their unique innervation patterns in the EB and BU using Gal4 driver lines. We identified five novel patterns in addition to the previously described six, which make up in total eleven R-neuron subclasses in this anatomical framework. Besides EB tangential elements, we also included columnar elements as well as non-DALv2 derived extrinsic ring neurons (ExR-neurons). Lastly, we utilized trans-Tango, an anterograde trans-synaptic labeling method, to address the connectivity between R-neurons and their targets. This reveals general principles of information flow within the EB network. Thus, our work will facilitate the generation and testing of hypotheses regarding circuit interactions within the EB and the rest of the CX.

713 Investigation of neural circuits that mediate acquisition of new knowledge. *D. Hattori* Department of Physiology, Department of Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX.

Animals acquire knowledge of the environment by learning, and use this knowledge to guide their behavior. Animals also show behavioral response to novel stimuli about which they have no prior knowledge. We have investigated the neural and behavioral correlates of novelty and familiarity in the olfactory system of *Drosophila*. Flies exhibit an alerting behavior upon presentation of a novel odor, which becomes suppressed upon familiarization. This behavior requires the activity of a class of mushroom body output neurons (MBONs) innervating the α'3 MB compartment. Calcium imaging experiments reveal that novel odors evoke strong activity in these MBONs that is rapidly suppressed upon repeated presentation of the same odor. This suppression of MBON response requires odor-evoked activity of the dopaminergic neurons that innervate the α'3 MB compartment. These data indicate that the α'3 compartment plays a causal role in the behavioral response to novelty and familiarity, as a result of dopamine-induced plasticity at the synapse between principal MB neurons, the Kenyon cells, and the MBONs.

The MB has long been implicated in classical conditioning, a form of supervised learning, in which odors activate Kenyon cells whereas unconditioned stimuli, such as reward and punishment, activate dopaminergic neurons. Our study shows that this circuit also supports detection of novelty and transition to familiarity, a form of unsupervised learning, by using the same mechanism of dopamine-induced plasticity, in which odors activate both Kenyon cells and dopaminergic neurons. In addition, previous studies as well as our new study indicate that the MB integrates flies' motivational states, such as hunger and satiety, to alter behavioral response to odors. Interestingly, mammalian neural circuits that support classical conditioning also exhibit these characters observed in the MB, suggesting that there may be a common operational principle of neural circuits involved in acquiring new knowledge. I will discuss our investigation of motivation-dependent behaviors as well as development of a bidirectional neural activity marker in these contexts.

714 Logic of an aminergic circuit in egg-laying. *Ethan Rohrbach*[1,2], Sonali Deshpande[1], James Asuncion[1,3], David Krantz[1] 1) Psychiatry, University of California, Los Angeles, Los Angeles, CA; 2) Neuroscience Interdepartmental PhD Program, University of California, Los Angeles, CA; 3) Medical Scientist Training Program, University of California, Los Angeles, CA.

Biogenic amines such as dopamine, serotonin, and octopamine regulate multiple behaviors in *Drosophila*. The specific organization of such neural systems and the mechanism of their circuit activity, however, remain unclear. To address these questions, we are studying the octopaminergic neural circuitry involved in regulating egg-laying. Previous studies in various insects including *Drosophila* suggest a relatively simple mechanism in which glutamate and proctolin cause contractions in oviduct muscles whereas octopamine promotes oviduct relaxation as mature eggs pass from ovary to uterus. These previous studies, however, have assessed the behavior of oviducts as a whole rather than as heterogeneous anatomical subregions. In addition, the localization and function of specific octopamine receptors have remained unclear. To establish a more accurate model of this tractable aminergic circuit we have used calcium imaging to visualize muscle activity in specific regions of the reproductive tract including the anatomically distinct lateral and common oviducts. In addition, we have developed molecular markers based on MiMIC insertions to determine the localization of each octopaminergic receptor. These MiMIC lines can also be employed to perform precise RNAi knockdowns of these specific receptors in the cells where they are expressed. Preliminary data indicate that octopamine has different effects in the lateral versus common oviducts. Furthermore, the two sites display markedly different profiles of octopamine receptor expression. Targeted receptor-RNAi experiments are beginning to elucidate if this distribution of specific octopamine receptors in the oviducts can explain the differences in the regulation of the common versus lateral regions. Expression of yet other octopamine receptors in local interneurons expressing the marker ppk suggest that some interactions may be mediated via previously unknown pathways. Our data provide fundamental information on the logic underlying aminergic neuromodulation of circuit function that may be applicable to more complex pathways in the fly and other systems.

715 Developmentally arrested precursors of pontine neurons establish an embryonic blueprint of the *Drosophila* central complex. *I.V. Andrade*[1], N. Riebli[2], B-C. Nguyen[1], J. Omoto[1], A. Cardona[2], V. Hartenstein[1] 1) Molecular, Cellular and Developmental Biology, UCLA, Los Angeles, CA; 2) Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, USA.

Serial electron microscopic analysis shows that the *Drosophila* brain at hatching possesses a large fraction of developmentally arrested neurons with a small soma, heterochromatin-rich nucleus, and unbranched axon lacking synapses. We digitally reconstructed all 802 "small undifferentiated" (SU) neurons and assigned them to the known brain lineages. By establishing the coordinates and reconstructing trajectories of the SU neuron tracts we provide a framework of landmarks for the ongoing analyses of the L1 brain circuitry. To address the later fate of SU neurons we focused on the 54 SU neurons belonging to the DM1-4 lineages, which generate all columnar neurons of the central complex. Regarding their topologically ordered projection pattern these neurons form an embryonic nucleus of the fan-shaped body ("FB pioneers"). FB pioneers survive into the adult stage where they develop into a specific class of bicolumnar elements, the pontine neurons. Later born, unicolumnar DM1-4 neurons fasciculate with the FB pioneers. Selective ablation of the FB pioneers altered the architecture of the larval FB primordium, but did not result in gross abnormalities of the trajectories of unicolumnar neurons, indicating that axonal pathfinding of the two systems may be controlled independently. Our comprehensive spatial and developmental analysis of the SU neurons adds to our understanding of the establishment of neuronal circuitry.

716 Neuronal communication keeps the circadian clock ticking at the right speed. *M. Schlichting*[1], M. Diaz[1], L.S. Baik[2], T.C. Holmes[2], M. Rosbash[1] 1) HHMI Brandeis University, Department of Biology, Waltham, MA; 2) Department of Physiology and Biophysics, University of California, Irvine, Irvine, CA.

Circadian clocks allow organisms to predict upcoming changes in illumination, i.e., day and night. Rhythmic organisms can thereby adjust their physiology, which gives them a fitness advantage. The underlying molecular mechanism in animals includes a conserved negative feedback loop. In *Drosophila*, Clock and

Cycle activate the transcription of *period* (*per*) and *timeless* (*tim*). Their protein products inhibit their own transcription by interacting with Clock/Cycle. This feedback loop is thought to be self-sustaining as it persists in constant darkness (DD). However, some studies question whether the feedback loop is cell-autonomous as they indicate that neuronal activity of discrete circadian neurons is important for proper free-running oscillations. To address this question, we used UAS-Kir to silence the entire brain clock network, which stopped clock neurons from producing action potentials as observed by electrophysiology. Not unexpectedly, we show here that silencing the network abolishes rhythmic behavior in light-dark (LD) as well as DD conditions. In contrast, PER immunohistochemistry indicates that its expression remains rhythmic in both conditions. Although silencing does not impact network synchrony in LD, individual neuron clusters adopt different speeds in DD. As suggested by the different speeds, a neuron-specific CRISPR/Cas9 strategy to knock out clock gene function in subsets of neurons shows that the normal circadian period of approximately 24h is a network property. Moreover, the 8 small ventral lateral circadian neurons (sLNvs or morning (M) cells) play a special role as a signal integrator from different brain regions. Consistent with this notion, the cycling amplitude of clock gene expression within these cells dampens rapidly in DD, an effect that was obvious at the mRNA as well as at the protein level. This suggests that a rather direct connection between neuronal firing and M cell clock gene transcription is necessary to sustain its circadian oscillations in DD.

717 Closely related *Drosophila* species as a model system to understand the neuronal mechanisms underlying behavioral evolution. *Yun DING*, Joshua LILLVIS, David STERN Janelia Research Campus, HHMI, Ashburn, VA.

Animals display an extraordinary diversity of behaviors. While the evolution of behavior in response to natural selection has been heavily studied historically, mechanistic understanding of how natural selection reshapes neural functions to encode adaptive behavioral changes remains rudimentary in any system. Progress in such knowledge requires the development of a tractable system allowing comparative studies at both the genetic and the neuronal level. We propose that closely related *Drosophila* species provides a rare opportunity to tackle this problem. First, *Drosophila* species, even the closely related ones, display numerous behavioral paradigms that are rich in natural variations. Second, *Drosophila* species are amenable to genetic manipulation, which is critical for mechanistic characterizations. Third, *D. melanogaster* has been a classical model to understand the neuronal basis of behavior, providing a necessary entry point for further cross-species comparison. Here, we focus on the evolution of *Drosophila* courtship songs to exploit the potential of *Drosophila* species as a neural-comparative model. We demonstrate that all major types of genetic manipulations, including transposon mediated transgene, *attB*/*P* integration, *CRISPR*/*Cas9* mediated genome editing, are feasible in a series of non-*melanogaster* species. Facilitated by these genetic manipulations, we can exploit neurogenetic reagents that label specific neuronal populations in *D. melanogaster* to target homologous neurons in closely related non-*melanogaster* species. This permits us to functionally compare homologous circuit elements across species using a combination of approaches including neural activation and inhibition, functional imaging and electrical recording, as well as single neuron transcriptome. In together, we provide a generalizable approach to dissect neural circuit function in an evolutionary context using closely related *Drosophila* species, priming the identification of neural substrate underlying behavioral evolution.

718 Investigation of *smoke alarm* in sensory neuron function and morphogenesis. *K.H. Fisher*, S.M. Mauthner, W.D. Tracey Gill Center for Biomolecular Sciences and Department of Biology, Indiana University, Bloomington, IN.

The detection and processing of sensory input is dependent on the proper function of many sensory neurons in the peripheral nervous system. *Drosophila* dendritic arborization (da) sensory neurons extensively cover the body wall and are grouped into four distinct classes based on morphology. Class IV da neurons are the larval pain-sensing neurons, called nociceptors, which encode and process noxious stimuli, and are required for nociceptive behavior. When presented with a noxious stimulus, larvae exhibit a highly stereotyped behavioral response. This behavior makes *Drosophila* a robust system for identifying genes important for nociception. In a genetic screen using tissue specific microarray analysis and thermal nociception assays, we identified genes showing enriched expression in nociceptive class IV neurons that are functionally important for thermal nociception behavior/responses. Of these genes, we identified a previously uncharacterized gene and named it *smoke alarm* (*smal*) due to a hypersensitive behavioral response to noxious thermal stimuli upon class IV specific RNAi knockdown. Analysis of class IV dendrite morphogenesis in *smal*-RNAi larvae revealed hyperbranched dendrites and an increase in isoneural crossovers. Investigation of *smoke alarm's* expression pattern revealed robust expression in class IV nociceptors, class I and class III sensory neurons, and chordotonal organs. With a newly generated null allele of *smal*, we are investigating structural differences in sensory neuron morphology and functional changes in nociceptive behavior. Finally, as Smal is homologous to a collagen binding protein, we are investigating its role in nociceptor-extracellular matrix interactions. Ongoing work will be presented.

719 Possible role for a eukaryotic translation initiation factor in behavioral plasticity. *I.M. Chin*, C. Vernier, Y. Ben-Shahar Department of Biology, Washington University in St. Louis, St. Louis, MO.

Recent studies suggest that protein translation plays a role in neuronal and behavioral plasticity at the developmental and physiological timescales. The mechanism by which such a general and fundamental cellular process supports complex phenotypic variation remains mostly unknown. Here we describe an RNAi screen of *Drosophila* genes homologous to loci within the human chromosome 7q11.23 region whose hemideletion causes Williams-Beuren Syndrome (WBS), a developmental disorder characterized by stereotypically hypersocial personality. We demonstrate that one of the fly WBS gene orthologs, *eIF4H1*, an ancillary modulatory component of the conserved, rate-limiting eIF4F eukaryotic translation initiation complex, may play a role in regulating social interactions in *Drosophila*. Specifically, we found that neuronal knockdown of *eIF4H1* altered the social behavioral response of male flies to conspecifics, measured by both social space displacement and male courtship. Together, these data suggest that the eukaryotic translation initiation complex could have an evolutionarily conserved role in the development and/or regulation of neural circuits underlying sociality across mammals and insects.

720 *Neuroligin3* is required for a response to the social environment in *Drosophila melanogaster.* *R.T. Yost*[1], A.M. Scott[2], A. Bao[1], R. Hakimjavadi[1], A. Lone[1], R. Dukas[2], A.F. Simon[1] 1) Department of Biology, University of Western Ontario, London, Ontario, CA; 2) Animal Behaviour Group, Department of Psychology, Neuroscience & Behaviour, McMaster University, Hamilton, Ontario, CA.

The social environment affects behaviour by altering molecular processes and neuronal functioning[1]. In *Drosophila melanogaster*, social isolation affects complex behaviours including interactions with others[2,3], as well as social spacing: the distance between flies[4]. The social environment is known to cause changes in gene expression[5,6], including in *Drosophila neuroligin3 (nlg3)*, an autism-associated post-synaptic cell adhesion protein[7]. *Neuroligin3* ensures proper synaptic formation, maturation, and function[7]. However, the involvement of *nlg3* in response to social isolation and how this alters social space is unknown. Using the social space assay, we observed that isolated flies had increased inter-individual distance. Using a measure of sociability[8], we found that isolated flies were less sociable than group reared individuals. Flies with a *nlg3* loss of function did not respond to social isolation in the social space assay. Furthermore, Western blot analysis of NLG3 protein levels revealed that NLG3 was unchanged after isolation. These results suggest NLG3 is required for a response to the social environment, but is probably downstream of a pathway directly responding to the social changes. This is the first evidence of the involvement of *Drosophila nlg3* influencing the response to environmental stimuli.

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721 Bright light and carbon dioxide effects on the *Drosophila* startle response. B.C. Capt[1], K. Pearman[2], *G.B. Call*[2] 1) Department of Biomedical Sciences, Midwestern University, Glendale, AZ; 2) Department of Pharmacology, Midwestern University, Glendale, AZ.

The *Drosophila* startle response is an escape mechanism mediated by the giant fiber, which receives sensory input from both the visual and mechanosensory pathways. Almost simultaneous activation of both the wing and leg muscles occur with giant fiber activation. We have developed a new assay to analyze the startle response that consists of a narrow climbing vial mounted on top of a vortex to stimulate a response in the fly that is captured by a high-speed video camera. Refinement of the new assay was needed as potential variables were recognized during its development. Due to the speed of the startle response, the use of a high-speed camera and a powerful light are required to allow for determination of the response. We hypothesize that the use of this very bright external light source is stimulating the *Drosophila* visual startle response pathway in some way, thus leading to increased startle rates when compared to other lighting conditions. Data collected through utilization of our assay demonstrates that three distinct responses are observed: a startle response (as explained above), a drop and no-response. A behavior is classified as a drop when the fly simply falls off the vial. A no-response behavior is when the fly holds on to the vial throughout the entire 2-second vortex event. The Ore-R flies, when exposed to the high-light condition demonstrate a startle response of 60%. However, Ore-R flies exposed to low lighting, infrared (IR) with standard room lighting and IR-only conditions experienced a startle response at approximately 30% providing evidence that the utilization of a very bright light increases ($P<0.05$) the *Drosophila* startle response. The results acquired from the low light and IR conditions in this assay potentially identify the portion of the startle response that is activating the mechanosensory pathway. Further experiments will be performed and presented at the meeting concerning this. Additionally, the utilization of CO_2 as an anesthetic has been shown to inhibit climbing, flight and other behaviors. Therefore, we wanted to determine the effects of CO_2 on the startle response in this new assay. Ore-R flies exposed to 100% CO_2 for 10 minutes and allowed to recover for 60 minutes appeared to startle less when illuminated with the intense external light when compared to the air-exposed control flies. This decrease in startle response caused by CO_2 was also observed when using IR-only illumination ($P<0.001$). Under both lighting conditions the no-response rate increased; providing evidence that CO_2 is having a behavioral effect. These results could indicate that CO_2 is affecting the mechanosensory pathway more than the visual pathway and further studies will be performed to determine this. This study provides evidence that the use of both intense external light and CO_2 affects the startle response behavior and could potentially have other undesirable effects in other behavioral assays.

722 Bidirectional opponent thermosensors orchestrate euthermic regulation via cross-inhibition. *Luis Hernandez Nunez*[2,3], Mason Klein[7], Paul Garrity[4,5,6], Aravinthan Samuel[1,2,3] 1) Department of Physics, Harvard University, Cambridge, MA; 2) Systems Biology Program, Harvard University, Cambridge, MA; 3) Center for Brain Science, Harvard University, Cambridge, MA; 4) National Center for Behavioral Genomics, Brandeis University, Waltham, MA; 5) Volen Center for Complex Systems, Brandeis University, Waltham, MA; 6) Department of Biology, Brandeis University, Waltham, MA; 7) Department of Physics, University of Miami, Coral Gables, FL.

Animals move away from excessively high or low temperatures and try to stay within a preferred range. In ectotherms, finding and staying in that range is particularly important to retain appropriate body temperature (euthermia). *Drosophila* larvae perform cold and warmth avoidance to escape unfavorable temperatures. Both avoidance behaviors are suppressed around 24°C (the euthermic range). The mechanisms that regulate *Drosophila* larva navigation to stay in the euthermic range are unknown. Here we show that the Ir21a-cold sensors -besides triggering cold avoidance at T<23 °C- are required to downregulate warmth avoidance progressively at T>25°C and suppress it at 23-25°C. We present new thermosensors and the thermoreceptors they use to respond to warmth. These sensors are required to downregulate cold avoidance at T<23°C and suppress it at 23-25°C. Our results reveal that the *Drosophila* larva, as mammals, uses peripheral cold and warmth sensors to perceive its environment. This study is the first to determine the computation of bidirectional opponent thermosensors signals that orchestrates motor outputs in a way that optimizes localization at ideal temperatures.

723 Loss-of-Function Variants in *IRF2BPL* are Associated with Neurological Phenotypes. *P.C. Marcogliese*[1], R.C. Spillmann[2], N. Stong[3], J.A. Rosenfeld[1], M.K. Koenig[4], D. Ortiz[5], H. Chung[1], Y. Wang[1], K. Riley[2], G. Mirzaa[6], D. Hemelsoet[7], B. Lee[1], S.F. Nelson[8], S. Yamamoto[1], M.F. Wangler[1], V. Sashi[2], L.D.M. Pena[9], H.J. Bellen[1,10], Undiagnosed Diseases Network 1) Department of Molecular and Human Genetics, Baylor College of Medcine, Houston, TX; 2) Department of Pediatrics, Duke University School of Medicine, Durham, NC; 3) Institute for Genomic Medicine, Columbia University Medical Center, New York, NY; 4) Department of Pediatrics, The University of Texas Health Science Center, Houston, TX; 5) University of Pittsburgh, Children's Hospital of Pittsburgh, Pittsburgh, PA; 6) Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, WA; 7) Department of Neurology, Ghent University Hospital, Ghent, Belgium; 8) Department of Human Genetics, University of California at Los Angeles, Los Angeles, CA; 9) Cincinnati Children's Hospital, Cincinnati, OH; 10) Howard Hughes Medical Institute, Houston, TX.

Interferon regulatory factor 2 binding protein-like (*IRF2BPL*) encodes a member of the IRF2BP family of transcriptional regulators. Currently the biological function of this gene is obscure, and the gene has not been associated with a Mendelian disease. We identified seven individuals who carry potentially damaging heterozygous variants in *IRF2BPL* and are affected with neurological symptoms. Five individuals who carry *IRF2BPL* nonsense variants resulting in a premature stop codon display severe neurodevelopmental regression, hypotonia, progressive ataxia, seizures, and a lack of coordination. Two additional individuals, both with missense variants, display global developmental delay and seizures and a relatively milder phenotype than those with nonsense alleles. The *IRF2BPL* bioinformatics signature based on population genomics is consistent with a gene that is intolerant to variation. We show that the fruit-fly IRF2BPL ortholog, called pits (protein interacting with Ttk69 and Sin3A), is broadly detected, including in the nervous system. Complete loss of pits is lethal early in development, whereas partial knockdown with RNA interference in neurons leads to neurodegeneration, revealing a requirement for this gene in proper neuronal function and maintenance. The identified *IRF2BPL* nonsense variants behave as severe loss-of-function alleles in this model organism, and ectopic

expression of the missense variants leads to a range of phenotypes. Taken together, our results show that IRF2BPL and pits are required in the nervous system in humans and flies, and their loss leads to a range of neurological phenotypes in both species.

724 Altered expression of relish influences the immune response and neurodegeneration in a Drosophila model of Machado-Joseph Disease. *Ethan Fenton*, John Warrick University of Richmond.

Machado-Joseph Disease (SCA-3), a trinucleotide repeat disorder, causes neurodegeneration through poorly understood mechanisms. In human neurodegenerative diseases, it has been suggested that inflammation may contribute to neurodegeneration. To test this hypothesis, we have modulated the expression of relish a nuclear transcription factor and Drosophila homolog of NF-kB, Relish, which, in part, regulates the immune response. Preliminary data suggest that increased expression of activated Relish increases degeneration, while decreased expression rescues degeneration. We suggest these changes in neurodegeneration occur due to modulation of the immune response.

725 Studying the Role of Trem2 in Drosophila models of Neurodegenerative Disorders. *Shuke Nie*[1], Mingkuan Sun[2], Liam Chen[3] 1) Department of Pathology, Johns Hopkins University, Baltimore, MD; Department of Neurology, Renmin Hospital of Wuhan University, Wuhan, China; 2) Department of Pathology, Johns Hopkins University, Baltimore, MD; 3) Department of Pathology, Johns Hopkins University, Baltimore, MD.

A common theme of neurodegenerative disorders discovered over the last decade is the important role played by neuroinflammation in the central nervous system. Microglia are activated after neuronal injury and in neurodegenerative diseases, and microglia-derived neuroinflammation has both beneficial and detrimental effects on neurons. Tiggering receptor expressed on myeloid cells 2(Trem2) is a key player in microglia function and mutant Trem2 involved in dysregulation of phagocytosis and neuroinflammation is associated with high risk of Alzheimer's disease. Here we studied the effect of of human wild type and R47H mutant Trem2 on the neuronal toxicities of several proteins implicated in the major neurodegenerative diseases, including tau, Htt and TDP-43 in *Drosophila*, aiming to assess the role of Trem2 in modulating protein aggregation and toxicity, and obtain optimal microglia-based therapeutic strategies for intervention. Our study reveals the timing and magnitude of microglial activation is a critical determinant of neuronal fate.

726 Targeted downregulation of *kdm4a* ameliorates *tau*-engendered defects in *Drosophila melanogaster*. *Sung Yeon Park*[1,3], Jieun Seo[2], Yang-Sook Chun[1,2,3] 1) Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine; 2) Department of Biomedical Sciences, Seoul National University College of Medicine; 3) Department of Physiology, Seoul National University College of Medicine.

Tauopathies such as Alzheimer's disease (AD) are characterized by the deposits of the beta-amyloid peptides (Ab) and neurofibrillary tangles of the hyper-phosphorylated tau protein. Given that the abnormal alteration of histone acetylation and methylation have been documented in AD patients with cause and effect relationship, we extended our investigation to the role of several JHDM (Jumonji histone demethylase) genes, which have never been studied in AD etiology. According to bioinformatics analysis, the expressions of JHDM1A, JHDM2A/2B, JHDM3A/3B were significantly increased in postmortem brain tissue with Alzheimer's disease than non-demented control, whereas JHDM1B mRNA level was significantly down-regulated in brain of Alzheimer's disease patients. Next, to directly identify the possible relationship between alterations in expression profile of JHDM genes and AD pathology in vivo, we examined whether tissue specific downregulation of JHDM *Drosophila* homologues (*kdm*) can affect tau-induced AD deficits using transgenic flies. We discovered that when any one of *kdm2, kdm3, kdm4a, or kdm4b* genes is silenced in the eye, the tau^{R406W}-engendered defects were ameliorated toward less severe phenotypes. But, in their CNS, uniquely, silencing of *kdm4a* ameliorated tau^{R406W} induced locomotion defects by reducing the amount of tau and restoring the heterochromatin loss. These results suggest that the specific alteration of *kdm4a* expression could be a potential therapeutic target in Alzheimer's disease.

727 The PINK1/Parkin pathway mediates dominant mitochondrial toxicity in CHCHD10-induced ALS-FTD. Yun-Jeong Choe[1], Minwoo Baek[1], J. Paul Taylor[2], *Nam Chul Kim*[1] 1) Pharmacy Practice and Pharmaceutical Sciences, College of Pharmacy, University of Minnesota, Duluth, MN; 2) Cell and Molecular Biology, St. Jude Children's Research Hospital, Memphis, TN.

Mutations in coiled-coil-helix-coiled-coil-helix-domain containing 10 (CHCHD10) have been identified as a genetic cause of amyotrophic lateral sclerosis and frontotemporal dementia. However, the disease-causing mechanism and the nature of mutations have not been understood. In this study, we generated a *Drosophila* model to study the function of CHCHD10 and disease-causing mechanisms. $dCHCHD10^{S59L}$ expression caused dominant toxicity in *Drosophila* eyes, motor neurons and muscles as well as HeLa cells while other mutants (R15L, P34S, G58R, and G66V) showed various effects in *Drosophila* and Hela cells. With the $dCHCHD10^{S59L}$ *Drosophila* model, we found that $CHCHD10^{S59L}$ is a dominant gain-of-function mutant, and the PINK1/Parkin pathway is crucial to generating mutant CHCHD10-dependent phenotypes. The genetic reduction of PINK1 and Parkin expression significantly rescued degenerative and mitochondrial phenotypes in *Drosophila* tissues and HeLa cells. In addition, PINK1 peptide inhibitors and mitofusin2 agonists successfully reversed $CHCHD10^{S59L}$-induced mitochondrial phenotypes. Our data indicate that chronic activation of the PINK1-mediated pathways by $CHCHD10^{S59L}$ generates dominant toxicity in our model systems although the PINK1/Parkin pathway is generally regarded as a guardian of neurodegeneration. Collectively, our results suggest PINK1-mediated pathways as potential therapeutic targets for the CHCHD10-induced degenerative diseases.

728 Proteomic analysis and genetic modifier characterization of mutant CHMP2B associated with Frontotemporal Dementia. *X. Zheng*, S.T. Ahmad Colby College, Waterville, ME.

Frontotemporal dementia (FTD) is a common neurodegenerative disease caused by mutations in multiple genes. A mutation in *CHMP2B* (*$CHMP2B^{Intron5}$*), which encodes a component of endosomal ESCRT-III complex, is associated with a hereditary type of FTD. Previous studies showed that misexpression of the protein in *Drosophila* eye using the Gal4-UAS system with *GMR*-Gal4 driver causes retinal degeneration. We used a proteomics-based approach to determine the effects of GMR-driven expression of $CHMP2B^{Intron5}$. Approximately 3500 unique proteins were identified, and among them approximately 2000 proteins were differentially present in the *Drosophila* heads expressing $CHMP2B^{Intron5}$. Gene Ontology (GO) analysis suggested that endosome transport, response to wounding, and cell surface receptor signaling pathway are significantly upregulated. We identified ten upregulated genes as potential modifiers of $CHMP2B^{Intron5}$ mediated defects and individually upregulated or downregulated those genes in $CHMP2B^{Intron5}$ flies. To characterize the modifier effect, we observed the shape and size of the eye and sectioned the retina to assess the level of internal structure disruption. We have identified and characterized three novel genetic modifiers of $CHMP2B^{Intron5}$. Thus, this study presented both a comprehensive picture and specific pathways of changes induced by $CHMP2B^{Intron5}$ expression.

729 Probing the role of glial endocytic genes on lipid droplet formation and Aβ42-induced neurotoxicity. *Matthew Moulton*[1,2], Jake Harland[1,2], Hugo Bellen[1,2,3] 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital, Houston, TX; 3) Howard Hughes Medical Institute at Baylor College of Medicine.

Mitochondria play a critical role in neuronal health during development and disease and mitochondrial dysfunction is associated with numerous neurological diseases. The study of genes affecting mitochondrial function has yielded important insight into neurological diseases such as Leigh Syndrome and Alzheimer's disease (AD). In compelling preliminary studies, our lab showed that elevated levels of reactive oxygen species (ROS), a byproduct of dysfunctional mitochondria, in neurons trigger the production of lipids that become peroxidized by ROS. These lipids are shuttled out of neurons and endocytosed by surrounding glia where they accumulate in lipid droplets (LD). While initially protective, the prolonged sequestration of peroxidized lipids in glia eventually leads to the demise of both glia and neurons. This discovery was first made in the lab using a fly model of neurodegeneration in the adult retina and confirmed

to be a conserved process in mice glia-neuron primary co-cultures and *in vivo*. Further studies demonstrated that the expression of human ApoE4 (the highest risk factor for the development of AD) in fly retinal glia reduces glial LD formation and promotes neuronal demise, while ApoE3 and, particularly the AD resistant isoform, ApoE2, both facilitate LD formation. Thus, ApoE4 confers a risk for heightened peroxidated lipids to pile up in neurons leading to neurodegeneration. Taken together, these data implicate a link between high-density lipoprotein (HDL) uptake, ROS, and neurodegeneration.

In AD brains, accumulation of the Aβ42 protein plays a major role in disease pathogenesis. This 42 amino acid peptide is lipophilic, with 18 hydrophobic residues, and binds to ApoE as well as the receptors for ApoE endocytosis. Thus, we hypothesize that HDL uptake and LD formation in glia may contribute to the clearance of Aβ42 *in vivo*. We seek to understand the role of endocytosis of HDL particles in LD formation and Aβ42 biology using our fly model. The findings of this study may help explain the link between dysregulation of lipids/Aβ42 and findings from genome wide association studies (GWAS) implicating endocytic genes as risk factors for AD. Here, we present the findings of a screen to identify endocytic genes that play a role in glial LD formation as well as document the intersection of LD formation and Aβ42 biology. We propose that Aβ42 and ROS synergize to promote neurodegeneration and that loss of endocytic machinery leads to an inhibition of LD formation and eliminates the protective effects of LDs in the brain.

730 *UQCRC1* regulates neurodegeneration in a fly model of Parkinsonism. *Yu-Chien Hung*[1], Wen-Chun Lo[1], Su-Yi Tsai[2], Po-Lin Chen[3], Chun-Hong Chen[3], Chin-Hsien Lin[4], Chih-Chiang Chan[1] 1) Graduate Institute of Physiology, National Taiwan Univ., Taipei, Taiwan; 2) Dept Life Science, National Taiwan Univ., Taipei, Taiwan; 3) National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Miaoli, Taiwan; 4) Department of Neurology, National Taiwan University Hospital, Taipei, Taiwan.

Ubiquinol-cytochrome c reductase core protein 1 (UQCRC1) is a subunit of complex III in mitochondrial respiratory chain. In previous studies, UQCRC1 has mostly been used as a mitochondrial marker. However, its role in mitochondria remains unclear. We have recently identified from patients using Whole-Exon Sequencing that the mutations in human *UQCRC1* associated with familial Parkinsonism. To reveal its endogenous function, we took RNAi approach against the fly ortholog of *UQCRC1*. Knockdown of fly *Uqcr-c1* caused locomotor defects, decreased the numbers of dopaminergic neurons, and led to age-dependent mitochondrial swelling. These defects resemble Parkinson-like phenotypes, and these phenotypes can be rescued by expression of human UQCRC1, indicating that the protein functions were evolutionary conserved. Morphologically, knockdown of *Uqcr-c1* in *Drosophila* flight muscles deteriorated the ultrastructure of mitochondria, suggesting that UQCR-C1 expression is important for mitochondrial maintenance. Finally, we knocked out UQCRC1 in human neuroblastoma SH-SY5Y cell, and observed a decrease in both growth rate and post-scraping cell migration. The molecular mechanism underlying UQCRC1-dependent neurodegeneration, and its functional relationship with other PD-associated genes *PINK1* and *parkin*, will be presented.

731 Mutation of *Gong* gene causes neurodegeneration in *Drosophila*. *Ye-Jin Park*[1], Hyeon-Pyo Shim[2], Sungkyung Kim[1], Gyunghee Lee[3], Tae-Yeop Kim[1], Mihwa Lee[1], Min-Cue Jo[1], Ah-Young Kwon[1], Seung-Hae Kwon[4], Jae H. Park[3], Sang-Hak Jeon[1] 1) Science Education, Seoul National University, Seoul, Korea; 2) Korea Institute for Curriculum and Evaluation, Chungcheongbuk-do, Korea; 3) Department of Biochemistry & Cellular and Molecular Biology, and Neuronet Research Center, University of Tennessee, Knoxville, USA; 4) Korea Basic Science Institute, Chuncheon Center, Korea.

Phosphatidylserine (PS) is clinically emerging as a therapeutic agent for improving cognitive defects that are resulted from neurodegenerative diseases. However, studies on the physiological roles of PS synthase (PTDSS) in the brain functions are scarce. In this study, we, for the first time, characterized the mutant phenotypes of the *Gongpo(Gong)*, a predicted *Drosophila* homolog of mammalian *PTDSS1*. *Gong* heterozygous mutant flies showed neurodegenerative phenotypes such as bang-sensitivity, locomotion defects, reduced life span, and excessive formation of vacuoles in the brain. When the *Gong* gene was knock-downed or was ectopically expressed in neurons and glia, both lethality and neurodegenerative phenotypes were observed only in glial-specific mutants. Furthermore, cytological inspection revealed that the mitochondrial cristae were collapsed and the swollen mitochondria were ruptured in the *Gong* heterozygotes. Consequently, the production of reactive oxygen species (ROS) was enhanced and apoptosis occurred actively in the heterozygous mutant's brain. Taking these results together suggests that mutant *Gong* gene dominantly causes mitochondrial dysfunction and destruction, and oxidative stress, which promotes cell death and eventually the neurodegenerative phenotypes. This study highlights a new mechanism of neurodegenerative diseases triggered by defective phospholipid metabolism in glial cells.

732 Investigating calcium as a mediator of Tau-induced neurotoxicity. *Rebekah Mahoney*[1,2,3], Maria Gamez[1,2,3], Bess Frost[1,2,3] 1) Department of Cell Systems and Anatomy, UT Health San Antonio; 2) Barshop Institute for Aging and Longevity Studies, UT Health San Antonio; 3) Glenn Biggs Institute for Alzheimer's and Neurodegenerative Diseases, UT Health San Antonio.

Calcium is required for the function of all cells in the body, including neurons. The pivotal role of calcium in so many neuronal processes dictates the need for precise regulation of its intracellular levels. Any dysregulation, however subtle, could lead to dramatic changes in normal neuronal function. Therefore, calcium is likely to have key roles in the cellular processes underlying aging-related changes in the brain, including normal age-associated memory impairments as well as more severe dementias, including Alzheimer's disease. Currently, although much is known of the role of calcium in the pathogenesis of Alzheimer's disease in mouse models, very little is known of the role of calcium in the pathogenesis of tauopathies, a group of neurodegenerative disorders characterized by the accumulation of hyperphosphorylated and aggregated tau protein in the brain. Central to this question we have investigated the role of calcium in the progression of tauopathies in the fruit fly *Drosophila* melanogaster. Preliminary studies using confocal microscopy suggest that pathological Tau reduces calcium levels, possibly through a decrease in the expression of the *Drosophila* BK channel homolog, Slowpoke, specifically within the neuron. We will further investigate Tau-mediated reduction of global calcium and will determine if pharmacologically increasing calcium levels along with genetic over-expression of neuronal Slowpoke alleviates Tau-induced neurotoxicity and increases healthspan and lifespan *in vivo*. These findings, along with current understandings of the role of Slowpoke in AD models, will hopefully advance our understandings of the role of calcium homeostasis in the pathogenesis of tauopathies and develop disease-modifying therapies.

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733 Muscleblind mitigates FUS toxicity by modulating stress granule dynamics and restoring SMN levels. Ian Casci[1,2], Karthik Krishnamurthy[3], Sukhleen Kour[2], Nandini Ramesh[1,2], Eric Anderson[2], Rogan Grant[2], Stacie Oliver[2], Lauren Gochenaur[2], Krishani Patel[2], Vadreenath Tripathy[4], Jared Sterneckert[4], Amanda Gleixner[5,6], Christopher Donnelly[5,6], Marc-David Ruepp[7], Piera Pasinelli[3], *Udai Pandey*[1,2] 1) Department of Human Genetics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA USA; 2) Division of Child Neurology, Department of Pediatrics, Children's Hospital of Pittsburgh, University of Pittsburgh Medical Center, Pittsburgh, PA USA; 3) Frances and Joseph Weinberg Unit for ALS Research, Department of Neuroscience, Farber Institute for Neuroscience, Thomas Jefferson University, Philadelphia, PA USA; 4) Department of Cell and Developmental Biology, Max Planck Institute for Molecular Biomedicine, Röntgenstrasse 20, 48149 Münster, Germany; 5) Department of Neurobiology, School of Medicine, University of Pittsburgh, Pittsburgh, PA; 6) Live Like Lou Center for ALS Research, Brain Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA USA; 7) Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, Maurice Wohl Clinical Neuroscience Institute, King's College London, London SE5 9NU, UK.

Pathogenic mutations in fused in sarcoma (FUS) lead to amyotrophic lateral sclerosis (ALS) in patients with varying ages of onset, progression and severity.

This suggests that additional, unknown factors may contribute to disease pathogenesis. We performed an unbiased genetic screen using a *Drosophila* model of ALS and discovered muscleblind as a novel modifier of FUS-mediated neurodegeneration *in vivo*. Muscleblind regulates cytoplasmic mislocalization of mutant FUS and subsequent accumulation in stress granules, dendritic morphology and toxicity in mammalian neuronal and human FUS patient iPSC-derived motor neurons. Interestingly, genetic modulation of endogenous muscleblind was sufficient to restore reduced SMN protein levels in axons expressing pathogenic mutations in FUS, suggesting a potential mode of suppression of FUS toxicity. Upregulation of SMN protein suppressed FUS toxicity *in vivo* indicating a link between FUS and SMN. Our data provide *in vivo* evidence that muscleblind is a dominant modifier of FUS-mediated neurodegeneration by regulating pathways involved in ALS pathogenesis.

734 Construction and characterization of novel *ninaE* alleles as a potential model for autosomal dominant retinitis pigmentosa. *M.E. Larsen*, J.C. Aldrich, S.G. Britt Neurology, Dell Medical School, Austin, TX.

Autosomal dominant retinitis pigmentosa is a disorder characterized by degeneration of rod photoreceptor cells in the retina leading to a gradual progressive loss of peripheral vision and night vision (and in some cases eventual blindness), and can be caused by mutations in numerous genes, including that encoding the primary visual pigment rhodopsin. Missense mutations affecting the chromophore binding site of rhodopsin are a common cause of this disorder. One such mutation, which replaces lysine 296 with glutamic acid (K296E), has been previously examined in the vertebrate visual system, and is linked to constitutive activation of rhodopsin followed by retinal degeneration. In order to further characterize the effects of this and similar mutations in a Drosophila model for retinal degeneration, we have constructed transgenic strains expressing mutated forms of Rhodopsin 1 (Rh1) — the functional ortholog of vertebrate rhodopsin encoded by *ninaE*. Flies expressing K319E (the *Drosophila* equivalent of vertebrate K296E), K319A or K319Q, in an otherwise *ninaE*-null background, lack deep pseudopupils. This suggests a reduction in levels of rhodopsin in the eye. Western blot analysis reveals an apparent elimination of mature Rh1 in flies as young as 24 hours post-eclosion in all three mutants (K319E, K319A, and K319Q). This is consistent with the lack of deep pseudopupil in the three mutant constructs and potentially indicative of defects in the processing or activation of Rh1. Work is underway to further characterize the retinal morphology of these mutants via confocal microscopy and the visual system response via electroretinogram recordings of electrophysiological light response, and to elucidate the nature of the Rh1 defect.

735 The palmitoyltransferase, *Patsas* is a novel retinal degeneration locus in Drosophila. *J.C. Aldrich*[1], J. L'Huillier[2], C. Flores[2], A. Heinen[2], S.G. Britt[1], N.J. Colley[2] 1) Department of Neurology, Dell Medical School, University of Texas, Austin, TX; 2) Department of Ophthalmology & Visual Sciences, School of Medicine & Public Health, University of Wisconsin, Madison, WI.

Mutations that disrupt the expression, function, or trafficking of Rhodopsin and other proteins involved in phototransduction can lead to progressive photoreceptor loss and retinal degeneration in both humans and flies. Such mutations underlie many heritable forms of visual impairment, including the dominant and recessive forms of retinitis pigmentosa. The Drosophila gene *Patsas* was identified in a screen for mutations that affect deep pseudopupil appearance—a phenotype closely linked to photoreceptor integrity in flies. Closer examination, using confocal and electron microscopy, reveals that homozygous *Patsas*$^{2\text{-}4388}$ mutants exhibit age-dependent loss of outer photoreceptors (R1-6), while inner receptors (R7-8) remain largely intact. Furthermore, western blot analysis indicates mutants have significantly reduced levels of Rh1 (the R1-6 opsin encoded by *ninaE*) upon eclosion and that these levels continue to decline as the flies age. *Patsas* encodes an S-palmitoyltransferase—a type of enzyme that catalyzes protein lipidation via the covalent attachment of one or more fatty acids (most often palmitic acid). Protein palmitoylation can serve a variety of functions including the facilitation of protein-protein interactions and trafficking between membrane compartments. Rh1 is predicted to be palmitoylated on two conserved cysteine residues located on its C-terminal tail. Consistent with this, results from an acyl-resin-assisted capture (acyl-RAC) assay demonstrate that Rh1 is most likely palmitoylated. To follow up on these results, we are using CRISPR to modify the putative cysteine residues in Rh1 and to make a clean knockout of *Patsas*.

736 Development of a Fly Model for Stargardt Disease Related Retinal Degeneration. *T.L. Jacobsen*, S.G. Britt Department of Neurology, Dell Medical School, University of Texas at Austin, Austin, TX.

Recessive Stargardt Disease (STGD) associated mutations in the human ABCA4 gene are the major cause of early onset retinal degeneration in the macula. Loss of ABCA4 function results in the accumulation of retinoid byproducts in the retinal pigment epithelium (RPE), which apparently leads to the death of the RPE and its associated photoreceptors. The human ABCA4 gene is a large, complex loci and hundreds of extant ABCA4 mutations contributing to STGD have been identified. Evidence suggests that the severity of STGD correlates with the degree of loss of function seen for a given ABCA4 variant, i.e., less ABCA4 function results in more severe phenotypes and earlier onset. Here we describe development of a model for STGD in the genetically robust and malleable Drosophila system. We are testing whether any native fly proteins display a function similar to ABCA4. Excess chromophore/retinaldehyde in the Drosophila retina can lead to photoreceptor degeneration (such as in *ninaE* null mutants), but whether there is any native fly protein that actively transports retinoids across photoreceptor membranes in a fashion similar to ABCA4 in vertebrates is unknown. To address this question, we are first looking at the phenotypic consequences in fly retinal tissue of knocking down the function of Drosophila members of the ABCA transporter family which are expressed in the adult fly head. Additionally, we are looking at the activity and phenotypic consequences when human ABCA4 protein is expressed in flies. Our goal is to determine whether ABCA4 can function in flies and possibly rescue mutant phenotypes associated with excess chromophore accumulation. If ABCA4 displays novel phenotypic effects outside of fly eye tissue, this may also present an avenue for examining ABCA4 function within the context of Drosophila.

737 Assessing the role of the p3 Peptide in Alzheimer's Disease Pathogenesis using a *Drosophila* model. *Joey Wong*[1], Thomas Finn[2], Cameron Kuo[1], Jeremy Lee[1] 1) Department of Molecular, Cell, and Developmental Biology, University of California, Santa Cruz, Santa Cruz, CA, 95064; 2) Department of Chemistry and Biochemistry, University of California, Santa Cruz, Santa Cruz, CA 95064.

Alzheimer's Disease (AD) is the leading cause of dementia, currently affecting 44 million people worldwide. AD is a neurodegenerative disease characterized by extracellular accumulation of plaques that are composed of peptides produced by proteolysis of the amyloid precursor protein (APP). APP is cleaved in alternative pathways. Among the fragments produced in one pathway is the 4.5 kD peptide amyloid beta (Aβ) and an alternative pathway produces p3, a 3kD peptide fragment that consists of amino acids 17 to 42 of Aβ. Aβ has been shown to disrupt normal neuronal function, eventually leading to neuronal death. However, little is known about the effects of p3 on neuron function and how these effects may differ or resemble those of Aβ, or whether p3 might contribute to, or modulate Aβ's effects on neuron function. Previous research has shown that, like Aβ, p3 can form amyloid fibrils *in vitro*; the amino acid sequence p3 shares with Aβ suggests overlapping mechanisms for peptide aggregation and neuronal toxicity. Moreover, the normal function of p3 is unknown.
We have generated a transgenic fly expressing the human p3 peptide. Using the ϕC31 integrase system, a DNA construct encoding p3 with a N-terminal secretory signal was integrated into the fly genome. We have confirmed the presence of the p3 peptide in the transgenic fly using polymerase chain reactions (PCR). As such, we have established the first *in vivo*, p3 fly model, with which lifespan, behavioral, and molecular analyses are being conducted, to assess whether AD-like pathogenesis is induced by the p3 peptide in comparison to flies that express Aβ. We are also determining the effects of co-expression of p3 and Aβ to assess whether the presence of p3 influences Aβ's deleterious effects.

738 Investigating Mitochondrial Transport in a *Drosophila* model of Alzheimer's Disease. *N. Vidyasagar*, S. Druinksy, A Stautz, M Mazeres, R Wilcox, S Davidson, J Lee Molecular Cell Developmental Biology, University of California: Santa Cruz, Santa Cruz, CA.

Alzheimer's disease (AD) is a major chronic neurodegenerative disease causing memory-loss, dementia and death and is estimated to affect over 40 million patients worldwide. The two hallmarks of this disease are amyloid plaques, largely composed of the peptide Aβ and neurofibrillary tangles composed of the Tau protein. Compromised axonal transport of mitochondria has been shown to be among the effects of these aggregates in vitro. A new in vivo system, has been established to study axonal transport of organelles in intact wing sensory neurons of live adult *Drosophila* (Vagnoni & Bullock, 2016) . Our study aims to utilize this system to perform live imaging of GFP tagged mitochondria in adult *Drosophila* models of Alzheimer's disease to assess whether axonal transport of mitochondria is affected in these models and how these effects change with age. We also aim to determine whether there is a relationship between defects in mitochondrial transport and other phenotypes in our AD model flies, including defective locomotion and reduced lifespan. To accomplish this goal, flies with pan-neuronal expression of human Aβ-42 or expression of a mutant version of human Tau subject to hyperphosphorylation and associated with compromised axonal transport were generated for mitochondrial imaging. Longevity assays indicate that Aβ-42 and MAP-Tau flies display a significantly shortened lifespan compared to controls, with Aβ-42 having the shortest lifespan. Locomotion assays utilizing the Rapid Iterative Negative Geotaxis (RING) assay indicate that Aβ-42 and MAP-Tau flies display significantly impaired locomotion in areas such as velocity, time taken to reach the top and proportion of flies that reached the top, with Aβ-42 flies displaying the most severe defects in climbing at 23 days after eclosion. Together, this data suggests that there is an age dependent progression of AD in our *Drosophila* models.

739 The microbiome's effect on the pathogenesis of Alzheimer's disease. *M. Zhu*, N. Broderick University of Connecticut , Storrs, CT.

Alzheimer's disease (AD) is the most common form of dementia, yet treatment remains elusive as little is known about the factors contributing to the disease. A newly proposed cause of AD is the pathogen hypothesis which states that an infection of the brain triggers the buildup of Aβ42 plaques and activation of microglia. In addition, the microbiome has been implicated as a potential factor in the onset of AD, but studies supporting a causative role are lacking. In order to understand whether bacteria can contribute to AD pathology and disease progression, we are using the model organism *Drosophila melanogaster,* in which we can express human Aβ42 in brains and generate axenic (germ free) flies in order to examine AD phenotypes in the absence of the microbiome. Moreover, we can also infect flies either systemically or orally to compare the effects of pathogens versus the microbiome on AD phenotypes. A previous study reported that intestinal infection with the non-lethal pathogen *Erwinia carotovora* (Ecc15) increased the severity of AD in flies, as measured in an increase in Aβ42 and shorter lifespans. We confirmed these results showing that Aβ42-expressing flies-orally infected with Ecc15 had shorter lifespans than uninfected controls. Our preliminary data also indicated that axenic flies expressing Aβ42 had longer lifespans than uninfected controls, suggesting that the microbiome may contribute to AD pathologies. In studying the role of pathogens, we found that intestinal infection with either Ecc15 or *Pseudomonas entomophila*, a lethal pathogen, did not lead to robust translocation of bacteria from the gut to the brains. One explanation how intestinal infections may indirectly lead to increased AD severity is that infection induces reactive oxygen species burst that damages tissues. Our results support this mechanism, as axenic flies have a decreased basal level of ROS production. To further understand these factors, we will infect pathogens directly into the fly hemolymph and assess AD phenotypes and lifespans of Aβ42 expressing flies. Future experiments will also quantify and observe activation of microglia. Altogether, this work will increase our understanding of Alzheimer's disease and the possible effects the microbiome may have on AD pathology.

740 Neuropathy-associated TRPV4 mutants cause CaMKII-dependent excitotoxicity and inhibit mitochondrial axon transport. *B. M. Woolums*[1], H. Sung[1], M. Tabuchi[1], K. Takle[2], B. McCray[1], J. M. Sullivan[1], M. Yang[1], C. Mamah[1], W. Aisenberg[1], B. Larin[1], D. N. Robinson[1], Y Xiang[2], M. N. Wu[1], C.J. Sumner[1], T.E. Lloyd[1] 1) Johns Hopkins University, Baltimore, MD; 2) University of Massachusetts Medical School, Worcester, MA.

Autosomal dominant mutations in the gene encoding TRPV4 cause Charcot-Marie-Tooth disease subtype 2C (CMT2C). CMT2C causing TRPV4 mutants cause increased calcium influx and cellular toxicity *in vitro*, and this gain of ion channel function is hypothesized to play an important role in CMT2C pathogenesis; however, it is unclear whether this gain of channel function occurs in neurons *in vivo,* if increased TRPV4 activity is sufficient to cause neurotoxicity, or what cellular processes are disrupted downstream of elevated calcium to confer disease. Here, we employ a novel *Drosophila* model of CMT2C in combination with primary mammalian neuron cell culture to address these questions. Expression of TRPV4^{R269C} in *Drosophila* neurons facilitates increased neuronal excitability and calcium influx, and significantly disrupts mitochondrial transport prior to the onset of synaptic degeneration. Furthermore, high expression of TRPV4^{R269C} causes progressive neuronal dysfunction and degeneration of both synapses and dendrites in our *Drosophila* model. Importantly, these phenotypes could be rescued by genetically or pharmacologically inactivating the TRPV4 ion channel pore, demonstrating the dependence of the phenotypes on TRPV4 ion channel function. We conducted a modifier screen in *Drosophila* CCAP neurons and identified CaMKII as a potent modifier of TRPV4^{R269C} mediated toxicity. Knockdown of CaMKII, a well characterized calcium dependent kinase, potently suppressed TRPV4^{R269C} mediated hyperexcitability and toxicity, suggesting that CaMKII is required for TRPV4 mediated neurotoxicity. We also report that the mitochondrial transport protein Miro enhances TRPV4^{R269C} mediated neurotoxicity in a manner dependent on the Miro calcium binding and GTPase domains. Finally, we also observe enhanced calcium responses and neurotoxicity in cultured primary mouse trigeminal neurons expressing TRPV4^{R269C}. Together, our data suggests neuropathy causing mutants sensitize the TRPV4 ion channel, resulting in neurotoxicity via increased neuronal calcium and the disruption of mitochondrial axon transport.

741 Functional Screen of Lysosomal Storage Disorder Toxicity with Alpha-Synuclein. *M. Yu*[1], I. Al-Ramahi[2,5], J. Botas[2,3,5], J. Shulman[1,2,4,5] 1) Department of Neuroscience, Baylor College of Medicine, Houston, TX; 2) Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 3) Department of Molecular & Cellular Biology, Baylor College of Medicine, Houston, TX; 4) Department of Neurology, Baylor College of Medicine, Houston, TX; 5) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX.

Parkinson's disease (PD) is the second-most common neurodegenerative disease, characterized by progressive motor and cognitive impairments. PD is thought to be highly heritable, yet the genetic basis for the majority of cases is not known. A set of 54 genes which cause lysosomal storage disorders (LSDs) have recently been linked to PD risk in human cohorts, including variants in the gene *GBA* which is found in 10% of PD patients. Our recent exome-wide analysis determined that burden of LSD gene variants was significantly associated with PD incidence in a cohort of 1156 participants, but our results did not determine the contribution of individual genes to disease risk. We therefore used an automated climbing assay to screen all conserved LSD genes for interaction with our disease model of neuronal alpha-synuclein overexpression, using RNAi-mediated knockdown. We have currently identified 10 strong candidate enhancers of alpha-synuclein toxicity, including the *GBA* homolog *Gba1b* and other genes involved in sphingolipid metabolism, for further validation.

742 Roles for cell-cell signaling in the spread of Aβ42-mediated pathology through the brain. *C. Yeates*[1], A. Sarkar[1], P. Deshpande[1], M. Kango-Singh[1,2,3], A. Singh[1,2,3,4] 1) Department of Biology, University of Dayton, Dayton, OH; 2) Premedical Program, University of Dayton, Dayton, OH; 3) Center for Tissue Regeneration & Engineering (TREND), University of Dayton, Dayton, OH; 4) Center for Genomic Advocacy (TCGA), Indiana State University, Terre Haute, IN.

Alzheimer's disease (AD) is a debilitating neurodegenerative disorder with no cure and few effective treatments. AD causes profound cognitive deficits and memory impairments. Here we use a Drosophila model of AD to study the interactions between diseased and healthy cells to better understand the spread of the disease through the brain.

Accumulation of the peptide amyloid beta into plaques is one of the characteristics of the disease. A 42 amino acid polypeptide, Aβ42, is a cleavage product of Amyloid Precursor Protein. Aβ42 tends to aggregate and forms oligomers, eventually making up the plaques seen in the disease. Human Aβ42 can be expressed in the developing retinal cells of fruit flies. In this study we use twin-spot MARCM (Mosaic Analysis with a Repressible Cell Marker) with the FLP/FRT

system to express Aβ42. This yields animals with GFP-negative WT cells adjacent to GFP-positive Aβ42-expressing cells in a heterozygous background. We found that populations of Aβ42-expressing cells are much larger than the adjacent populations of WT cells. This suggests that cell-cell signaling between the two populations may be either interfering with the survival of WT cells or inducing cell death at a later stage in development. Previous research has implicated Aβ42 in the aberrant activation of pathways leading to cell death. Here we present evidence that signaling between Aβ42-expressing cells and adjacent WT cells mediates neurodegeneration.

743 Role of Lunasin in Alzheimer's Disease. *P. Deshpande*[1], N. Gogia[1], S. Brochers[1], A. Singh[1,2,3,4] 1) Department of Biology, University of Dayton, Dayton, OH; 2) Premedical Program, University of Dayton, Dayton, OH; 3) Center for Tissue Regeneration & Engineering (TREND), University of Dayton, 300 College Park Drive, Dayton, OH; 4) Center for Genomic Advocacy (TCGA), Indiana State University, Terre Haute, IN.

Alzheimer's Disease (AD), a common form of dementia and an age related progressive neurodegenerative disorder, manifests as memory loss and reduced cognitive ability. One of the hallmarks of AD is formation of the Amyloid-beta 42 (hereafter Aβ42) plaques, which triggers oxidative stress due to aberrant signaling and finally results in the death of neurons. However, the exact mechanism causing cell death is still not well understood. We misexpressed high levels of human Aβ42 protein in the developing fly retina, which mimics AD like neuropathology. Recently, we found that a plant protein Lunasin can ameliorate Aβ42 mediated neurodegeneration in the eye by blocking c-Jun N-terminal kinase (JNK) signaling pathway. It is known that Immune deficiency (IMD) pathway, Nuclear factor-κB (NF-κB) signaling pathway, Toll receptor pathway and JNK pathway crosstalk with each other in neurodegeneration. Here we test the role of IMD and NF-κB pathway in Aβ42 mediated neurodegeneration. Loss of function of Relish (Rel), a member of and NF-κB and its downstream gene- Diptericin rescues the small glazy eye phenotype. Our working model is that Lunasin might down-regulate JNK signalling pathway which in turn downregulates IMD pathway to ameliorate Aβ42 mediated neurodegeneration.

744 Validation of Potential Therapeutic Targets of Alzheimer's Disease Among Cross-Species Modifiers of Tau. *H. Chester*, J. Kim, B. Bleiburg, M. Avalos, I. Al-Ramahi, M. de Haro, H. Zoghbi, J. Botas Baylor College of Medicine, Houston, TX.

Alzheimer's disease (AD) is the most common neurodegenerative disease with a total of 44 million people affected worldwide. Individuals with AD suffer from cognitive decline, memory loss, mood and behavior changes. Two neuropathological hallmarks of AD are extracellular plaques and intracellular neurofibrillary tangles, which are composed respectively of amyloid-β and hyperphosphorylated tau. Accumulation of these two proteins contributes to the widespread death of cholinergic neurons. The presence of tau tangles strongly correlates with the severity of neurodegeneration in AD and has also been implicated in a variety of other neurodegenerative disorders, collectively termed tauopathies. Overexpression of wild type tau in a murine model is sufficient to induce tau hyperphosphorylation and neurodegeneration while decreasing tau levels ameliorates both neuronal loss and behavioral deficits caused by tau accumulation. The detrimental nature of tau accumulation on neuronal function and success of decreasing tau levels in mouse models of AD provides further evidence for exploring new mechanisms to lower levels of tau and decrease its neurotoxicity.

Using a Drosophila model to identify genetic modifiers of tau allows us a high throughput method to study the effect of modifier genes on both protein accumulation and tau-induced neuronal dysfunction. A parallel screen of approximately 6500 genes from the druggable human genome in both Daoy cells overexpressing tau and a *Drosophila* model expressing human tau generated a list of candidate genes that when knocked down, decreased tau levels and suppressed tau induced neuronal dysfunction. Characterization of these genes provides greater understanding of pathways that may play a role in AD pathogenesis.

Further validation of candidate genes in immortalized human cell lines and *Drosophila* confirmed both a reduction in tau levels as well as improved motor performance. Translation of these results into a mammalian model system is vital in order to select which genes are viable therapeutic targets for further studies. We are currently knocking down candidate genes in both wild type and P301S primary mouse neurons by lentiviral infection with validated shRNAs. Genes capable of reducing neuronal tau levels in mouse primary neurons are good candidates for studying the mechanism of tau reduction as well as effect on mouse behavior.

745 Comparative analysis of two dSOD1 knock-in ALS models. *K. Russo*[1], A. Spierer[2], A. Held[3], K. Wharton[3] 1) Neuroscience, Brown University, Providence, RI; 2) Ecology and Evolutionary Biology, Brown University, Providence, RI; 3) Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the progressive decline in motor function, with the ultimate loss of motor neurons. The cellular origin and the underlying molecular basis of dysfunction is poorly understood. Genetic models of ALS can be used to provide insight into the cellular pathways altered by specific patient alleles. More than 100 mutations in the SOD1 (superoxide dismutase-1) gene are associated with ALS. We knocked-in two synonymous mutations, $dSod1^{G85R}$ and $dSod1^{A4V}$, into conserved residues of the endogenous dSod1 locus by CRISPR-Cas9 gene editing therefore maintaining gene dosage. These two mutations represent the two classes of SOD1 mutations, those that eliminate the superoxide scavenging function (dismutase) of SOD1, and those that do not, respectively. Phenotypic analyses reveal that $dSod1^{G85R}$ pharates fail to eclose from the pupal case. However, more than 90% of $dSod1^{A4V}$ pharates eclose as viable adults and live more than 2 weeks with a dramatic loss in viability by ~3 weeks. This shortened lifespan and detection of climbing deficits enabled a focused analysis of the progressive loss of motor function. We have identified genetic suppressors of the $dSod1^{G85R}$ eclosion deficit and are evaluating their ability to suppress $dSod1^{A4V}$ associated defects. Those genes that suppress both $dSod1^{G85R}$ and $dSod1^{A4V}$ dysfunction identify cellular pathways that modify both types of SOD1 mutations. These results will clarify commonalities of ALS-inducing mutations and will inform a greater understanding of the underlying molecular mechanisms responsible for the ultimate degeneration of motor neurons.

746 Glycolysis upregulation is neuroprotective as a compensatory mechanism in ALS. *E. Manzo*[1], I. Lorenzini[2], D. Barrameda[1], A. O'conner[1], J. Barrows[1], A. Starr[2], T. Kovalik[2], D. Shreiner[1], A. Joardar[1], J.C. Lievens[3], R. Bowser[2], R. Sattler[2], D.C. Zarnescu[1] 1) Molecular and Cellular Biology, University of Arizona, Tucson, AZ; 2) Neurology, Barrow Neurological Institute, Phoenix, AZ; 3) Aix-Marseille Université, CNRS, Centre de Recherche en Neurobiologie et Neurophysiologie de Marseille, Marseille, France.

Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig's Disease, is a fatal neurodegenerative disorder affecting upper and lower motor neurons. TAR DNA-binding protein 43 (TDP-43) is found in cytoplasmic inclusions in 97% of ALS patients. Our lab has previously shown that overexpression of either wild type or mutant human TDP-43 in motor neurons of *Drosophila* induces motor deficits and reduces lifespan. Using this model we performed global metabolomics profiling and identified several significant changes consistent with alterations in glucose metabolism and long chain fatty acid transport. Based on our metabolomics data, we hypothesize that altering glucose metabolism through genetic and dietary intervention can provide protection against neurodegeneration. Our data indicate that a high sugar diet improves locomotor and lifespan defects caused by TDP-43. Consistent with these findings the overexpression of human glucose transporter GLUT-3 in motor neurons improves locomotor function and rescues defective synaptic vesicle recycling at the

neuromuscular junction caused by TDP-43. These results suggest that increased glucose availability is protective in degenerating motor neurons. Furthermore, Pfk mRNA - a key indicator of glycolytic activity - is significantly upregulated in TDP-43 expressing flies and iPSC motor neurons with TDP-43 pathology. Moreover, over-expression of Pfk is sufficient to rescue TDP-43 induced locomotor toxicity in *Drosophila*, while the knockdown of Pfk aggravates TDP-43 induced impairments. Taken together, our findings in fly and human tissue point to upregulated glycolysis as a compensatory mechanism, which allows neurons to counter TDP-43 proteinopathy.

747 Expression of LL-37, a human antimicrobial peptide, attenuates the neurotoxicity of Aβ$_{42}$ in a *Drosophila* model of Alzheimer's disease. *S. Gutierrez*[1], M. Kaur[1], B. Baird[1], T. Kurman[1], A. Barron[2], J. Lee[1] 1) UC Santa Cruz; 2) Stanford University School of Medicine.

Alzheimer's disease (AD) is the most common form of dementia and is characterized by amyloid plaques in the brain composed primarily of the Aβ peptide. Although Aβ's function is unknown, recent studies indicate that it has antimicrobial properties and might have similar biophysical functions to LL-37, a human antimicrobial peptide. Previous *in vitro* studies have shown that LL-37 and Aβ are direct binding partners, but it is still a question if they are binding partners *in vivo.* Also, it is unknown what the consequences are of an interaction between LL-37 and Aβ with respect to Aβ's effects on neuronal function.

We have developed a *Drosophila* model for *in vivo* experimentation on the effects of co-expressing human LL-37 and human Aβ$_{42}$ peptides. Adult longevity and pre-adult developmental assays were conducted with four groups of flies: flies that only expressed Aβ$_{42}$ (AD model flies), flies that only expressed LL-37, flies that co-expressed Aβ$_{42}$ and LL-37, and wild type flies. Results showed that while Aβ$_{42}$-expressing adult flies had significantly shorter lifespans than wild type adult flies, adult flies co-expressing both Aβ$_{42}$ and LL-37 lived longer, i.e. co-expression had a rescue effect on their lifespan. In addition, co-expression had a rescue effect on pre-adult mortality compared to flies only expressing Aβ$_{42}$.

The expression of Aβ$_{42}$ was quantified to confirm expression in equal levels between flies that only express Aβ$_{42}$ and the flies that co-express Aβ$_{42}$ and LL-37. To quantify Aβ$_{42}$ expression, quantitative PCR with primers specific for Aβ$_{42}$ was used to quantify Aβ$_{42}$ mRNA levels.

These data suggest that LL-37 interactions with Aβ, *in vivo*, partially ameliorate the effects of Aβ on neuronal function. Memory and climbing assays, along with histological analyses are being conducted to more fully assess the effects of LL-37 on Ab in neurodegeneration and on Aβ's deleterious effects on behavior.

748 Late-breaking news: Autophagy goes on strike! – Rampant immune response kills neurons! *A.K. Shukla*[1], Joshua Spurrier[2], Edward Giniger[1] 1) National Institute of Neurological disorders and stoke, National Institute of Health, Bethesda, MD; 2) Yale University, New Haven, CT.

Innate immunity is central to the pathophysiology of neurodegenerative disorders, but it remains murky why immunity is altered in the disease state, and whether changes in immunity are a cause or a consequence of neuronal dysfunction. Here we identify a molecular pathway that links innate immunity to age-dependent loss of dopaminergic neurons in Drosophila. We find, first, that altering the expression of the activating subunit of the Cdk5 protein kinase (Cdk5α) causes severe disruption of autophagy. Second, this disruption of autophagy is both necessary and sufficient to cause massive hyperactivation of innate immunity, particularly anti-microbial peptides. Finally, it is the upregulation of immunity, in turn, that induces the age-dependent death of dopaminergic neurons. Given the dysregulation of Cdk5 and innate immunity in human neurodegeneration, and the conserved role of the kinase in regulation of autophagy in humans, this sequence is likely to have direct application to the chain of events in human neurodegenerative disease.

749 Temperature sensitive SMA-causing point mutations lead to SMN instability, locomotor defects and premature lethality. *A. Raimer*[1], A. Spring[2], M. Edula[3], S. Singh[3], A.G. Matera[3,4] 1) Curriculum in Genetics and Molecular Biology, UNC, Chapel Hill, NC; 2) UNC SPIRE Fellows Program, UNC, Chapel Hill, NC; 3) Department of Biology, UNC, Chapel Hill, NC; 4) Department of Genetics, UNC, Chapel Hill, NC.

Spinal Muscular Atrophy (SMA) is the leading genetic cause of death in small children with a carrier frequency of 1:50. This progressive neurodegenerative disease is caused by reduction in levels of Survival Motor Neuron (SMN) protein. Although most SMA patients are homozygous for *SMN1* deletions, a small percentage of patients are heterozygous for the deletion and a missense mutation. Whereas the genetics and physiology of SMA are well defined, the molecular mechanism that leads to disease remains unclear.

We have developed an allelic series of SMA patient-derived missense mutations in *Drosophila Smn,* covering all three functional domains of the protein. These animals recapitulate the full range of phenotypic severity observed in human SMA. We have identified a subset of alleles in the Tudor domain that is temperature sensitive. Specifically, at higher temperatures, these Tudor mutants display defects in locomotion, viability and longevity when compared to other mutants in the series. These phenotypes correlate with a marked reduction in SMN protein levels at non-permissive temperatures.

To investigate the mechanism underlying the reduction in SMN protein levels at higher temperatures, we inhibited translation in *ex vivo* larval tissues using cycloheximide and measured changes in SMN stability over time. We found that SMN levels remain stable at permissive temperatures, whereas the mutant protein is differentially sensitive at non-permissive temperatures. We hypothesize that the SMN Tudor domain plays a unique role in the protein's stability; in contrast, mutations in the YG-box oligomerization domain are similarly lethal and yet relatively insensitive to changes in temperature.

In conclusion, these studies identify a novel mechanism of disease etiology for a subset of Tudor domain mutations. Although each domain of SMN is capable of contributing to disease pathology, its impact on SMN folding and function is distinct. Moreover, these temperature-sensitive alleles provide a powerful resource for forward genetic screens aimed at identifying SMN-interacting proteins and pathways.

750 Oxidation Resistance 1 (OXR1) is associated with a novel neurodevelopmental disorder. *J. Wang*[1], J. Rousseau[3], E. Kim[2], Y-T. Cheng[1], W. Bi[1], J. Rosenfeld[1], L-J. Wong[1], P. Campeau[3], H. Bellen[1] 1) Baylor College of Medicine, Houston, TX; 2) Rice University, Houston, TX; 3) Université de Montréal, Montréal, Canada.

Personalized medicine with tailored diagnoses and therapeutics has been the looming goal of cutting-edge medicine. The practical execution of this goal requires a multidisciplinary system. Here we demonstrate an approach towards personalized medicine. Close partnership with patients, physicians, and basic scientists can successfully lead to personalized diagnoses. Together, we make progress towards a tailored therapeutic approach using WES and WGS, MARRVEL and other databases, and studying phenotypes in patient fibroblasts and genetically engineered fruit flies.

By re-analyzing sequencing data from patients with unknown neurological diseases, we identified four families affected by a neurodevelopmental disease with potentially pathogenic variants in the gene *Oxidation Resistance 1 (OXR1)*. *OXR1* defends cells against oxidative stress but the mechanism is unknown. Patients homozygous for two variants of *OXR1* have developmental delay, seizures, speech problems, hypotonia, cerebellar abnormalities, and intellectual disability. By examining patient fibroblasts, we observe lysosome/autophagy-like sub-cellular abnormalities that are being investigated.

In parallel, we generated a fruit fly model where the homolog of *OXR1, mustard*, is knocked out. The mutant flies die as emerging adults and the lethality can be rescued by human *OXR1* cDNA. Using tissue-specific knock-down, we found that the lethality is associated with severe neurological defects. By using

immunoprecipitation-mass spectrometry of the HA tagged OXR1 protein expressed in flies, we found four proteins that may help us understand how *OXR1* and *mustard* contribute to oxidative stress resistance and neuronal health.

751 Investigating Zika virus interactions with host cellular pathways using *Drosophila*. *Brooke Hull*[1,2], Michael Harnish[2], Nichole Link[2], Angad Jolly[2], Silvia Balcazar[3], Priya Shah[4], Hugo Bellen[2,5,6], Shinya Yamamoto[2,6] 1) Postbaccalaureate Research Education Program (PREP), Graduate School, Baylor College of Medicine, Houston, TX; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Summer Undergraduate Research Training Program, Baylor College of Medicine, Houston, TX; 4) Department of Chemical Engineering, University of California Davis, Davis, CA; 5) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX; 6) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX.

Spread of Zika virus (ZIKV) is linked to increases in neurological diseases such as Guillan-Barré syndrome and congenital microcephaly, yet the molecular mechanisms are mostly unknown. Recent work suggests increased pathogenicity of the South American ZIKV strain is associated with mutations that occurred in pre-Membrane protein (prM) and NonStructural protein 1 (NS1) compared to ZIKV strains isolated in Central Africa and Southeast Asia. To better understand how ZIKV may cause neurological phenotypes in humans, we are using *Drosophila* to assess the interactions between ZIKV proteins and molecular and cellular pathways of the host cell. Furthermore, we wish to use a functional screening platform in flies to assess the role of new mutations that arose in the ZIKV genome during evolution. We generated GAL4/UAS system based transgenic lines that encode one of the ten proteins and two non-coding RNAs encoded in the ZIKV genome to assess the consequences of over-expressing these factors in vivo. Interestingly, using the sequences from the Puerto Rican strain, we found that most factors exhibited a scorable phenotype when expressed in the nervous system or developing tissues, providing us with a functional assay. Some phenotypes such as microchaeta loss and increased density of microchaeta suggest that ZIKV proteins can modulate neurodevelopment through a potential link to the Notch signaling pathway. We are in the process of reverting the mutations in the Puerto Rican prM and NS1 sequences to the more ancient and less pathogenic Cambodian strain sequences to determine if either of these mutations behave differently when tested in *Drosophila*. This study demonstrates the value of *Drosophila* in functional characterization of viral factors and disease-associated mutations to begin to unravel the mechanism of ZIKV mediated neurodevelopmental and neurological disorders.

752 Forward genetic screen in *Drosophila* to identify novel regulators of dopamine dynamics. *S.L. Deal*[1], E.S. Seto[2], M. Lagarde[3], J.L. Salazar[3], S. Yamamoto[1,3,4] 1) Program in Developmental Biology, Baylor College of Medicine (BCM), Houston, TX; 2) Department of Pediatrics, BCM, Houston, TX; 3) Department of Molecular and Human Genetics, BCM, Houston, TX; 4) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX.

Neuronal communication is indispensable for proper brain function. As major components of neuronal signaling, neurotransmitter fine-tuning is critical to this process. Dysregulation of the neuromodulator dopamine (DA), for example, leads to social and behavior impairments in animal models and neurological and neuropsychiatric disorders in humans. Thus, understanding the cellular and molecular mechanisms that regulate DA synthesis, secretion and metabolism (dynamics) is paramount to finding better treatments for patients with DA-related disorders. However, very little is known about the genes and pathways that fine-tune DA levels *in vivo*. Since core components of DA synthesis and secretion are highly conserved, we performed a forward genetic screen in *Drosophila*. Using tissue-specific RNAi and chemical mutagenesis/clonal analysis, we identified 123 genes that affect cuticle pigmentation, a DA-dependent phenotype. 85% of these genes are conserved between flies and humans. Knockdown of many of these genes in DArgic neurons led to alterations in basal activity measured by Drosophila Activity Monitors (DAM), suggesting a role for them in DA regulation in the brain as well as epidermal cells.

Interestingly, a number of genes isolated from this screen have been linked to human neurodevelopmental, neurological and psychiatric disorders including mitochondrial disorders, autism and schizophrenia. Out of these hits, we are studying the role of *Ubiquitin Specific Protease 7* (*USP7*) and its allosteric activator *GMP Synthase* (*GMPS*) in DA regulations *in vivo*. While there is extensive research on this deubiquitinase complex in the cancer field, only recent studies have found that mutations in *USP7* are associated with a neurodevelopmental disease. We are currently dissecting the functional role of USP7 and GMPS in DA regulation and analyzing the impact this may have on patients affected by USP7-related disorders.

753 The K^+-dependent Na^+/Ca^{2+} exchanger Nckx30C is implicated in temperature-sensitive paralysis and neurodegeneration in *Drosophila*. *S. Lye*, H. Bolus, E. Cytron, S. Chtarbanova Department of Biological Sciences, University of Alabama, Tuscaloosa, AL.

Na^+/Ca^{2+} exchanger families are involved in the regulation of intracellular calcium signaling, and have been suggested to have implications on the pathogenesis of neurodegeneration and seizure disorders. Fruit flies have been successfully used as a model for various human pathologies including seizures and neurodegenerative diseases. Previous studies have established a connection between mutations linked to defective behaviours such as temperature-sensitive (TS) paralysis and neurodegeneration. However, the exact mechanisms underlying these conditions are not fully understood. In an unbiased genetic screen for TS paralytic mutants, we identified the *Drosophila* line 426 that exhibits a seizure-like phenotype at 38°C. We demonstrate that in addition to the TS paralysis, 426 flies exhibit early onset locomotor defects, age-dependent neurodegeneration and reduced lifespan. We mapped the mutation in 426 flies to the *Drosophila* gene *Nckx30C*, which encodes a K^+-dependent Na^+/Ca^{2+} exchanger with enriched expression in brain tissue. Nckx30C is homologous to mammalian Solute Carrier Family 24 (SLC24) proteins of which pathophysiological involvement and function in the brain is poorly understood. We found that additional *Nckx30C* mutants also exhibit TS paralysis and age-dependent neurodegeneration, suggesting a role for this gene in neuropathology. Moreover, neuron- but not glia-specific knockdown of *Nckx30C* using RNAi recapitulates the TS-paralytic and defective locomotor phenotypes observed in 426 flies. Our studies in *Drosophila* reveal a novel role for the *Nckx30c* gene in TS-paralysis and neurodegeneration. Mutants identified in our screen could serve as a valuable model to decipher the function of SLC24 proteins in channelopathies and neuopathology.

754 A comprehensive functional screen of pathogenic neurofibromatosis type 1 (*NF1*) missense mutations. *J.A. Walker*[1], T.R. O'Meara[1], B.S. Leger[1], S.R. Ching[1], R.J. Drake[1], T. Charitou[1], S. Paul[2], S. DuBois-Coyne[2], M. Brown[2], A. Veraksa[2] 1) Center for Genomic Medicine, Massachusetts General Hospital, Boston, MA; 2) University of Massachusetts, Boston, MA.

Neurofibromatosis type 1 (NF1) is a common disorder with autosomal-dominant inheritance affecting 1 in ~3,500. Symptoms include benign but often disfiguring peripheral nerve associated tumors (neurofibromas), as well as malignant tumors. While the tumor suppressor role of *NF1* has received much attention, NF1 is a multi-system disorder whose non-tumor symptoms contribute significantly to its morbidity, including skeletal and vascular abnormalities, pigmentation defects, reduced overall growth and cognitive deficits. No effective therapy for any NF1 symptom yet exists.

NF1 encodes neurofibromin, a large (~320 kDa) protein, the only known function of which is to serve as a Ras-specific GTPase-activating protein (GAP). However, pathogenic missense mutations are found throughout *NF1*, suggesting other parts of the highly conserved protein are also essential for its function. We hypothesize that amino acid substitutions outside of the GAP domain may disrupt important novel functional domains, disrupt interactions with other proteins, or alter the regulation, stability or subcellular localization of neurofibromin – any of which could perturb its activity and contribute to the varied clinical symptoms of NF1.

We have investigated the molecular and cellular consequences of patient *NF1* missense mutations using a *Drosophila*model of NF1. *Nf1* transgenes bearing

conserved pathogenic missense mutations were tested for their ability to rescue established mutant *Nf1* phenotypes: increased Ras signaling, reduced systemic developmental growth, aberrant circadian rhythms and defective cognitive function. Affinity purification and mass spectrometry was used to determine whether *Nf1* mutations alter protein interactions. Altered subcellular localization of mutant transgenic neurofibromin in fly neurons was assessed using confocal microscopy.

We have identified several *Nf1* missense mutations in two regions outside of the established GAP domain that fail to rescue *Nf1* phenotypes, including Ras signaling. Further genetic and mechanistic experiments underline the importance of these novel domains in the normal function of neurofibromin. These mutations are subsequently being modeled in human induced pluripotent stem cells using CRISPR/Cas9 gene editing. This study, combining functional testing *in vivo* in*Drosophila,* with subsequent validation in human cells, allows us to correlate cellular and molecular phenotypes to specific patient-derived *NF1* mutations in different regions of neurofibromin. This knowledge may aid the discovery of new biomarkers and therapeutic targets for NF1.

755 Characterization of metazoan tricRNA biogenesis factors in neurological disease. *C. Schmidt*, L. Min, J. Giusto, M. McVay, A. G. Matera University of North Carolina Chapel Hill, Chapel Hill, NC.

Mature tRNAs are generated by multiple post-transcriptional processing steps, which can include intron removal. Recently, our lab discovered a new class of circular RNAs, formed by intramolecular ligation of excised tRNA introns. We term these molecules tRNA intronic circular (tric)RNAs. To investigate the mechanism of tricRNA biogenesis, we generated reporters that replace the endogenous introns of *Drosophila* or human tRNA genes with the Broccoli fluorescent RNA aptamer. Using these reporters, we showed that several known tRNA processing factors, such as the RtcB ligase and components of the TSEN endonuclease complex, are involved in tricRNA biogenesis. Depletion of these factors inhibits tRNA intron circularization. We also identified Clipper endonuclease as a likely tricRNA turnover factor. Furthermore, we found that that RNAi mediated depletion of the *Drosophila* CLP1 kinase orthologue (cbc) results in increased tricRNA levels. These findings support a model wherein cbc acts as a negative regulator of tRNA processing *in vivo*. Interestingly, mutations in both *CLP1* and human TSEN complex members and have been shown to cause neurological diseases such as pontocerebellar hypoplasia. To determine if disease phenotypes are conserved in *Drosophila*, we characterized mutations in *cbc* and the fly ortholog of human *TSEN54* using viability and locomotion assays, and also determined larval brain volume. We observed that mutations in both *cbc* and *TSEN54*resulted in reduced viability, developmental delays, altered larval locomotion, and reduced brain lobe volume. Currently, we are determining if reduced brain size is due to programmed-cell-death by staining brains for apoptosis markers. Effects of tissue-specific loss of cbc expression will also be presented. In summary, our work identifies the major players in *Drosophila* tricRNA biogenesis, and provides the first *in vivo* evidence for a negative regulator of tRNA splicing. Additionally, the results reveal conserved phenotypes of tRNA processing defects in vertebrates and invertebrates, and highlight *Drosophila* as a genetically tractable system for studying RNA-based neurological diseases.

756 A rescue based screen to functionally assess *de novo* missense variants lined to Autism Spectrum Disorders using *Drosophila*. *J.C. Andrews*[1,2], Paul Marcogliese[1,2], Samantha Deal[1,2], Hillary Graves[1,2], Yu-Hsin Chao[1,2], Sharayu Jangam[1,2], V. Hemanjani Bhavana[1], Xi Luo[1,2], Ning Liu[1,2], Hongling Pan[2], Hsiao-Tuan Chao[1,2,4,5], Pei-Tseng Lee[1,2], Shinya Yamamoto[1,2,3,5], Michael Wangler[1,2,3,4,5] 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX; 3) Program in Developmental Biology, Baylor College of Medicine, Houston, TX; 4) Department of Pediatrics, Baylor College of Medicine, Houston, TX; 5) Department of Neuroscience, BCM, Houston, TX.

Autism spectrum disorders (ASDs) are complex neurodevelopmental disorders primarily characterized by varying degrees of impaired social communication, repetitive and restricted behavior patterns and atypical responses to sensory stimuli. While the disease mechanism(s) underlying ASD remain unclear, there is strong support for a substantial genetic component. Currently, both disease heterogeneity and an expanding list of candidate genes have made assessing their functional significance challenging. In order to functionally annotate potential disease-causing variants, we are conducting a functional screen in flies to analyze ASD candidate genes from the Simon's Simplex collection (SSC). The SSC contains whole-exome sequencing data from 2,500 families with a proband with non-syndromic ASD. The majority of probands possess *de novo* missense variants, which we have categorized for study based on independent genomic data from non-Autism cohorts, potential gene expression in the fly CNS, and the availability of *Drosophila* reagents. Subsequently, we have selected 126 *Drosophila* Mi[MIC] lines with insertions between coding exons for conversion to loss-of-function T2A-GAL4 lines. We successfully converted 111 Mi[MIC] lines to T2A-GAL4 insertions. 49/111 of these T2A-GAL4 lines are homozygous lethal, and rescue experiments using human cDNA are currently underway. The remaining 62/111 T2A-GAL4 lines are currently being evaluated for alterations in stereotyped patterns of behavior. Together with the parallel over-expression based screen (see poster by Harnish et al.), our rescue based screen provides an assay system to assess the function of human variants linked to ASD and other diseases.

757 The influence of antioxidant SOD2 on autophagy in a *Drosophila* model for MJD/SCA3. *H. Ragoowansi*, J. Warrick University of Richmond, Richmond, VA.

Spinocerebellar Ataxia Type-3 (SCA3) is one of the most common dominantly inherited ataxias. It is caused by an expansion of the CAG repeat in the ataxin-3 protein. The extended polyglutamine sequence causes the diseased protein to form aggregates and leads to neuronal dysfunction and death. It has been suggested that antioxidants may be helpful in slowing disease progression because they remove damaging reactive oxygen species from the cells. However, data from our lab suggests that increased SOD2 antioxidant expression increases the disease progression and toxicity. We hypothesize this effect may be due to reduced autophagy. To test this hypothesis, we visualized Ataxin-3 and LC3 in a *Drosophila* model of SCA3 disease. We increased the levels of SOD2 antioxidants in the flies and compared them to flies with endogenous levels of SOD2. We found that the LC3, normally found in the membranes of autophagosomes, and Ataxin-3 proteins co-localize in nuclear aggregates. We found the co-localization of the two proteins to be enhanced in 1- and 7-day old flies with upregulated antioxidant levels. This finding may indicate a dysfunction in autophagosome formation and/or function. To further analyze autophagic function, we are looking at Ref2 (*Drosophila* homologue to mammalian p62) and Lamp2 presence and localization in flies with endogenous levels of antioxidants and increased levels of SOD2 antioxidant.

758 A screen to identify drugs that suppress seizures in Dup15q syndrome suggests serotonin pathway modulation may be therapeutic. Jungsoo Han[1], Tracy Peters[2], Avani Alapati[3], Glen Palmer[2], *Lawrence T. Reiter*[1,4,5] 1) Neurology, UTHSC, Memphis, TN; 2) Clinical Pharmacy, UTHSC, Memphis, TN; 3) Rhodes College; 4) Pediatrics, UTHSC, Memphis, TN; 5) Anatomy and Neurobiology, UTHSC, Memphis, TN.

Debilitating seizures that become pharmacoresistant to common anti-epileptic drugs (AED) are a hallmark of Dup15q syndrome, especially among individuals with isodicentric duplications of chromosome 15q11.2-q13.1. Recently, our lab generated a novel fly model of Dup15q that recapitulates this seizure phenotype through over-expression of *Dube3a* in glial cells where *UBE3A* is bi-allelically expressed in mammals, not neurons where expression is restricted to the maternal copy of the gene. To repurpose previously approved drugs and identify compounds that reveal mechanistic insight to seizures in Dup15q syndrome, we conducted a medium throughput screen of 1280 small molecules (95% previously approved for use in humans) from the Prestwick Chemical Library. We designed a variation of the fly bang sensitivity assay (BSA) to determine if any adult flies expressing Dube3a in glial cells (*repo>Dube3a*) could be rescued by treatment in the food with each compound (1 mM in DMSO). Flies of the genotype *repo*-GAL4/TM3,*Sb* were crossed to UAS-*Dube3a* homozygous

animals and the offspring raised entirely on food with 1 mM of drug in DMSO or 0.1% DMSO alone control food at 25°C. Crosses were screened 88 vials at a time. Adult flies were removed after 3 days and newly emerging animals were aged for 4 days prior to the BSA. Flies were mechanically stressed for 10s to induce seizures (vortexing) and then immediately sorted for seizure vs non-seizure using a custom rig that allows flies that are not seizing to be separated from seizing flies. These two groups were scored for genotype in order to identify and count *repo>Dube3a* animals in both the seizure and non-seizure groups. In the primary screen, we identified 13 potential drug candidates that can suppress seizures in *repo*>Dube3a flies. We have now done dilution curve secondary screening on 4 of these compounds and identified two compounds that suppress seizures in *repo>Dube3a* animals. Two compounds, serotonin and mirtazapine, are both involved in regulation of serotonin receptors. We are in the process of validating other serotonin agonists and antagonists as well as the remainder the primary screen compounds in dose response experiments. Identification of new AEDs specific for the Dup15q syndrome from previously FDA approved compound libraries may prove useful for treatment of individuals with Dup15q epilepsy in the very near future.

759 Transcriptomic and proteomic profiling of glial or neuronal *Dube3a* overexpression reveals common molecular changes in gliopathic epilepsy. *K. Hope*[1,2], D. Johnson[3], D. Lopez-Ferrer[4], D. Kakhniashvili[5], L. Reiter[1,6,7] 1) Neurology, University of Tennessee Health Science Center, Memphis, TN; 2) 2Integrated Biomedical Science Program, University of Tennessee Health Science Center, Memphis, TN; 3) Molecular Bioinformatics Core, University of Tennessee Health Science Center, Memphis, TN; 4) Thermo Fisher Scientific; 5) Proteomics and Metabolomics Core, University of Tennessee Health Science Center, Memphis, TN; 6) Department of Pediatrics, University of Tennessee Health Science Center, Memphis, TN; 7) Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, TN.

Epilepsy affects millions of individuals worldwide and 30-50% of these cases are treatment resistant. Duplication 15q syndrome (Dup15q) is caused by duplications of chromosome 15q11.2-q13.1 and results in a high rate of treatment resistant epilepsy. We recently generated a *Drosophila melanogaster*Dup15q model that recapitulates the seizure phenotype when *Dube3a* (the fly *UBE3A* homolog) is overexpressed in glial cells, but not neurons, implicating glia in Dup15q epilepsy. Here we investigated the differences between *Dube3a* overexpression in glia (*repo>Dube3a*) versus neurons (*elav>Dube3a*) using global transcriptome analysis through RNA-sequencing and global protein analysis through mass spectrometry of whole fly head extracts. We identified 851 transcripts differentially regulated in *repo>Dube3a* heads/brains, including an upregulation of glutathione S-transferase (GST) genes that occurred cell autonomously within glial cells which was not observed in *elav>Dube3a* flies. We reliably measured approximately 2,500 proteins at the peptide level by proteomics, most of which were also quantified at the transcript level by RNAseq. Combined transcriptomic and proteomic analysis revealed an enrichment of 21 synaptic transmission genes downregulated at both the transcript and the protein level in *repo>Dube3a*, including *Synapsin*, *Sap47*, and *Syx1a*, indicating synaptic proteins change in a cell non-autonomous manner in *repo>Dube3a* flies. In order to test if these molecular changes were specific to Dup15q or were common among other glial driven seizure types, we identified 6 additional glial-driven, bang-sensitive seizure lines. We found an upregulation of GSTs in 4 out of the 6 gliopathic seizure lines driven by *repo*-GAL4. These data suggest GST upregulation is common among gliopathic seizures and may ultimately provide insight for treating epilepsy.

760 Defective cortex glia and neuronal cell body interaction leads to photosensitive epilepsy. *G. Kunduri*[1], Daniel Turner-Evans[2], Yutaka Konya[3], Yoshihiro Izumi[3], Kunio Nagashima[4], Stephen Lockett[5], Joost Holthuis[6], Takeshi Bamba[3], Usha Acharya[7], Jairaj Acharya[1] 1) Cancer and Developmental Biology Laboratory, National Cancer Institute, Frederick, MD; 2) Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA 20147; 3) Department of Metabolomics, Kyushu University, Fukuoka, Japan; 4) Electron Microscopy Laboratory, National Cancer Institute, Frederick, MD 21702; 5) Optical Microscopy and Analysis Laboratory, Frederick National Laboratory for Cancer Research, Frederick, MD 21702; 6) Molecular Cell Biology Division, University of Osnabrüeck, Germany; 7) Department of Molecular, Cell and Cancer Biology, University of Massachusetts Medical School, Worcester, MA 01605.

Seizures induced by visual stimulation (photosensitive epilepsy; PSE) represent a common type of epilepsy in humans, but lack of experimental models has hindered efforts to delineate the molecular mechanisms that underlie PSE. Here, we show that failure of cortex glia to encapsulate neuronal cell bodies leads to PSE in *Drosophila*. The cortex glial membranes are severely compromised in Ceramide phosphoethanolamine synthase null mutants and fail to encapsulate the neuronal cell bodies in the *Drosophila* neuronal cortex. Human sphingomyelin synthase 1 that synthesizes ceramide phosphocholine (sphingomyelin) instead of ceramide phosphoethanolamine rescues cortex glial abnormalities and PSE, underscoring the evolutionarily conserved role of these lipids in glial membranes. Further, we show compromise in plasma membrane structure underlies the glial cell membrane collapse in *cpes* mutants and leads to PSE phenotype.

761 Identification of novel combinatorial drug therapies to treat tuberous sclerosis complex. *C.R. Baxter*, B.E. Housden Living Systems Institute, University Of Exeter, Exeter, Devon, GB.

Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder affecting 1 in 6,000 births. TSC is caused by a mutation of the TSC1 or TSC2 genes, which produce components of the TSC complex. The TSC complex is an upstream inhibitor of mTOR activity, a key regulator of cell growth. When TSC function is lost, high levels of dysregulated mTOR activity can lead to the formation of benign tumours in a wide range of tissues, resulting in a variety of symptoms, including, seizures, learning disabilities and brain, kidney, skin and lung tumours. Preliminary data demonstrates that synthetic lethal screening of wild-type and TSC mutant *Drosophila* cell lines can lead to the identification of potential therapeutic drug targets. Using this approach, we have already identified several clinically-approved drugs that cause selective reduction in viability of TSC cells compared to wild-type cells and may be suitable for repurposing to treat TSC. Following on from this, I am using variable dose analysis (VDA), a highly sensitive method for genetic interaction screens (GI screens), to identify combinations of drug targets that kill TSC cells more effectively and more specifically than any single drug used alone. I am focussing on identifying targets that can be inhibited using existing clinically-approved drugs so that my results will be rapidly transferrable to clinical use. I will present my results so far from these synergy screens as well as my future plans to characterise synergistic combinations of clinically-approved drugs for the treatment of TSC.

762 Screening for inhibitors of human oncogenic KRAS using a *Drosophila melanogaster* model. *S. Kairamkonda*[1], AB Chougule[1], A Lennek[2], P Liaw[2], S Yanofsky[2], W Garland[2], JH Thomas[1] 1) Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, Lubbock, TX; 2) Tosk, Inc., Sunnyvale, California.

Extrinsic cell proliferation signals are frequently mediated through RAS signaling. In mammals, enhanced activity of the RAS-RAF-MEK-MAPK pathway leads to uncontrolled cell proliferation and cancer. Approximately 30% of human cancers have *RAS* gain-of-function mutations and 85% of these mutations are found in the *KRAS* gene. The most common *KRAS* mutation found in human cancer patients is $KRAS^{G12V}$. We constructed a transgenic *Drosophila melanogaster* model of the human mutant $KRAS^{G12V}$ gene under *UAS* transcriptional control. KRAS was expressed in the wings to facilitate scoring of any abnormal phenotypes caused by expression of human mutant *KRAS*. Of the many wing drivers tested, the MS1096 *GAL4* driver line was chosen because its expression of $KRAS^{G12V}$ produces severely crumpled wings. We characterized this model and found that expression of $KRAS^{G12V}$ in the wing disc caused increased cell proliferation. Furthermore, the phenotype was suppressed by mutations in the genes of the endogenous Drosophila RAS-RAF-MEK-MAPK pathway. To test whether our model could be used for identifying potential drugs, we treated these flies with trametinib, an inhibitor of MEK. Trametinib treatment rescued the defects caused by human $KRAS^{G12V}$. We screened for small molecules that reversed the observed wing defects and found several effective compounds. These are now undergoing further analysis. We also developed another Drosophila model for the oncogenic human mutation $KRAS^{G12D}$, which also produced a crumpled wing phenotype.

However, trametinib treatment did not rescue this defect. To determine whether *KRAS*^{G12D} is acting through a signal transduction pathway other than the RAS-RAF-MEK-MAPK pathway, we are using RNAi-mediated knockdown and chemical inhibition experiments to test for a role of other signal transduction pathways. These studies are expected to further our understanding of the molecular mechanisms of mutation-specific functions of oncogenic *KRAS*, and aid in the discovery of new drugs that target specific *KRAS* mutations in human cancers.

763 Role of miR-133 in changing tissue microenvironment for tumorigenesis. *I. Datta*[1], Y.C. Huang[1], Y. Tamori[2], N. Liu[3], W.M. Deng[1] 1) Cell and Molecular Biology, Florida State Universitty, Tallahassee, FL; 2) National Institute of Genetics, Japan ; 3) Chinese Academy of Sciences, Shanghai, China.

Tissue microenvironment plays a crucial role in tumorigenesis. In the Drosophila wing disc, the tumor 'hotspot' for neoplastic Tumor Suppressor Gene (nTSG) mutations is the hinge area, and the pouch region is the tumor 'coldspot'. Pro-tumor cells in the pouch region undergo apoptosis and basal extrusion due to epithelial organization of the tissue and it forms the 'coldspot'. Endogenous growth-promoting JAK/STAT signaling is high in the hinge area and the epithelial organization drive the pro-tumor cells to undergo apical delamination into the lumen thus allowing tumor formation. We have identified microRNA-133 (miR-133) as a molecule which converts the 'cold spot' of pouch area to a tumor 'hot spot' where it induces tumorigenesis. Although miRNA-133 downregulates JAK/STAT, it however promotes JNK signaling in the wing disc to induce tumors in the wing disc pouch region. miR-133 targetome analysis lead to the discovery of 5 possible target genes. Of these 5 targets, Rim and Rau are likely responsible for the conversion of the tumor 'coldspot' to a 'hotspot', which is similar to miR-133 overexpression. This shows that microRNAs and their targets and regulatory pathways can affect tumorigenesis by changing the tissue microenvironment. This in vitro tissue model will help shed light on the newly emerging role of microRNA in tumor progression.

764 The tissue-intrinsic local microenvironment makes a decisive influence on tumor invasiveness. R. Kobayashi, Y. Fujita, *Y. Tamori* Institute for Genetic Medicine, Hokkaido University, Sapporo, Hokkaido, JP.

Transformed mutant cells evolve through a multistep process in which they become tumorigenic and invasive. Although a number of causative genetic backgrounds for malignant transformation have been discovered, the critical mechanism that makes a decisive influence on the invasiveness of tumor cells remains elusive. Here, we show through analysis of transformed cells mutant for conserved neoplastic tumor-suppressor genes (nTSGs) and oncogenic Ras (Ras^{V12}) in *Drosophila* wing imaginal disc epithelia that invasiveness of the tumor cells depends on the tissue-intrinsic local microenvironment.

We have previously shown that nTSG-deficient cells become tumorigenic specifically when they are localized at the folded hinge regions of wing imaginal discs. In these "tumor hotspots" where cells constitute robust structures on their basal side, nTSG-deficient cells deviate from the apical side of the epithelium, and initiate tumorigenic overgrowth by exploiting endogenous JAK/STAT signaling activity. Conversely, in the wing pouch area that we dubbed "tumor coldspots," nTSG-deficient cells are extruded toward the basal side and undergo apoptosis.

When Ras^{V12} is ectopically expressed in the nTSG-deficient cells (nTSG-Ras^{V12} clones), the hotspot-derived nTSG-Ras^{V12} clones showed tumorigenic overgrowth and formed ball-shaped benign tumor masses at the apical side of epithelial layer as nTSG-deficient cells without Ras^{V12} do. At the tumor coldspots, however, the basally extruded nTSG-Ras^{V12} clones survived and showed invasive behaviors including MMP1 upregulation and intense protrusions, indicating that invasiveness of the nTSG-Ras^{V12} clones differ between coldspots and hotspots despite their homogeneous genetic background. This study provides evidence that not only tumor initiation but also malignant progression are highly dependent on the tissue-intrinsic local microenvironments.

765 A Novel Role for MICOS Complex *CHCHD6* in Establishing Cardiac Structure and Function, with Possible Implications for Hypoplastic Left Heart Syndrome. *K. Birker*[1], R. Bodmer[1], G. Vogler[1], T. Olson[2], J. Theis[2] 1) Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA; 2) Mayo Clinic, Rochester, MN.

Hypoplastic left heart syndrome (HLHS) is a severe birth defect that accounts for up to 4% of congenital heart diseases (CHD). HLHS is thought to be a complex, multifactorial genetic disease, however, our ability to understand the genetic complexities and pathogenic mechanisms leading to this disease is limited due to a lack of identified associated genes (e.g. *NKX2-5*/*tinman*). Therefore, there is a great need to uncover additional genes that contribute to the molecular, cellular, and morphological processes underlying HLHS. A candidate gene list was generated by collaborators from the Mayo Clinic (Rochester, Minnesota) based on whole genome sequencing of a patient with sporadic HLHS and their unaffected parents and siblings. We use *Drosophila* as a cardiac model system to functionally test candidates. Heart function and structure are determined using high-speed video microscopy and immunohistochemical techniques upon spatially targeted gene knockdown (KD). Of the 10 candidate HLHS genes initially tested, cardiac-specific KD of coiled-coil-helix-coiled-coil-helix-domain-containing protein 6 (*CHCHD6*) in *Drosophila* results in drastically compromised heart contractility, indicative of a potentially critical role for CHCHD6 in the adult heart. Additionally, KD of *CHCHD6* results in altered filamentous Actin-based sarcomeric myofibrillar structure, as defined by poorly labelled sarcomeric Z-lines. CHCHD6, previously unknown to be associated with heart disease, is part of the MICOS complex in the inner mitochondrial membrane and functions to maintain cristae morphology and respiratory complex assembly. KD of other MICOS complex components, such as *Mitofilin/Mic60*, also show significantly reduced contractility, however sarcomeric filamentous Actin appears normal. Since HLHS is a congenital disease, we examined *CHCHD6* mutant embryos for cardiac-specific defects during heart morphogenesis and did not observe any overt cardiac defects. These results support the suitability of the *Drosophila* heart to functionally test candidate HLHS genes, including combinations. We are continuing to examine interactions with these and other emerging candidate genes to identify novel gene functions and pathways that are likely to contribute to HLHS. In the future, further validation of novel candidate genes, genetic interactions, and causal pathways can possibly lead to the targeted prevention of HLHS, post-natal risk of heart failure in HLHS, as well as other CHDs.

766 Multi-Model System Approach to Identifying Atrial Fibrillation Genes and Mechanisms. *J.N. Kezos*, M. Yu, A. Colas, K. Ocorr Development, Aging and Regeneration, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA.

Atrial fibrillation (AF) is the leading cause of heart failure and stroke in aging humans, but the genetic/molecular mechanisms remain unclear. Meta-analyses of GWAS studies have identified common variants as well as rare variants that increase AF susceptibility in the general population. This growing list of putative AF causing genes suggests that the underlying cause is multifactorial and involves networks of interacting genes. A barrier to understanding AF mechanisms is the lack of experimental platforms enabling rapid large-scale exploration of gene function in cardiac tissue. To overcome this we combine two unique model systems, the genetically tractable *Drosophila melanogaster* cardiac model and hPSC-atrial-like CMs (ACMs). We previously demonstrated age-related cardiac arrhythmia in wildtype flies and in flies with mutations in KCNQ and hERG/seizure voltage-dependent K^+ channels suggesting that cardiac arrhythmia phenotypes can develop in response to combined genetic and aging insults, as in humans. We take advantage of the short lifespan of *Drosophila* and the ability to manipulate genes specifically in the heart in order to examine gene networks in an aging-dependent manner. This system enables rapid characterization of cardiac-specific gene loss or gain of function at the whole organ level. As proof of principle we tested 20 genes that have been genetically linked to AF via cardiac-specific RNAi knockdown (KD). High-speed video recordings of hearts in semi-intact flies at different ages permitted quantification of various cardiac parameters including diastolic and systolic intervals and arrhythmia. KD of putative AF-related genes *Pnr*, *Ptx1*, and *Sh* lengthened systolic intervals whereas KD of *SclA* significantly shortened systolic intervals, suggesting increases and decreases, respectively, in the underlying action potentials. We also observed increased bouts of arrhythmic activity compared to hearts from similarly aged wildtype flies. These results paralleled action potential lengthening and shortening seen with KD of these same genes in ACMs. We have now initiated an experimental pipeline using RNAseq data from human atrial tissue to identify

genes linked to AF that will be tested in a high throughput manner in ACMs and in fruit flies, allowing us to identify potential gene-regulatory networks underlying AF pathogenesis.

767 Functional assessment of de novo missense variants linked to Autism Spectrum Disorders through an overexpression based screen in *Drosophila*. *J. Michael Harnish*[1], Samantha Deal[1], Paul Marcogliese[1], Jonathan Andrews[1], Hilary Graves[1], Sharayu Jangam[1], Venkata Shavana[1], Michael Wangler[1,2,3], Shinya Yamamoto[1,2,3] 1) Human and Molecular Genetics, Baylor College of Medicine, Houston, TX; 2) Developmental Biology, Baylor College of Medicine, Houston, TX; 3) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX.

Autism Spectrum Disorders (ASD) encompass a complex, increasingly prevalent spectrum of neurodevelopmental disorders. Numerous recent efforts have uncovered gene variants associated with ASD. A subset of *de novo* mutations unique to probands have been considered to participate in the pathogenesis of ASD. Although several compelling variants have been identified, we have yet to understand the functional consequence of most ASD associated rare variants. Functional consequences of missense mutations are difficult to predict, even with state-of-the-art bioinformatics tools, thus necessitating *in vivo* studies. Here we report an over-expression based screen in *Drosophila* to assess functional consequences of genetic variants linked to ASD. We prioritized 132 *de novo* missense variants in 92 genes from the Simons Simplex Collection, a cohort of over 2,500 simplex families, for functional studies. We generated UAS-human cDNA transgenic lines containing reference or ASD-linked variant alleles and ectopically expressed them using ubiquitous (Tubulin-), wing (nubbin-) and eye (GMR-) GAL4 drivers. Upon over-expression of reference cDNA we observed scorable phenotypes for 36 genes (39%). We observed a difference between reference and variant transgenic lines in 23 cases examined so far. Variants that exhibit a weaker phenotype than the reference cDNA are likely loss-of-function alleles (amorph, hypomorph), whereas variants with stronger phenotypes are likely gain-of-function alleles (hypermorph, antimorph, neomorph). Interestingly, some hits were linked to genes that have been linked to Mendelian disorders such as *Pyruvate Carboxylase* (*PC*) that causes Leigh necrotizing encephalitis, suggesting that different alleles may present as distinct diseases. Together with parallel efforts using UAS-human cDNA transgenes in rescue experiments (see poster by Andrews et al.), our over-expression based screen provides a high-throughput assay to assess the function of hundreds of variants linked to ASD and other diseases.

768 Modeling a premature aging syndrome caused by a mutant form of Barrier-to-Autointegration Factor (BAF) in Drosophila. *S.Cole. Kitzman*, Tyler Weaver, Rebecca Cupp, Tingting Duan, Catherine Musselman, Pamela Geyer Biochemistry, University of Iowa, Iowa City, IA.

The nuclear lamina is a protein meshwork that lies beneath the nuclear envelope and contributes to transcription, DNA replication and genome integrity. Dysfunction of proteins embedded in nuclear lamina causes many human diseases, including premature aging or progeroid syndromes. Recently, an atypical progeria syndrome was identified, called Néstor-Guillermo Progeria Syndrome, wherein patients develop hair loss, thin skin, bone loss and stiff joints, but do not develop cardiovascular or metabolic disease. This syndrome is caused by a mutation in the human *BANF*1 gene, a gene encoding a conserved chromatin protein that binds DNA, the nuclear lamina LEM-Domain (LEM-D) proteins, lamins, and histones. In Néstor-Guillermo Progeria Syndrome, mutant *BANF1* generates an Ala12Thr substituted Barrier-to-autointegration Factor (BAF). We are using Drosophila BAF to understand the molecular pathogenesis of this early aging syndrome. Drosophila BAF is 65% identical to that of human BAF in amino acid sequence, including conservation of Ala at position 12. First, we are using bacterially purified wild-type and mutant BAF proteins to test for effects on DNA binding and their interactions with LEM-D proteins, lamins, and histones. Second, we used CRISPR to engineer the progeroid mutation into the endogenous *baf* gene. We are currently defining *in vivo* effects of this mutation on development. Together, our experiments will provide insight into how altered BAF function contributes to early aging phenotypes.

769 The structural and functional analysis of the Orc6 protein – *Drosophila* model of the Meyer-Gorlin syndrome. *M. Balasov*[1], K. Akhmetova[1], G. Zhu[2], I. Chesnokov[1] 1) Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL; 2) Division of Life Science, Hong Kong University of Science and Technology, Hong Kong, China.

The origin recognition complex (ORC) is important for DNA replication in eukaryotic cells. ORC is also involved in other cell functions. The smallest ORC subunit, Orc6, has an active role in both DNA replication and cytokinesis. Orc6 in *Drosophila* and human has a DNA binding activity on its own and is important for DNA binding of whole six-subunit ORC. We solved the structure of Orc6 alone and in a complex with DNA. These structural and subsequent functional analyses revealed that Orc6 in eukaryotes has a homology with transcription factor TFIIB. We propose that Orc6 may position the whole complex at the origins of DNA replication similar to the role of TFIIB in positioning transcription pre-initiation complex at the promoter.

The cytokinesis function of *Drosophila* Orc6 is achieved through its interaction with the septin protein Pnut, which together with Sep1 and Sep2 forms a septin complex. Septins are weak GTPases that can assemble into large filaments. The binding of Orc6 to Pnut stimulates septin complex filament formation. Mutational analysis revealed that both N-terminal and C-terminal domains of Orc6 are important for this activity of the protein.

The C-terminus of Orc6 contains a highly conserved motif important for the interaction of the protein with the core ORC. A mutation found in patients with Meier-Gorlin syndrome (MGS) maps to the same segment in Orc6, destabilizing the interaction of *Drosophila* and human Orc6 with the core Orc1-5 subcomplex. An additional new mutation in N-terminus of Orc6 was recently identified in some patients with Meier-Gorlin syndrome. In order to study the effects of MGS mutations in animal model system we introduced N- and C-terminal MGS mutations in Orc6 and established *Drosophila* models of Meier-Gorlin syndrome. Obtained flies die at third instar with abnormal chromosomes and DNA replication defects, however, the lethality can be rescued by elevated expression of mutant Orc6 protein. Rescued MGS flies are unable to fly and display planar cell polarity defects. To probe the effect of specific mutations in human Orc6 directly in live animal we have created live *Drosophila* model where *Drosophila orc6* is replaced with the human gene.

770 The RNA export factor, Nxt1, is required for maintenance of muscle integrity, and for normal expression of mRNAs of genes that also generate circular RNAs. *Helen White-Cooper*, Kevin van der Graaf School of Biosciences, Cardiff University, Cardiff, GB.

The RNA export pathway is essential for export-competent mRNAs to pass from the nucleus into the cytoplasm, and thus is essential for protein production and normal function of cells. *Drosophila* with partial loss of function of *Nxt1*, a core factor in the pathway, show reduced viability and male and female sterility. The male sterility has previously been shown to be caused by defects in testis-specific gene expression, particularly of genes without introns. Here we describe a specific defect in growth and maintenance of the larval mucles, leading to muscle degeneration in *Nxt1* mutants. RNAseq revealed reduced expression of mRNAs of many genes in *Nxt1* mutant muscles. Genes under-expressed in the mutant typically have long introns, and most normally encode circular RNAs in addition to mRNAs. Circular RNAs are alternative products made from the same pre-RNA as mRNAs. They are common, stable, and have a variety of functions. Our data on *Nxt1* links the mRNA export pathway to a global role in mRNA expression of genes that also produce circular RNAs.

Muscular atrophy diseases in humans have been linked to RNA metabolism, particularly splicing, however this is the first report of a specific role for the RNA export pathway in maintenance of muscle integrity. Intriguingly, despite the widespread defect in gene expression, the muscle degeneration in *Nxt1* mutant larvae was rescued by increased expression of a single gene, the costamere component *tn (abba),* in muscles. *tn* is the *Drosophila* homologue of human *Trim32*, mutations of which cause limb girdle muscular dystrophy 2H. It will be very interesting to further examine the role of *Nxt1* in production of circular RNAs, and the role of circRNAs in muscular dystrophies.

771 Altered metabolism in Trim32 deficient muscle. *S. Bawa*[1], DS. Brooks[1], BV. Geisbrecht[1], JM. Tennessen[2], ER. Geisbrecht[1] 1) Biochemistry and Molecular Biophysics, Kansas State University, Manhattan, KS; 2) Department of Biology, Indiana University, Bloomington, Indiana 47405.

TRIM32 is a member of tripartite motif (TRIM) protein family involved in multiple biological processes, including differentiation, muscle physiology and regeneration, and tumor suppression. Mutations in two different domains of the ubiquitously expressed TRIM32 protein give rise to two clinically separate diseases, one of which is Limb-girdle muscular dystrophy type 2H (LGMD2H). Uncovering the muscle-specific role of TRIM32 in LGMD2H pathogenesis has proven difficult as neurogenic phenotypes, independent of LGMD2H pathology, are present in *TRIM32 KO* mice. We previously established a platform to study LGMD2H pathogenesis using *Drosophila melanogaster* as a model. Loss of *Drosophila* TRIM32, encoded by the *thin (tn)* gene, recapitulates clinical features observed in LGMD2H patients, including impaired locomotion and muscle degeneration. Here we show that LGMD2H-causing mutations in the NHL domain are molecularly and structurally conserved between fly and mammalian TRIM32. Using a proteomics approach to identify proteins that physically interact with this NHL domain, we find that TRIM32 binds to the Aldolase (Ald) and Phosphoglycerate mutase (Pglym). Strikingly, these glycolytic proteins are also found at the Z-disk, consistent with dTRIM32 localization. Loss of TRIM32 reduces glycolytic enzyme levels and protein localization at their sites of action. Metabolomics analysis reveals a reduction in pyruvic acid and lactic acid, indicative of a decrease in glycolytic flux. Consistent with a role for glycolytic intermediates in glycolysis-driven biomass production, nutritional supplementation of amino acids in *tn-/-* mutants restores muscle mass. Here we have uncovered a novel mechanism underlying muscle disease pathogenesis that may have broader relevance to other disease states.

772 BAG-3/Starvin and its role in muscle cell proteostasis. *F. Naeem*, D. Brooks, M. Stetsiv, E. Geisbrecht Biochemistry and Molecular Biophysics, Kansas State University, Manhattan, KS.

Proteostasis is important for maintenance and homeostasis of the cellular proteome in normal and stressed cells such as muscles and neurons. When this system fails, protein misfolding and/or aggregation can result in neurodegenerative diseases and protein aggregate myopathies. *Drosophila starvin (stv)* encodes for the co-chaperone BAG-3 protein, a key player in multiple biological processes, including development, apoptosis, cytoskeletal organization, and autophagy. In a specific type of selective macroautophagy, BAG-3 mediates the transport and sequestration of client proteins, one example of which is Filamin (Fil). Tension exerted by contractile muscle tissue requires continuous folding and refolding of individual Ig-like domains in Fil, eventually damaging the ability of the protein to sense and transmit mechanical strain. BAG-3 is instrumental in recycling Fil and likely other misfolded muscle proteins. Our analysis of *stv* mutant alleles in addition to RNAi approaches have uncovered novel muscle degeneration phenotypes. Moreover, we have developed a sensitized genetic background approach assay to uncover other genes that function with *stv* to maintain normal muscle structure and function with the long-term goal of understanding additional genes that contribute to BAG3-mediated human myopathies.

773 Transcriptomic Profiling of High Sugar-Induced Obesity resistant and susceptible Drosophila Genetics Reference Panel Lines. *Sumit Patel*[1], Urska Cvek[2], Phillip Kilgore[2], Matthew Talbert[1] 1) Biology, University Of Louisiana Monroe, Monroe, LA; 2) Health Center, Louisiana State University, Shreveport, LA.

From our previous Genome Wide Association Study (GWAS) of obesity-induced mortality on a high sugar diet (HSD) utilizing the Drosophila Reference Panel (DGRP), there was a substantial variation of lifespan among the 193 DGRP lines. Utilizing 3 obesity resistant: DGRP 721, 821 and 913, and susceptible: DGRP 100, 832 and 911 lines from the top 10% of the long lived and short lived of the 193 lines on the obesogenic diet, we were able to perform whole-body RNA sequencing of the 6 lines (3 replicates each) after 7-day exposure to a normal diet and the HSD. Differential expression analysis was performed between the normal diet and HSD for each of the 6 lines to identify any significant differences in the gene expression patterns. The differentially expressed genes with FDR-adjusted $p<0.05$ from each genotype were submitted to DAVID (Database for Annotation, Visualization and Integrated Discovery) to group genes into annotation clusters according to similar function, relatedness and nature. High sugar-susceptible lines displayed an overrepresentation of differentially expressed genes involved in processes such as antimicrobial function, ion channel transport and biosynthesis of antibiotics. High sugar-resistant lines indicated an overrepresentation of differentially expressed genes associated with CHK, transcription, DNA replication and synaptic vesicle secretion/the cell junction. Further analysis is pending, and physiologically relevant genes will be identified that deserve transgenic validation in the future.

774 Impacts on lifespan and energy homeostasis for genes identified by genome-wide association study of High Sugar Diet mortality in *Drosophila melanogaster*. *Sumit. Patel*, Matthew Talbert Biology, University Of Louisiana Monroe, Monroe, LA.

From our previous Genome Wide Association Study (GWAS) of obesity-induced mortality on a high sugar diet (HSD) utilizing the Drosophila Reference Panel (DGRP), there was variation of lifespan among the 193 DGRP lines. HSD was by provided by using sucrose in a disproportionate amount to yeast in their solid diet, providing it in a 5:1 w/v ratio in our case. The DGRP analysis pipeline determined associations between polymorphisms and lifespan on a HSD. The most significant lifespan associated polymorphisms with p-values ranging from $1.7 X10^{-8}$ to $1.0X10^{-5}$[M1] were within loci of genes involved in: neural processes, behavior, development, and apoptosis, among other functions. In the present study, we utilized the GAL4-UAS system to perform a whole-body RNAi knockdown of the physiological relevant genes, *rdgA, olf413, caps, axo and mub,* that had the highest number of polymorphisms associated with lifespan in our GWAS. We evaluated the impact of knockdown on lifespan and energy homeostasis upon HSD and normal diet. Overall, the trend was for lifespan of knockdowns to be reduced on HSD compared to the control lines. There was an increased triglyceride storage level and starvation resistance across the knockdowns when exposed to HSD. Interestingly, lifespan was not consistently reduced by HSD relative to normal diet, but triglyceride content was always increased and feeding quantity was always decreased. This may suggest that this diet formula is inducing fat storage and feeding quantity change in a way that varies by genotype, perhaps as a set of protective mechanisms, and through this modulating lifespan.

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775 Tumor-induced systemic inflammation dirupts blood-brain barrier leading to premature death of hosts. *J. Kim*, D. Bilder University of California-Berkeley, Berkeley, CA.

Whereas malfunction of local organs through direct contact with tumors is a well-characterized cause of host death, how systemically secreted tumor-derived factors impact host lifespan is still unclear. To investigate this, we transplanted malignant epithelial tumors into the abdomens of adult Drosophila hosts. Tumor-bearing hosts showed widespread activation of Jak/STAT signaling, and exhibited very premature death. Jak/STAT activity was particularly high in the glial cells of the brain, which comprised the blood-brain barrier (BBB). Strikingly, tumor-bearing hosts displayed disruption of BBB function. We found that Jak/STAT signaling in the BBB was necessary and sufficient for the leaky BBB and lifespan reduction. Further, knocking down the sole Jak/STAT signaling receptor in the BBB rescued BBB function and extended the lifespan of tumor-bearing hosts. Therefore, BBB dysfunction is a novel phenotype caused by tumor-induced Jak/STAT activation that leads to the premature death of hosts. Understanding this mechanism might provide a hint for new cancer theapies.

776 Cigarette smoke exposure selectively affects survival of male *Drosophila* larvae. *K. Sirocko*[1], K. Uliczka[1], H. Angstmann[1], S. Papenmeier[1], S. Bartel[2], B. Hammer[2], S. Krauss-Etschmann[2] 1) Division of Invertebrate Models, Priority Research Area Asthma & Allergy, Research Center Borstel, Germany; 2) Division of Early Life Origins of Chronic Lung Diseases, Priority Research Area Asthma & Allergy, Research Center Borstel, Germany, Airway Research Center North

(ARCN), Member of the German Center for Lung Research, Germany.

Introduction/Background

Prenatal cigarette smoke exposure (CSE) is a major risk factor for later development of asthma. Moreover, epidemiological studies indicate that an enhanced risk of asthma may be propagated across generations. The fruit fly *Drosophila melanogaster* has already been successfully used to unravel molecular mechanisms underlying abnormalities of airway epithelial structure and function related to asthma, thereby emerging as a useful model to study conserved mechanisms of transgenerational epigenetic inheritance.

Aims/Objective

In the present project, we aim at establishing a transgenerational *Drosophila* smoking model to identify highly conserved mechanisms mediating parental smoke-induced alterations in airway epithelial cells.

Methods

Wild type larvae were exposed to mainstream cigarette smoke (CS) generated by a smoking robot. Cotinine levels were assessed in whole larvae by ELISA. The *Cyp18a1* expression (homologue to human Cyp1a1) was measured both in whole larvae and isolated airways by qPCR. RNA-Seq was performed on isolated airways from CS-exposed larvae. Enriched biological themes were identified using the DAVID software. Larval viability was determined by counting numbers of living larvae, pupae, and emerging adults. The uptake of CS into the airways was monitored by using the GFP reporter strain hsp70-GFP.

Results

While cotinine levels remained unchanged in whole larvae, *Cyp18a1* expression was significantly increased in the same specimen up to 60 minutes after CSE. In isolated airways, *Cyp18a1* expression was increased 3-fold upon CSE in both sexes. Fluorescent images of hsp70-GFP-larvae demonstrated that CS enters the airways through the posterior breathing openings. Transcriptome analysis revealed that genes predominately involved in cytochrome P_{450} metabolism (e. g. *Cyp6a2*, *Cyp307a2*), glutathione metabolism (e.g. *GstD4*, *GstD5*, *GstD8*), and oxidative stress response (e. g. *hsp70*, *hsp40*, *hsp27*) were strongly upregulated in both sexes at a similar level. However, mortality rate only of male but not female CS-exposed larvae was significantly enhanced.

Conclusion

We established a juvenile *Drosophila* smoking model which is characterized by a sex difference in sensitivity to CSE. In prospective studies, we are interested in identifying developmental and immunity-related pathways mediating inter- and transgenerational changes of CSE.

777 Using mitochondrially-targeted restriction enzymes to study the response to mitochondrial DNA double strand breaks in Drosophila muscle. *A.N. Spierer*, D.M. Rand Ecology and Evolutionary Biology, Brown University, Providence, RI.

The mitochondrion is responsible for key cellular functions (e.g. aerobic metabolism, nutrient sensing, and apoptosis, among many others). For these functions and others to occur, mito-nuclear coordination is required between all 37 mitochondrial genes and ~1200 nuclear gene products. Damage to nearly all these genes in the mitochondrial (mtDNA) or nuclear (nDNA) genomes can negatively impact mitochondrial function, underscoring the importance of studying how the cell maintains the integrity of both genomes.

While the mechanisms surrounding maintenance of nDNA integrity are relatively well known, those surrounding mtDNA are poorly understood. MtDNAs are constantly exposed to damaging byproducts of aerobic respiration called reactive oxygen species (ROS). These molecules are a major cause of oxidative damage and are established to cause mtDNA double strand breaks (mtDSB). When a circular mtDNA molecule incurs an mtDSB, it is typically degraded. Repair can occur, but it does so with a higher mutation or deletion rate than nDNA. Mitochondrial dysfunction can occur when the fraction of healthy or intact mtDNAs drops below a critical threshold within a cell. Despite the known dangers and implications of mtDSBs, the mtDSB response and how the cell maintains mtDNA integrity are poorly studied compared to that of the nuclear genome.

Accordingly, we developed a Drosophila model to study how muscle cells maintain mtDNA integrity by creating mtDSBs. We tested several muscle-specific Gal4 lines driving the expression of a restriction enzyme (MitoRE) targeted to the mitochondrion. Our expression of the MitoRE allows us to mimic the induction of mtDSBs, while controlling for the tissue and break site.

Our findings suggest that the MitoRE controlled by localized vs. systemic muscle-Gal4 drivers can have differential effects on whole organism traits (lifespan, climbing performance, and flight performance). We also observed differences in OXPHOS complex activity and mtDNA:nDNA ratios between control (Gal4) and experimental (Gal4-UAS) flies.

We are also conducting a modifier screen using the Drosophila Genetics Reference Panel (DGRP) lines. Our aim is to identify nuclear loci that enhance or suppress the impact of mtDSBs using the MitoRE system. Initial analyses pairing our Gal4-UAS system with 17 DGRP lines suggest that there will likely be significant genetic variation to identify candidate loci. As we expand to the entire DGRP collection, our end goal is to identify and validate candidate loci that rescue the effects of mtDSBs and help maintain mtDNA integrity.

778 Examining The Role of L-2HG in Promoting Renal Cancer in a Drosophila Model. *Yasaman Heidarian*, Jason Tennessen Biology, Indiana University, Bloomington, IN.

L-2-hydroxyglutarate (L-2HG) is an oncometabolite that accumulates in Clear Cell Renal Cell Carcinoma (CCRCC), the most common form of renal cancer. In vitro studies have shown that L-2HG promotes the proliferation, migration and invasion of CCRCC cells, however, the molecular mechanisms by which L-2HG contributes to renal cancer remains poorly understood. To address this deficiency, we have mutated the *Drosophila* homolog of L-2HG dehydrogenase (*L2HGDH*), which encodes the enzyme responsible for degrading L-2HG and is commonly mutated in CCRCC tumors. Metabolomic characterization of *L2HGDH* mutant adults revealed significant elevation of L-2HG, lactate, and several other metabolites that are aberrantly regulated in mammalian cells lacking L2HGDH. Examination of Malpighian Tubules in *L2HGDH* mutants, however, failed to uncover aberrant cellular growth in renal cells. Intriguingly, whole body mouse *L2HGDH* mutants also do not develop renal tumors, thus demonstrating that L-2HG interacts with other oncogenic pathways to induce tumor formation in kidney. We are now examining potential genetic interactions between *L2HGDH* mutations and candidate tumor suppressors and oncogenes within the fly renal system.

779 Fly pharmacology: Smac mimicry in a novel polycystic kidney disease model. *C. Millet-Boureima*[1], R. Chingle[2], W.D. Lubell[2], C. Gamberi[1] 1) Concordia University, Montreal, CA; 2) University of Montreal, Montreal, CA.

Polycystic Kidney Disease (PKD) is an incurable genetic disorder affecting 12.5 million people around the world. PKD causes cystic degeneration of the kidneys for which there are no effective drugs due in part to incomplete mechanistic knowledge of the cystic process. Dialysis and kidney transplants are currently the only available treatments, for half of the patients who reach kidney failure.

Mutations in the *Bicaudal C (BicC)* gene cause renal cysts in vertebrates and flies alike.

Second mitochondrial activator of caspases (Smac)-mimics can induce apoptosis in cancer cells. Smac-mimics were previously found to curb cystic cell proliferation in a rat model of PKD. Smac-mimics were synthesized and previously shown to induce cell-death in cultured breast cancer cells. In a first-in-kind cystic fly model of PKD, we have now found that administration of these novel Smac-mimics rescued the fly cystic phenotype indicating a similar mechanism of renal cystic degeneration in flies and vertebrates.

780 Candidate therapeutics for N-glycanase 1 deficiency identified through small molecule screens in multiple model organisms. *J. Mast*, M. Prangley, T. Portillo Rodriguez, E. Perlstein Perlara PBC, South San Francisco, CA.

Perlara PBC, is a public benefit company committed to discovering small molecule therapeutics for rare genetic diseases using genetic model organisms such as flies, worm, and yeast in high-throughput drug discovery screens. This parallel, multi-model whole animal screening approach leverages shared evolutionary pathways and increases the probability of rapidly identifying potential therapeutics. We have worked closely with Grace Science Foundation to find such a therapeutic for N-glycanase 1 (NGLY1) deficiency. NGLY-1 deficiency is a congenital disorder of glycosylation caused by mutations in NGLY1 that lead to developmental delay, movement disorders, hypotonia, and peripheral neuropathy in patients. Similarly, flies homozygous mutant for the ortholog of NGLY1 (*Pngl*) are developmentally delayed during larval stages, exhibit partial pupal lethality, and are small as adults. Flies heterozygous for *Pngl* are more sensitive to bortezomib, a proteasome inhibitor, than control larvae – also leading to a delay in larval growth. To find chemical modifiers of *Pngl*, we conducted a bortezomib-sensitized small molecule screen for compounds effecting the larval size phenotype in these heterozygous flies. We screened two chemical libraries – first, a smaller 2560 compound repurposing library comprised of FDA approved drugs, compounds that have reached clinical trial stages, drugs available in the EU or Japan, natural products, and other compounds, and second, a 20,000 compound library of novel material. Across these two libraries, we identified 46 compounds that significantly rescued the *Pngl* heterozygote larval size phenotype. Critically, 33 of these molecules also rescued larval size defects in *Pngl* homozygotes. We cross-validated our "hit" molecules and compounds identified from a parallel *C. elegans* screen, finding 12 compounds that ameliorate NGLY1-deficiency phenotypes in both of these models. These compounds are likely affecting conserved pathways and are prime candidates for developing therapeutics for NGLY1-deficiency patients

781 A platform for high-throughput drug discovery in flies. *M. Prangley*, T. Portillo Rodriguez, J. Mast, E. Perlstein Perlara PBC, South San Francisco, CA.

Perlara PBC (a Public Benefits Corporation) was established with the goal of discovering small molecule therapies for rare genetic diseases. To accomplish this, we engineer models (often with specific patient alleles) of these disorders in flies, worms and yeast, and identify phenotypes amenable for high-throughput screening. We have developed an automated platform for whole-fly screening of large chemical libraries in 96-well plates. For our current projects, we screen two chemical libraries – first, a smaller 2560 compound library comprised of FDA approved drugs, compounds that have reached clinical trial stages, drugs available in the EU or Japan, natural products, and other compounds for the purpose of rapidly repurposing compounds for patients, and second, a 20,000 compound library of novel material to identify innovative treatments. We dispensed these chemical libraries into 96-well plates using a Labcyte Echo liquid handling device that uses acoustic vibrations to dispense precise minute liquid volumes. These drugs are then mixed in-well with fly media. Larvae or embryos either homozygous and heterozygous for our gene of interest are rapidly sorted and using fluorescent balancer chromosomes and dispensed into wells using a large-particle Biosorter. We have also developed multiple image-based high-throughput endpoint assays for screening; measuring larval size, larval lethality, delayed time to pupation, and pupal lethality, and adult activity. More nuanced phenotypes such as eye roughness, larval locomotor behavior, shock sensitivity, pigmentation, and others can be used as verification for smaller subsets of promising compounds.

782 Application of *Drosophila* cell-based CRISPR technologies to studying the NGLY1 rare disease gene ortholog *Pngl*. *R. Viswanatha*[1], R. Brathwaite[1], S. Knight[1], G. Amador[1], J. Zirin[1], N. Perrimon[1,2], C. Chow[3], S.E. Mohr[1] 1) Genetics, Harvard Medical School, Boston, MA; 2) Howard Hughes Medical Institute; 3) University of Utah.

CRISPR pooled-format cell screening facilitates relatively rapid identification of genes required for growth of a given cell line or resistance to a toxic treatment. In this study, we are using CRISPR pooled format screen technology in Drosophila cells to uncover biological activities relevant to the rare human disease NGLY1. NGLY1 is a cytoplasmic deglysoylation enzyme involved in endoplasmic reticulum (ER)-associated degradation (ERAD), a process critical to clearing the ER of misfolded proteins during ER stress. We are taking two specific approaches. First, we are developing knockout cell lines that will be used in a synthetic lethality screen, with the goal of identifying genetic interactors with Pngl, the Drosophila ortholog of human NGLY1. Second, we are screening a panel of ER stress-inducing toxins such as tunicamycin to identify Drosophila genes that when knocked out, confer resistance to one or more of these ER toxins. Once candidates have been identified using the cell-based approach, they will be subjected to in vivo analyses including to test interactions with NGLY1 deficiency model flies. Through this work, we hope to deepen our understanding of NGLY1 and ER functions. This information has the potential to uncover potential therapeutic strategies for treatment of NGLY1.

783 Survival Motor Neuron (SMN) regulates immune system and fat body function by regulating Traf6, NF-kB, and Tor activity. *Ashlyn M. Spring*[1,6], Amanda C. Raimer[2,3], Dominique Brown[4], Helen Dinh[5], Tori Rosen[6], Jennifer Zepeda-Martinez[4], Sarah Gill[6], A. Gregory Matera[2,3,4,5,6] 1) SPIRE Postdoctoral Fellowship Program; 2) Integrative Program in Biological and Genome Sciences; 3) Curriculum in Genetics and Molecular Biology; 4) SPIRE Summer Undergraduate Research Experience; 5) SURE-Research Experience for Undergraduates; 6) Department of Biology, University of North Carolina, Chapel Hill.

Spinal muscular atrophy is a common neurodegenerative disorder that primarily impacts motor neurons in young children and adults. SMA is caused by strongly reduced levels of Survival Motor Neuron (SMN) protein, which is encoded by two genes in humans (*SMN1* and *SMN2*) and by a single homolog, *Smn*, in fly. SMN is ubiquitously expressed and canonically functions in the assembly of small nucleolar ribonucleoproteins (snRNPs) that carry out mRNA splicing. Additionally, SMN has been implicated in assembly and regulation of other RNP complexes and is linked to translational regulation, RNA trafficking, endocytosis, autophagy, cytoskeletal regulation, and cellular signaling. We employ two types of SMA modes in the fly by reducing SMN levels or function through 1) RNAi-mediated *Smn* knockdown or 2) expression of one of fourteen transgenic point mutant *Smn* constructs in an otherwise *Smn* null background. In both cases, we can recapitulate classical phenotypes of SMA including reduced lifespan and impaired locomotor function. These SMA models also display signs of aberrant immune activation and fat body dysfunction. Larvae for all of our transgenic SMA models develop melanotic masses at highly elevated levels and undergo developmental delay and arrest. Tissue specific *Smn* knockdown reveals separable, tissue-specific functions for *Smn* in both the immune system and fat body. An ongoing candidate-based enhancer/suppressor screen indicates that SMN is mediating these phenotypes through inhibition of the UBA1/Ben/Traf6 ubiquitylation complex and its downstream targets in the NF-kB and TOR pathways. These findings provide the first information about the cellular and molecular functions of SMN in immune, metabolic, and endocrine systems.

784 Serpin *scca1* dysregulation in *Drosophila* airways enhances their vulnerability to asthma risk factors. *H. Angstmann*[1], K. Uliczka[1], T. Roeder[2], S. Krauss-Etschmann[3], C. Wagner[1] 1) Division of Invertebrate Models, Priority Research Area Asthma & Allergy, Research Center Borstel, Germany; 2) Molecular Physiology, Institute of Zoology, Christian-Albrechts-University of Kiel, Germany; 3) Division of Early Life Origins of CLD, Priority Research Area Asthma & Allergy, Research Center Borstel, Germany.

Bronchial asthma is a chronic inflammatory airway disease caused by a complex interaction between an individual's genetic make-up and the environment. Along this line, genetic studies have shown that variants in the **ser**ine **p**eptidase **in**hibitor gene (serpin) *scca1* are associated with asthma. However, the

functional role of these variants (GV) is unknown.
Therefore, we aim at deciphering the pathophysiological role of *scca1* GV in asthma. To achieve this, we are exploring whether *scca1* GV increase the susceptibility towards common risk factors (hypoxia, tobacco smoke) and whether they affect airway epithelial barrier functions (structure, integrity and immune defence).
The fly's orthologous of *scca1* have been identified based on amino acid similarity and the presence of one serine protease inhibitor domain. By using the Gal4/UAS system, fly models (overexpression/RNAi models) dysexpressing *scca1* in the airway epithelium were successfully established. To figure out if *scca1* dysregulation affects airway morphology, number and length of secondary branches in 1st and 3rd instar larvae were assessed. 3rd instar larvae were then exposed to hypoxia (HE: 5% O_2, 2h). Larval crawling activity (a measure for hypoxic stress) was determined every ten minutes during hypoxia. Developmental period and mortality were assessed by counting the number of pupae and adult flies at different time points after HE.
Airway-specific overexpression of *scca1* led to an increased number of secondary branches whereas its downregulation caused an increase in their length. Moreover, dysexpression of *scca1* resulted in a high vulnerability towards hypoxic stress within the first 30 minutes of exposure. Additionally, an exposure to acute hypoxia significantly increased adult but not pupal mortality of animals with reduced *scca1* expression. While *scca1* dysexpressing animals generally showed a developmental delay of pupae as well as adults, additional exposure to hypoxia does not affect the developmental period of both stages.
In summary, we developed a *Drosophila* model for *scca1,* which is characterised by an altered airway structure. These airway alterations could be responsible for an impaired adaptability towards environmental stressors. Upcoming experiments will cover a more detailed analysis of molecular mechanisms underlying pathophysiological alterations of the airways due to *scca1* dysregulation.

785 Exploring Parallels in the Mechanisms of Hearing and Deafness Between *Drosophila* and Mammals. *Daniel Sutton*[1], Jonathan Andrews[2], Andrew Groves[1], Shinya Yamamoto[1,2] 1) Baylor College of Medicine, Houston, TX; 2) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX.

The auditory system of the fly, Johnston's organ (JO), is located within the second segment of each antenna and is comprised of ~200 stretch receptor units called scolopidia. These scolopidia contain mechanosensitive neurons that respond to gravity and sound from vibrations of the outermost antennal segment. Although JO is morphologically distinct from the mammalian cochlea, orthologs of some human deafness genes play conserved functions. While many studies have attempted to draw parallels between vertebrate and fly hearing mechanisms, comprehensive study in *Drosophila* exploring the expression pattern and functions of orthologs of vertebrate deafness genes has not been pursued.

We first used DIOPT (DRSC Integrative Ortholog Prediction Tool) to generate a comprehensive list of fly genes that are orthologous to human and mouse deafness genes, and identified genetic tools to assess the expression pattern of these genes and proteins within JO. We then explored whether these genes were expressed in specific cell types within JO using T2A-GAL4 and UAS-GFP lines, and tested whether certain proteins are enriched in specific structures within the scolopidia in pupal JO. In addition to validating previous findings that Myosin VIIa, Non-muscle myosin II, and Protocadherin-15 orthologs are expressed in JO, many other conserved deafness associated genes or proteins are also expressed. Interestingly, orthologs of Usher syndrome proteins Cadherin-23 and Whirlin were also expressed in JO and partially colocalize with the Myosin VIIa and Protocadherin-15 orthologs, suggesting a potential functional link. The finding that many Usher syndrome proteins are co-expressed within JO suggests a molecular conservation between the Usher complex in humans and flies. The extent of these interactions and whether loss of function of these genes in *Drosophila* is sufficient to cause deafness has not yet been explored. The expression of other deafness gene orthologs within JO further indicates that the fly can be used as a model to quickly and efficiently study mechanisms of deafness.

786 Identification of genetic modifiers in a fly model of Alzheimer's disease co-expressing Aβ42 and tau. *M. Singh*, Jean Geste, Diego Rincon-Limas Department of Neurology, University of florida, Gainesville, FL.

Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder characterized by dementia and cognitive decline due to progressive cerebral cortical atrophy. Brains of AD patients are characterized by accumulation of microscopic extracellular amyloid-beta (Aβ) plaques and intracellular neurofibrillary tangles composed of hyperphosphorylated tau. The deposition of Aβ42 which is one of the fragments of amyloid precursor protein (APP) has been known to play role initiating the events leading to the formation of amyloid and subsequently hyperphosphorylation of tau. However, animal models expressing either Aβ42 or tau individually do not mimic the complexity of the human condition. Indeed, recent evidences suggest that Aβ42 and hyperphosphorylated tau interact to modulate neurotoxicity in AD. To shed light into their concerted roles in AD pathogenesis and to discover pathways mediating Aβ42 and tau interactions, we generated transgenic flies co-expressing Aβ42 fused to a signal peptide and wild type tau. Overexpression of Aβ42 or tau in Drosophila using the UAS-Gal4 system causes mild to moderate rough eye. In comparison, co-expression of Aβ42 with tau causes severe roughening and reduction of the eye size. The level of neuronal cell death in eye tissues was also significantly enhanced in flies co-expressing Aβ42 and tau. To unbiasedly identify genetic targets and biological pathways mediating Aβ+tau interactions, we are currently performing a genetic screen using transgenic RNAi flies from Vienna Drosophila Resource Centre (VDRC). Among 1700 RNAi lines screened so far, we have identified 60 genetic modifiers (8 suppressors and 52 enhancers) involved in various molecular and biological functions such as DNA binding, Kinase activity, protein folding and membrane transport activity. Our preliminary findings illustrate the value of our Aβ+tau flies to identify novel candidate genes and molecular pathways that could modulate AD pathogenesis. This work is supported by grant 7AZ12 from the Florida Department of Health Ed and Ethel Moore Alzheimer's Disease Research Program to DERL.

787 Multiple pathways contribute to human TDP-43 toxicity in flies. *Deepak Chhangani*[1], Lorena de Mena[1], Edward HoLostalo[1], Nho Cao[1], Abdullah Afridi[1], Jada Lewis[2], Pedro Fernandez-Funez[3], Diego Rincon-Limas[1] 1) Department of Neurology, McKnight Brain Institute, Gainesville, FL; 2) Department of Neuroscience, University of Florida, Gainesville, FL; 3) Department of Biomedical Sciences, University of Minnesota, Duluth, MN.

TAR DNA-binding protein 43 (TDP-43) is associated with neurodegenerative conditions including Amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), Frontotemporal dementia (FTLD) and Chronic traumatic encephalopathy (CTE). This highly-conserved DNA/RNA binding protein is primarily distributed in nuclei. However, it translocates to the cytoplasm and gets hyperphosphorylated and ubiquitinated, which may subsequently impart protein aggregation. Despite considerable efforts to investigate the physiological role of TDP-43, we still have a very limited understanding of the molecular and cellular mechanisms of underlying TDP-43 proteinopathies. A key challenge, therefore, is to identify critical proteins and pathways mediating TDP-43 neurotoxicity. To shed light on this issue, we searched for genetic modifiers of neurotoxicity in transgenic flies expressing human TDP-43^{M337V}, a prevalent mutation in TDP-43 proteinopathies. These flies display a reliable eye phenotype, thus, we crossed them with a library of 6,261 RNAi strains obtained from the Vienna Drosophila Resource Center. We identified 300 modifiers of mutant TDP-43 toxicity in a primary screen. After eliminating genes that have dominant effect on eye phenotype, we validated 80 robust suppressors, 60 enhancers and 25 lethals. Furthermore, we found that many suppressors are linked to transcription, mRNA elongation, splicing and nucleocytoplasmic shuttling. In addition, we also found many modifiers linked to other functions such as neurogenesis, syntaxin binding, mitochondrial transport, ubiquitin activity, phosphatidylinositol signaling, and protein quality control. In summary, this loss-of-function screen has led

to the identification of several genes and molecular pathways not previously known to be associated with TDP-43 pathologies. This work was supported in part by grants R21NS096647 and R01AG059871 (DERL), and Florida Department of Health 5AZ04 (DERL and JL).

788 Screens in Drosophila and cell line models implicates *GPR21* as a suppressor of neurodegeneration. *M. Avalos*[1,2], I. Al-Ramahi[1,2], M. Rousseaux[1,2], G. Vazquez-Velez[1,3], C. Lasagna-Reeves[1,2], M. de Haro[1,2], A. Perez[1,2], H. Zoghbi[1,2,3,4,5], J. Botas[1,2,3] 1) Department of Genetics and Genomics, Baylor College of Medicine, Houston, TX; 2) Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital, Houston, TX; 3) Department of Developmental Biology, Baylor College of Medicine, Houston, TX; 4) Department of Pediatrics, Baylor College of Medicine, Houston, TX; 5) Howard Hughes Medical Institute Baylor College of Medicine, Houston, TX.

In neurodegenerative diseases such as Alzheimer's and Parkinson's, toxic protein aggregates build up over time leading to numerous downstream pathologies at the molecular and cellular level. Because of the complex array of factors involved in triggering neuronal death, therapeutic interventions that ameliorate the levels of toxic protein have been proposed as a way to treat neurodegeneration. That reduction of tau in a Aβ-induced neurotoxicity mouse model of Alzheimer's disease is neuroprotective is evidence in support of this proposal. Screens in a human medulloblastoma-derived cell line (daoy cells) have assessed which genes are likely to be significant modulators of toxic protein levels. Parallel screens for genetic modulators of neurodegeneration in *Drosophila* models, whereby genes with human homologs are knocked down by RNAi, have also produced many candidate suppressors of disease phenotypes associated with expression of toxic proteins in Alzheimer's, Parkinson's, or Huntington's. These cross-species unbiased screens have uncovered genes, such as *NUAK1* in tau neurodegenerative models, that modulate levels of disease-driving proteins. Among these hits is *GPR21*, a Gq coupled and constitutively active orphan GPCR. It's expressed in the brain (including cortical neurons and hippocampus), shows no evidence of loss-of-function intolerance in humans, and was shown to reduce α-synuclein, APP, and tau levels in daoy cells, indicating a previously unknown interaction between GPR21 and protein clearance mechanisms. Additionally, neurodegenerative phenotypes are suppressed in two quantitative motor-performance (climbing) assays in *Drosophila* models of neurodegeneration where full-length mHTT or N-terminal mHTT is expressed in the fly CNS. *GPR52*, a close paralog of *GPR21*, modulates levels of mHTT in striatal cells and its *Drosophila* homolog suppresses HD phenotypes. These data point to a link between *GPR21* and neurodegeneration, though it is unclear which neurodegenerative disease may be most affected by *GPR21* modulation.

789 Traumatic injury induces stress granules formation and perturbs nucleocytoplasmic transport in Drosophila. Eric Anderson[1], Lauren Gochenaur[1], Aditi Singh[1], Rogan Grant[1], Andres Morera[2], Jacob Schwartz[2], Christopher Donnelly[3], *Udai Pandey*[1] 1) Department of Pediatrics, University of Pittsburgh Medical Center, Pittsburgh PA; 2) Department of Chemistry and Biochemistry, University of Arizona, Tucson AZ ; 3) Department of Neurobiology, University of Pittsburgh Medical Center, Pittsburgh PA.

Traumatic brain injury (TBI) has been predicted to be a predisposing extrinsic factor for ALS and several other neurodegenerative disorders. We examined the contribution of traumatic brain injury (TBI) as an extrinsic factor and investigated if TBI influences the susceptibility of developing neurodegenerative symptoms in vivo. We found that traumatic injury leads to the induction of stress granules (SGs) in the Drosophila brain. The degree of SGs induction directly correlates with the level of trauma in flies. Furthermore, we found that the level of mortality is directly proportional to the number of traumatic hits. Interestingly, trauma-induced SGs are ubiquitin, p62 and TDP-43 positive, and persistently remain over time suggesting that SGs might be aggregates and exert toxicity in our fly models. TDP-43 pathology has been observed in ALS/FTD and several other related neurodegenerative diseases. Importantly, mild and repetitive trauma in flies expressing ALS-linked genes such as FUS and expanded G4C2 repeats increased mortality and locomotion dysfunctions suggesting that mild trauma aggravate neurodegenerative symptoms associated with ALS. Furthermore, we found elevated levels of high molecular weight ubiquitinated proteins and p62 in animals expressing ALS-causing genes with TBI, suggesting that TBI may lead to defects in protein degradation pathways. We observed that genetic and pharmacological induction of autophagy enhanced the clearance of SGs and promoted survival of flies in vivo. Finally, we performed proteomic analysis of the Drosophila brains and identified several candidate neuronal proteins that become altered in response to traumatic injury. Particularly, we observed that nucleoporin proteins were significantly upregulated in response to traumatic injury in the fly brains. We found that these nucleoporins especially FG repeat containing proteins are responsible for TDP-43 pathology and neurodegenerative symptoms *in vivo.* Currently, we are examining nucleoporin proteins as potential genetic modifiers of traumatic injury in our fly models and we expect to better understand the role of traumatic injury in ALS pathogenesis.

790 Synergistic effect of Notch and RhoGEF-2 in the follicular epithelium. *Caique Costa*, Allison Jevitt, Yi-chun Huang, Yang Sheng-An, Wumin Deng Department of Biological Sciences, Florida State University, Tallahassee, FL.

The Notch Intracellular Domain (NICD) is the active form of the transmembrane receptor Notch, which is established after contact with its ligands from neighboring cells. This contact results in a proteolytic cleavage of Notch and consequent release of NICD into the nucleus. Where it regulates the expression of specific target genes. Notch can work as a tumor suppressor or as an oncogene with respect to cellular context. As a tumor suppressor, low levels of NOTCH1 has been shown to spontaneously induce the formation of basal cell carcinomas in mice. As an oncogene, some types of human lymphoblastic leukemias have been reported with overexpression of ICN1, the Notch1 active form. During *Drosophila* oogenesis, Notch acts in follicle cells to trigger a switch from active mitosis to endocycle. However, when overexpressed by itself, Notch, is not sufficient to drive tumorigenesis in *Drosophila* follicle cells, which leads us to hypothesize that NICD overexpression (OE) needs to work in synergy with misregulation in other pathways to drive tumor formation. For instance, Notch can have tumorigenesis potential in mouse intestinal cells depending on interaction with the normal Wnt signaling pathway. Thus, the goal of this study is to investigate the tumorigenesis potential between NICD OE and developmental and growth related pathways in *Drosophila* egg chambers follicle cell. To address this, we performed a genetic screen to investigate the interaction of NICD OE with knockdown or overexpression of candidate genes and how this might contribute to tumorigenesis. Out of 51 genetic interactions analyzed, only one of these, a member of the Ras homologous (Rho) family of GTPases, RhoGEF-2, is a guanine exchange factor and cytoskeleton regulator. RhoGEF-2 and NICD OE results in apoptosis and multilayer formation at the posterior ending of egg chambers at stage 8. It is speculated that this may be due to the role of Rho in cell behavior, morphology, and regulation of structural cell proteins such as actin and microtubules. Thus, aberrant activity of Rho and Notch may synergistically disrupt the normal harmony of critical cell pathways and lead to apoptosis and multilayer formation.

791 Downregulation of *CHMP2B* mitigates TDP-43 neurotoxicity in both *Drosophila* and mammalian models. *X. Sun*, Kuili Tian, Haiqiong Wang, Shuang Zhang, Yanshan Fang Interdisciplinary Research Center on Biology and Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, CN.

A pathological hallmark of amyotrophic lateral sclerosis (ALS) and frontal temporal dementia (FTD) is abnormal cytoplasmic inclusions that contain TDP-43 protein (Neumann et al., 2006). In addition, mutations in the gene encoding TDP-43 can cause ALS and FTD. To understand the mechanisms underlying TDP-43 neurotoxicity, we generated transgenic flies to express human TDP-43 in the fly eye and conducted RNAi screens for genes that can modify TDP-43-induced

eye degeneration. In the screen, one of the modifier genes we found is *Charged multivesicular body protein 2b* (*CHMP2B*), a component of the endosomal sorting complex required for transport (ESCRT).

The ESCRT machinery plays an important role in many cellular events, including the regulation of multivesicular body formation in the endosomal lysosomal pathway, cytokinesis, and viral budding (Henne et al., 2011). CHMP2B is a major component of the ESCRT-III complex and is also a known causal gene of ALS and FTD. In this study, we find that downregulation of *CHMP2B* in flies dramatically suppresses TDP-43-induced eye degeneration. Moreover, using an inducible pan-neuronal driver (*elav*-GS) to downregulate *CHMP2B* in adult fly neurons significantly mitigates the age-dependent climbing defects and extends the shortened lifespan of TDP-43 flies.
Further experiments show that RNAi-*CHMP2B* does not affect the mRNA or protein levels of TDP-43 in flies, indicating that the modifying effect of RNAi-*CHMP2B* is not due to an alteration of the expression levels of the TDP-43 transgene. Next, we extend our study of CHMP2B and TDP-43 to the mammalian system using human 293T cells and mouse motor neuron-like NSC-34 cells. Consistently, siRNA knockdown of CHMP2B also suppresses TDP-43-mediated viability decline in the mammalian cells, whereas CHMP2B overexpression remarkably enhances TDP-43 cytotoxicity. The ongoing experiments are to verify the effect of *CHMP2B* in primary neurons and *in vivo* TDP-43 mouse models, as well as to elucidate the mechanism by which CHMP2B modifies TDP-43 neurotoxicity. Together, our study may reveal a molecular link between the two ALS and FTD causal genes TDP-43 and CHMP2B, and cast novel insights to the understanding of the pathogenesis of ALS and FTD.

792 Decreased tricarboxylic acid cycle (TCA) in *Staphylococcus aureus* increases survival to innate immunity. *A. Page*, K. Kluthe, K. Carlson, A. Nuxoll Biology, University of Nebraska at Kearney, Kearney, NE.

The tricarboxylic acid (TCA) cycle is a key component in producing ATP. With the *Staphylococcus aureus* genome, resistance and virulence are easily transferred from one strain to another, making antibiotic resistance a major public health problem. *S. aureus* accounts for approximately 80,000 infections every year, resulting in 11,000 deaths. While resistance is heavily studied, antibiotic tolerance is not. Persister cells are a subpopulation of dormant cells tolerant to antibiotic killing. This subpopulation is thought to be the underlying cause of many chronic and relapsing infection. Recent work has revealed that persister formation in *S. aureus* is dependent on lowered ATP levels. Through whole genome analysis, central metabolism was identified as an essential part of persister formation. However, the underlying mechanism of persister formation is still unknown, as is the implications of persisters in terms of antibiotic tolerance. An organism's first line of defense is the innate immune system, including antimicrobial peptides (AMPs). AMPs are a key component of both the human and *Drosophila* innate immune system. Challenging *S. aureus* with the AMPs, LL-37 and hBD-3 revealed several logs of killing. Deletion of TCA cycle genes resulted in 100-fold more survival compared to wild type. Currently, experiments are being performed using a *Drosophila* model for infection. Preliminary data suggests that deletion of TCA cycle genes induces persister formation, and persisters pose a challenge for the innate immune response. The project described was supported by grants from the National Center for Research Resources (5P20RR016469) and the National Institute for General Medical Science (8P20GM103427), a component of the National Institutes of Health.

793 Using MARRVEL.org for human genetics and cross-species data collection. *J. Wang*, D. Mao, S. Yamamoto, Z. Liu, H. Bellen Baylor College of Medicine, Houston, TX.

In the age of sequencing data, interpreting human genetic variants is a major challenge. Model organisms are increasingly used to understand the biological consequences of genetic changes. Hence, there is an unmet demand for quickly assessing variants in human genes using model organisms like Drosophila. To address these challenges and take advantage of the large volume of previously generated data and valuable work in both human genetics and model organisms, it is critical to provide a concise and easily accessible summary view of the available knowledge. A multidisciplinary aggregation of resources is needed to facilitate the functional annotation of human genes. This should permit us to determine if a variant in a human gene is pathogenic or potentially pathogenic, and hence indicate further study of potentially pathogenic variants in model organisms.

To this end, we created a website, MARRVEL (Model organism Aggregated Resources for Rare Variant ExpLoration), available at http://marrvel.org/. MARRVEL is the first publicly available resource that integrates information from human genetic databases as well as from across multiple model organisms in a human information-centric manner. MARRVEL aggregates information from important human genetic databases including control population and disease population data to facilitate analysis of variants of unknown significance that are potentially pathogenic. These public human databases include OMIM, ExAC, Geno2MP, DGV, and DECIPHER. Next, MARRVEL displays a concise summary of what is known about the human gene's homologs across yeast, worm, fly, fish, rat, and mouse via databases such as SGD, WormBase, FlyBase, ZFIN, and MGI. In addition, protein alignment of the best homologs is provided with the functional domains highlighted, accomplished via DIOPT (http://www.flyrnai.org/diopt).

MARRVEL facilitates the analysis of human variants with the wealth of publicly available human genetics data and provides a summary of valuable accumulated knowledge across model organisms. Cross-discipline database integration accessible to model organism scientists and clinicians can assist the functional annotation of the human genome.

794 Dissecting the Genetic Basis of Variation in Cocaine and Methamphetamine Consumption in *Drosophila melanogaster*. *BM Baker*[1,2], CA Highfill[1,2], RRH Anholt[1,2], TFC Mackay[1,2] 1) Department of Genetics and Biochemistry, Clemson University; 2) Clemson Center for Human Genetics, Clemson University.

Studies on *Drosophila melanogaster* can identify genetic and transcriptional networks that underlie variation in voluntary consumption of cocaine and methamphetamine to serve as a blueprint for subsequent studies on humans. Exposure to these psychostimulants in flies results in behavioral and physiological effects that resemble those observed in humans. We derived an outbred advanced intercross population (AIP) from 37 of the sequenced inbred wild-derived lines of the *Drosophila melanogaster* Genetic Reference Panel (DGRP). These lines are maximally genetically divergent, have minimal residual heterozygosity, are not segregating for common inversions, and are not infected with *Wolbachia pipientis*. We assessed voluntary consumption of sucrose, methamphetamine-supplemented sucrose and cocaine-supplemented sucrose and found significant phenotypic variation in the AIP, in both sexes, for consumption of both drugs. We performed whole genome sequencing and extreme QTL mapping on the top 10% of consumers for each replicate, sex and condition, and an equal number of randomly selected flies. We evaluated changes in allele frequencies genome-wide among high consumers and the control flies and identified 3,033 variants associated with increased consumption that reside in 1,963 genes, enriched for genes associated with nervous system and mesoderm development. We assessed the effects of ubiquitous RNA interference (RNAi) on consumption for 22 candidate genes, of which 14 showed a significant increase or decrease in consumption. Extensive recombination in the AIP generates increased statistical power compared to genome-wide association analysis of the DGRP and illustrates the polygenic genetic architecture that underlies variation in cocaine and methamphetamine consumption. Supported by NIH grant U01DA041613.

795 Investigating the Impact of Genetic Factors on Fly Microbiome. *Khursana Duty*, Hui-Min Chung Biology, University of West Florida, Pensacola, FL.

Gut microbiota, also known as intestinal flora, is the community of microbes residing in the intestinal tract. The balance of this diverse microbial community

ties to a number of health benefits. The influence of microbiota on animal metabolism, nutrition, and immune and endocrine systems has been long acknowledged. In the last decade, studies have shown the microbiota effects on central nervous system as well. Gut microbiota influences neurological outcomes, such as mood, behavior, and certain neurological disorders (Sampson, 2015). Intestinal microbes affect development of the nervous system by altering neurochemical pathways (Neufeld et al., 2011). It is unclear whether microbial composition is acquired through various exposures throughout life or somehow controlled by the host's genetic makeup.

This research will examine whether the host's genetic background plays a role in shaping the microbial community composition using *Drosophila melanogaster*. This is a simple model that shares similar cellular function and tissue organization with the human. The *Drosophila* genome is well understood; its genes can be matched to equivalent human genes. Many genes associated with disease in humans are also found in the fruit fly, such as *pink1* (mutation associated with Parkinson's disease) and *psn* (mutation associated with Alzheimer's disease) genes.

The current objective of this research is (1) to examine whether the wild type gut microbiota differs from that of the *pink1* and *psn* flies; (2) determine whether restoring partial *psn* activity in *psn* flies will change the microbiota composition to resemble that of wild type flies. We hypothesize that (1) mutant flies' gut microbe composition will differ from that of the wild type, and (2) the microbiota composition of the *psn* mutant flies can be altered if the *psn* gene activity is restored.

Preliminary results reveal significant differences in the gut microbial community composition between the wild type flies and the mutant flies. The wild type fly gut microbiota is more diverse compared with the *pink1* flies. *Pink1* fly gut has high abundance of Pasteurellales bacteria, whereas the wild type fly gut has high abundance of Bifidobacteriales and Rhodospitillales bacteria. *Psn* fly gut has much lower abundance of Lactobacillales bacteria compared with wild type fly gut.

796 Characterizing the in vivo effects of Mitochondrial RNase P complex mutations. *M. Saoji*, R. T. Cox Biochemistry, USUHS, Bethesda, MD.

Mitochondria contain double stranded circular DNA (mtDNA) that encodes for 13 proteins involved in oxidative phosphorylation, 2 ribosomal RNA (rRNA) and a complete suite of transfer RNAs (tRNAs). Loss of any of these products can lead to mitochondrial disease. Like bacteria, mtDNA is transcribed as polycistronic RNA. In order for each critical product to be available for mitochondrial function, the m:tRNAs, mt:rRNAs and mt:mRNAs must be efficiently and properly excised. The Mitochondrial Ribonuclease P (mtRNase P) complex processes the 5′ end of the mt:tRNAs in humans. Human mtRNase P is a three-protein complex composed of a mtRNase P protein (MRPP) 1, 2 and 3. MRPP3 is the catalytic endonuclease while MRPP1, a methyltransferase and MRPP2, a dehydrogenase, are required for the catalytic activity of MRPP3. In humans, mutations in each of the three proteins are linked to severe mitochondrial diseases. Our lab studies the Drosophila homologs of mtRNase P, Roswell (MRPP1), Scully (MRPP2) and Mulder (MRPP3), to understand the effect of mutations on complex stability, in-vivo mt:tRNA processing and overall mitochondrial health. Work from our lab has shown that each of these proteins is required for survival. Loss of each protein is associated with mitochondrial deficits partly due to reduced mt:tRNA processing. We are currently characterizing the various mutants of Roswell, Scully and Mulder to determine their effect on the levels of other two proteins of the complex. We are also determining how these mutations affect the catalytic ability of the complex to process the 5′ ends of different mt:tRNA. Finally, we are interested in exploring the effects of tissue specific knockdown of individual mtRNase P proteins on the overall health of the fly. Using this study, we strive to parse out the individual contribution of the three mtRNase P proteins for proper functioning of the complex in vivo. Since the human diseases resulting from decreased function of the process exhibit complex symptoms, this careful analysis should shed light on disease etiology.

797 Early muscle abnormalities are alleviated by pharmacological inhibition of the angiotensin-converting enzyme in a *Drosophila* model of Alzheimer's disease. J. Thomas[1], R. Ingram, III[1], L. Okumu[1], G. Gorman[1,2], P. Abadir[3], *P. Jumbo-Lucioni*[1,4] 1) McWhorter School of Pharmacy, Samford University, Birmingham, AL 35229; 2) Pharmaceutical Sciences Research Institute, Samford University, Birmingham, AL 35229; 3) Division of Geriatric Medicine and Gerontology, Johns Hopkins School of Medicine, Baltimore, MD 21224; 4) Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL 35294.

Evidence supporting a protective role of angiotensin-converting-enzyme inhibitors (ACEis) against Alzheimer's disease (AD) has been inconsistent. Attempts to understand mechanisms have focused on cognitive deficits but failed to look at other ACEi-sensitive tissue like muscle. Muscle loss appears early and has been associated to the severity of cognitive decline in AD. ACEis like lisinopril have shown to delay loss in muscle mass and strength that are characteristic of normal aging. Like vertebrates, orthologues of human ACE are present in *Drosophila* and the activity of the fly ACE, AnCE, is inhibited by lisinopril.Our objective was to test the effects of lisinopril administration on age-dependent muscle deficits in a fly model of AD. Overexpression of the human amyloid precursor protein and the β-site APP-cleaving enzyme was targeted neuronally using the tissue-specific driver, *elav-gal4,* in our AD model. We characterized climbing speed of 7,14 and 21-days old virgin male flies treated or not with 1mM lisinopril. We found that, like vertebrates, AD flies were significantly movement impaired compared to controls at all ages (*p-value*≤0.05). Lisinopril caused a drastic improvement in the physical performance of both treated cohorts, control and AD flies, after 7 days of treatment (*p-value*=0.03). At day 14, lisinopril significantly ameliorated the locomotion deficits in AD flies (*p-value*=0.04). We assayed the total hydrogen peroxide levels (H_2O_2) in muscle from 14-days old flies and found that lisinopril strikingly decreased H_2O_2 levels in treated AD flies compared to their untreated counterparts (*p-value*=0.02). Lisinopril bioavailability was confirmed in whole flies by LC/MS/MS. These results are the first to reveal that 14-day lisinopril intervention positively impacts muscle performance traits in a *Drosophila* AD model.

798 Generation and *in vivo* characterization of Human *NOTCH* transgenes to study the functional impact of disease-associated variants in *Drosophila*. *A. Phillips*[1], H.K Graves[2], P.C Marcogliese[2], J.L Salazar[2], S. Yamamoto[2,3,4,5] 1) Rice University , houston, TX; 2) Department of Molecular and Human Genetics, Baylor College of Medicine (BCM), Houston, TX; 3) Program in Developmental Biology, BCM, Houston, TX; 4) Department of Neuroscience, BCM, Houston, TX; 5) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX.

Notch signaling is a well-studied signaling pathway that is conserved throughout metazoan evolution. While the *Drosophila melanogaster* genome encodes a single Notch receptor, the human genome encodes four paralogs (NOTCH1-4). Variants in human *NOTCH* genes have been found to cause diverse Mendelian disorders and have been identified in various types of cancer. Some Notch related diseases in human are thought to be caused by loss-of-function mutations (amorphs or hypomorhs), whereas others are considered to be due to gain-of-function mutations (hypermorphs, antimorphs or neomorphs). Classification of *NOTCH* mutations into different functional classes has primarily been performed using mammalian cell based signaling assays. Some groups have utilized knock-in mouse models to assess the functional consequences of *Notch* variants to understand their effects *in vivo*. However, considering the number of identified missense variants found in human *NOTCH* genes is increasing at a rapid pace due to utilization of whole-exome sequencing in clinical settings, we need a higher-throughput assay system to experimentally determine the functional consequence of disease associated NOTCH variants *in vivo*.

We generated a series of transgenic constructs and *Drosophila* lines that allow the expression of full length human NOTCH1-4 using the phiC31 transgenesis system. Unfortunately, not all of these transgenes were able to activate Notch signaling *in vivo* in flies, and NOTCH1 seemed to act in a dominant negative manner. We hypothesized that this may be because the human NOTCH proteins are not compatible with fly Delta or Serrate ligands, so we generated a series of NOTCH1-4 transgenic lines that can function in a ligand-independent manner (S2-dependent form, S3-dependent form, intracellular domain only form). Interestingly, NOTCH1 and NOTCH2 proteins that do not require ligands for their activation were able to strongly activate Notch signaling in flies, while

NOTCH3 and NOTCH4 were able to do so, but in a weaker manner. These results suggest that the intracellular domains of human NOTCH proteins have the ability to functionally interact with Mastermind and Suppressor of Hairless to activate fly Notch signaling *in vivo*. We are currently introducing several disease-linked *NOTCH1* and *NOTCH2* mutations into these constructs to assess their functions *in vivo*.

799 Visualizing gene expression and 3D genome architecture during embryogenesis. *Alistair Boettiger* Developmental Biology, Stanford University, Stanford, CA.

Establishment of different cell types during development requires precise interactions between genes and distal regulatory elements. However, our understanding of what these interactions look like in three dimensions, how they vary across cell types in complex tissue, and how they relate to transcriptional state remain limited. I will describe optical reconstruction of chromatin architecture (ORCA), a microscopy approach to follow the path of DNA in intact nuclei. I will describe an application of this method to the study the structure of the Bithorax-Complex in individual nuclei of cryosectioned *Drosophila* embryos with nanoscale accuracy and genomic resolution as high as 2 kilobases. To score gene expression in the same individual cells, we quantified up to ~30 RNA species in parallel. ORCA enabled visualization of predicted cell-type-specific boundaries between active and Polycomb-repressed DNA in this locus. Unexpectedly, we also observed Polycomb-independent boundaries which further partitioned the active, but not repressed, portions of the locus in a cell-type-specific manner. Deletion of these boundary regions corresponded with ectopic enhancer-promoter contacts, aberrant gene expression, and developmental defects. Together, our results illustrate a powerful approach for high-resolution, single-cell DNA domain analysis *in vivo* on hundreds of thousands of individual cells. The data reveal extensive 3D chromatin organization that changes with cell identity, and demonstrate that boundary elements contribute to formation of physical domains in *Drosophila*.

800 Genetic approaches to study protein complex in *Drosophila*. *J. Ni* School of Medicine, Tsinghua University, Beijing, Beijing, CN.

Gene editing including Loss-of-function and gain-of-function of one or multiple targets is the most powerful method to address the mysterious mechanism behind the genetics and development. Equipped with all sorts of genetic tools and tremendous resource, *Drosophila melanogaster* is advantageous to other model organisms. Proteins encoded by two or more genes can have partially overlapping or redundant functions, and thus modulate one of them may result in little or no effect on functional or phenotypic analyses. To increase efficiency and versatility, we developed the next generation targeting activator system and transgenic RNAi system in *Drosophila*. Several case illustrations will be provided of how the two systems can be used to study the synthetic developmental effect.

801 A high-throughput screening platform to identify yeast metabolites that alter signaling pathways in *Drosophila melanogaster*. *L.N. Tran*[1], S. Lacefield[2], W.D. Tracey[1,2] 1) Gill Center for Biomolecular Science, Indiana University, Bloomington, IN; 2) Department of Biology, Indiana University, Bloomington, IN.

Metabolites, once thought of as mere cellular byproducts, are now understood to have many direct effects on cellular processes, though the interplay between metabolism and cell signaling mechanisms remains largely unknown. *Drosophila melanogaster* is an ideal system to study metabolites as potential signaling molecules in human disease progression as 1) there are many well-established mutant strains useful for studying signaling pathways in human disease and 2) we can systematically search for metabolites that suppress disease pathways through dietary supplementation of modified yeast strains. Since there are thousands of yeast strains with unique metabolome profiles available, we sought to develop an efficient and systematic method of identifying metabolites that might interact with key signaling pathways. Using a robotic biosorting system, we cultured *Drosophila* larvae in a 48-well plate format with food that includes the live yeast strain BY4741. We tested the viability of the flies on two food recipes, one that is molasses-based and one that is cornmeal-based, and determined that a food recipe consisting of cornmeal, sucrose, and live grown yeast is sufficient for the normal development of *Drosophila* larvae to adulthood. Moreover, we found that adult eclosion rate improved as the concentration of supplemented yeast increased. We are now testing additional conditions to further optimize the growth and viability of cultured flies. Future efforts to screen the yeast libraries for metabolites that modulate fly signaling pathways using this high-throughput assay in *Drosophila* disease models, may provide important insight into metabolites that have conserved pharmacological effects on human diseases.

802 A DREaMR system that integrates gene knockout, a drug-inducible reporter and an inducible mutant rescue in *Drosophila* and *Aedes*. *Jieyan Chen*, Junjie Luo, Yijin Wang, Yinpeng Zhan, Nick DeBeaubien, Adishthi Gurav , Craig Montell Neuroscience Research Institute, UC Santa Barbara, Santa Barbara, CA.

Combining multiple transgenic components can be time consuming and is sometimes unattainable in organisms, such as the insect disease vector, *Aedes aegpypti*, which do not have balancer chromosomes. Here, we developed a system, which we call DREaMR (**D**rug-induced **Re**porter **a**nd **M**utant **R**escue) that allows us to simultaneously accomplish three important goals without the need for combining separate genetic elements. These include creating a: 1) mutant allele, 2) drug-inducible gene rescue, and 3) reporter to determine the expression pattern of the protein, thereby eliminating the requirement for generating antibodies. We integrate a DREaMR cassette into the promoter or coding [CM1] region of the target gene so that: 1) the insertion disrupts the native gene, 2) an rtTA (reverse tetracycline-controlled transactivator) is expressed under the control of the target gene promoter, and 3) a TRE (tetracycline regulatory element) is fused to a tagged version of wild type rescue gene. We knocked in a DREaMR cassette in the *Drosophila white* gene and found that the DREaMR system was effective. The flies exhibited a white-eye phenotype, and we rescued the phenotype upon feeding the animals doxycycline. To show that we can apply this system to another organism, we focused on *Aedes*. We generated DREaMR knockins targeting *Aedes kh* and *yellow*, which are homologous to *Drosophila cinnabar* and *yellow*, respectively. We found that the DREaMR knocked into *kh* displayed a white-eye mutant phenotype, and that we could restore their eye color in the presence of doxycycline. We also knocked a DREaMR cassette into the *Aedes yellow* gene, which caused a yellow body phenotype. We suppressed this phenotype upon addition of doxycycline. Having validated the DREaMR system, we are now using it to generate male sterile mosquitoes, which involves releasing sterile males that render females sterile, since female mosquitoes mate only once. We generated *B2T* (homologous to the male sterile gene *βTub85D* in *Drosophila*) mutant *Aedes*, and verified that the mutant males are sterile. We will test a DREaMR cassette inserted into the *Aedes B2T* gene, which will allow us to maintain fertile *B2T* males in the lab by inducing the rescue construct with doxycycline. In summary, we developed a simple but elegant system that allows drug-controllable genetic manipulation in both *Drosophila* and mosquito *Aedes* without the needs for any genetic crosses.

803 Simple design of complex multigene regions using *Drosophila* Modular Cloning (DMoClo). *David Loehlin* Biology Dept., Williams College, Williamstown, MA.

Rigorous dissection of the structure and function of genome regions faces many technical hurdles. It would be useful to have methods for straightforward

assembly of multi-gene constructs that is modular, extendible to many experimental questions, and minimizes error.

I describe an implementation of the Modular Cloning method for Drosophila, termed DMoClo. This is based on (and compatible with) the yeast MoClo system from John Dueber's lab. In DMoClo, complex multi-gene constructs are designed and assembled from libraries of part plasmids using golden gate cloning in a hierarchical, low-error process. In the first step, genes or other elements are assembled from off-the-shelf or custom parts: -connector-promoter-CDS-terminator-connector-backbone-. In a second step, multiple genes are assembled into a premade or custom Drosophila transformation vector: -connector-gene1-con-gene2-con-gene3-con-etc-vector-. This hierarchical step makes it easy to test modifications to any element of interest in constructs containing 6 or more genes.

This method was used by two undergraduate students in my lab to build complex constructs for testing hypotheses about the structure and function of duplicate genes in a few weeks. We first redesigned and built a number of popular Drosophila transformation vectors. Second, we used them to build multigene constructs and tools for engineering segmental duplications. Third, to make the system adaptable for the many unique design needs of Drosophila researchers, we developed a set of helper plasmids for PCR-free generation of novel transformation vectors, allowing use of arbitrary visible markers, transposon ends, recombinase sites, bacterial origins, insulators, etc.

804 Inferring chromosome-wide recombination rates from pooled sequencing of marker selected progenies. *A. Mantha*, D. Bachtrog, K. Wei Integrative Biology, University of California Berkeley, Berkeley, CA.

Genetic recombination promotes genetic variation through crossover events where homologous chromosome pairs physically exchange chromosomal segments during meiosis. Alfred H. Sturtevant pioneered the study of recombination rates by scoring the frequency of recombinants between linked genes with visible markers across the X chromosome, thus creating the very first genetic linkage map. Since then, assaying the number of recombinants between two markers (visible or molecular) remains the de facto way to measure recombination rates. Recent extension of this approach uses whole genome sequencing of large numbers of recombinant individuals to infer where crossover occurred. While this method provides sensitive genome-wide estimates, it requires hundreds to thousands of library preparations. Here, we present a new and efficient method to estimate recombination rates across a chromosome by pooled sequencing of marker selected individuals from a typical two generation recombination backcross. We conducted backcrosses with the ebony (e) sepia (se) double mutant on chromosome 3 generating a total of 3351 progenies, 799 of which are recombinants between e and se. We selected and pooled all se- individuals (n = 1559) regardless of the e phenotype, so the allele frequency (AF) of the e- se- chromosome equals 1 at the se locus in this pool. Crucially, the AF is expected to decreases distally due to increases in the number of recombinants, and eventually return to the Mendelian ratios of 0.75 at 50 cM; i.e. the rate of decrease is a function of the recombination rate. After accounting for reference allele bias and CG bias, AF inferred from the pooled sequences decreases towards 0.75 as the physical distance from the selected locus increases, as expected. Based on the AF at e (0.8655), we infer a genetic distance of 26.9 cM from se, which closely approximates the distance calculated from the recombinant counts. This reveals the efficacy of estimating genetic distance between the selected locus, se, and, extensively, any locus on the chromosome; the recombination rate can then be calculated from the genetic distance across the chromosome. Our results are a proof-of-principle that pooled sequencing of individuals selected at a locus allows inference of recombination rates, due to the relationship between AF decay and genetic distance. Depending on the marker used for selection and the chromosome, our method can create chromosome-wide recombination maps in as little as one library preparation of one pool.

805 RNAi and CRISPR screening resources at the DRSC. *S.E. Mohr*[1], R. Tao[1], S. Knight[1], G. Amador[1], R. Viswanatha[1], Y. Hu[1], A. Comjean[1], P. Merckaert[1,2], J. Zirin[1], N. Perrimon[1,3] 1) Dept Gen, Harvard Med Sch, Boston, MA; 2) University of Paris-Sud; 3) Howard Hughes Medical Institute.

Many research projects can benefit from harnessing the advantages of cultured cell screens as part of an overall strategy aimed at uncovering novel in vivo and disease-relevant gene functions. The Drosophila RNAi Screening Center (DRSC) has been supporting genome-wide RNAi screens in Drosophila cultured cells for more than fifteen years. To date, we have supported more than 200 projects by the community. Over the years, we have added several additional RNAi libraries, other types of screening reagents, and new strategies for production of modified cell lines. We continue to support both off-site and on-site arrayed-format RNAi screens, including high-content image-based screens, and have recently added CRISPR pooled-format screening to the suite of technologies we offer. We are also using state-of-the-art approaches such as machine learning to improve CRISPR pooled screen reagent libraries, and using both plasmid and synthetic RNA-based strategies to test arrayed-format CRISPR-based screening. Recently supported screens include a variety of assays designed to contribute to fundamental understanding in Drosophila and for rapid translation to mammalian systems.

806 A toolbox for tissue-specific CRISPR-mediated deletion of circadian clock genes in *Drosophila*. *R. Delventhal**[1], M. Pantalia*[1], R. O'Connor*[1], M. Ulgherait[1], H. Kim[1], J. Canman[2], M. Shirasu-Hiza[1], *authors contributed equally 1) Dept. of Genetics & Development, Columbia University Irving Medical Center, New York, NY; 2) Dept. of Pathology & Cell Biology, Columbia University Irving Medical Center, New York, NY.

Circadian rhythms regulate key aspects of physiology and disease in all organisms. These rhythms are driven by the oscillating transcriptional activity of circadian regulators over a 24-hour period, often referred to as the molecular clock. Classically, studies examining how the molecular clock regulates animal behavior have relied upon ablation of subsets of circadian neurons or rescue and RNAi-knockdown of core circadian regulators. Here we report the design and validation of genetic tools that target two of the key molecular clock genes, *timeless* and *period*, for CRISPR-mediated, tissue-specific gene disruption in *Drosophila*. We created *UAS-gRNA* lines expressing multiple, gene-targeting guide RNAs (gRNAs) that, when combined with *UAS-Cas9* and a tissue-specific *Gal4* driver, will disrupt the targeted gene only in the *Gal4*-expressing cells of interest. We show that these tools can recapitulate a null mutant phenotype and also demonstrate how these tools provide a unique opportunity to uncover novel insights into the circadian regulation of behavior. Furthermore, we verified the efficiency of the CRISPR-mediated gene targeting on the level of both RNA and protein. Taken together, our work demonstrates the efficacy and potential of these tools for advancing our understanding of the cell-specific roles of key clock genes in regulating diverse biological processes.

807 A new CRISPR/Cas9 based screening method for isolating randomly induced recessive lethal mutations in a gene of interest by phenotype selection within the F1 progeny of a single genetic cross. W. A. Ng, A. Ma, *B. H. Reed* Dept Biol, Univ Waterloo, Waterloo, ON, CA.

We have developed a method for selecting randomly induced recessive lethal mutations in a gene of interest using an F1 visible screen. Our method takes advantage of the ability to over-express a gene of interest using CRISPR/Cas9 mediated targeted over-expression. In essence, the screening strategy is based upon the idea that if over-expression of a wild-type allele can generate a phenotype, then over-expression of a newly induced loss-of-function allele will lack this phenotype. This method also depends on the use of CRISPR/Cas9 based mutagenesis to recover alleles of the gene of interest that are refractory to CRISPR/Cas9 mediated over-expression. As a proof-of-principle, we used this method to select EMS induced mutations of our gene of interest (*hindsight*, *hnt*). From approximately 45,000 F1 progeny we successfully recovered 8 new EMS induced loss-of-function *hnt* alleles over a period of two weeks. This new method can, in theory, be applied to any circumstance (and in any species) where CRISPR/Cas9 mediated targeted over-expression is associated with lethality or a visible phenotype.

808 Efficient gene knock-ins in *Drosophila* using homology-independent insertion of universal donor plasmids. *Justin Bosch*, Ryan Colbeth, Jonathan Zirin, Norbert Perrimon Genetics Department, Harvard Medical School, Boston, MA.

Site-specific insertion of DNA into an endogenous gene (knock-in) is a powerful method to study gene function. However, traditional methods for knock-in require laborious cloning of long homology arms for homology-directed repair. Here, we report a simplified method in *Drosophila* to insert large DNA elements into any gene using homology-independent repair. This method employs CRISPR-Cas9 and non-homologous end joining (NHEJ) to linearize and insert donor plasmid DNA into a target genomic cut site. The inclusion of commonly used elements such as GFP on donor plasmids makes them universal, abolishing the need to create gene-specific homology arms and greatly reducing user workload. Using this method, we show robust gene-specific integration of donor plasmids in cultured cells and the fly germ line. Furthermore, we demonstrate its usefulness for gene function analysis by fluorescently tagging endogenous proteins, disrupting gene function, and generating reporters of gene expression. This method simplifies the generation of site-specific large DNA insertions in *Drosophila* cell lines and fly strains, and better enables researchers to dissect gene function in vivo.

809 Large scale sgRNA libraries for in vivo gene overexpression and knockout by CRISPR-Cas9. *J.D. Zirin*[1], Y Hu[1], B. Ewen-Campen[1], L. Lu[1], D. YangZhou[1], R. Colbeth[1], E. Vogt[1], C. Villalta[1], S. Van Nest[1], C. Cavers[1], A. Comjean[1], S. Kondo[2], J. Ni[3], S. Mohr[1], N. Perrimon[1,4] 1) Dept of Genetics, Harvard Medical School, Boston, MA; 2) Invertebrate Genetics Laboratory, National Institute of Genetics, Mishima, Shizuoka, Japan; 3) Tsinghua Fly Center, Tsinghua University, Beijing, China; 4) Howard Hughes Medical Institute, Boston, MA.

The Transgenic RNAi Project (TRiP) (https://fgr.hms.harvard.edu/) has established a new in vivo functional genetics platform for production of reagents based on CRISPR-Cas9. Here we present our libraries of publicly available sgRNA fly stocks for either gene overexpression or gene cutting, and discuss the pros and cons of these new tools relative to previous technologies. Stocks in the TRiP-CRISPR Overexpression (TRiP-OE) library express sgRNAs targeting upstream of a gene transcription start site. Gene activation is triggered by co-expression of catalytically dead Cas9 (dCas9) fused to an activator domain, either VP64-p65-Rta (VPR) or Synergistic Activation Mediator (SAM). Stocks in the TRiP-CRISPR Knockout collection (TRiP-KO), express one or two sgRNAs targeting the coding sequence of a gene or genes, allowing for generation of indels in both germline and somatic tissue. Researchers can view what is currently available or in production, and submit new nominations for both TRiP-OE and TRiP-KO stock production through our sgRNA Stock Tracking System (http://www.flyrnai.org/tools/grna_tracker/). As with our earlier RNAi libraries, TRiP-CRISPR stocks are distributed by the Bloomington Drosophila Stock Center, which lists available stocks on their guideRNAs page (http://flystocks.bio.indiana.edu/Browse/RNAi/sgrna.php).

810 Targeting adult courtship behavior to prevent the spread of CRISPR/Cas9 based gene drives. *P. Chennuri*, J. Zapletal, M. Erraguntla, M. Lawley, Z. Adelman, K. Myles Texas A&M University, College Station, TX.

Multiple studies have demonstrated the potential of CRISPR/Cas9 gene drives to suppress or replace insect pest populations. Genetic control strategies based on these types of systems are intended to be self-sustaining, spreading indefinitely through a target population. In contrast to self-limiting genetic control systems that remove themselves from the target population, CRISPR/Cas9 gene drives are theoretically less tractable, which renders such strategies more controversial and riskier. Although the development of variant alleles resistant to nuclease-cutting appears to be the main technical hurdle to truly self-sustaining nuclease-based gene drives, this was recently addressed in laboratory studies targeting the *Anopheles gambiae doublesex* gene, preventing selection of Cas9-resistant variants through functional constraint of the target sequence. Although development of a gene drive capable of collapsing populations of the human malaria vector is a laudable goal, this study has intensified debate regarding the safety of gene drive research. Previous studies have shown that *Drosophila* males hemizygous for mutations in the *yellow* gene exhibit defects in courtship behavior, placing them at disadvantage when mating with wild type females. With knowledge of these results, we investigated whether courtship behavior could be targeted to prevent the spread of a nuclease-based gene drive element upon premature release. We demonstrated defective male courtship behavior and copulation success in flies carrying a CRISPR/Cas9 gene drive targeting the *yellow* gene. The same gene drive construct quickly reached negligible levels of prevalence in population studies introducing both gene drive males (within 3 generations) and females (within 7 generations) into wild type populations. We propose that, in addition to the primary CRISPR/Cas9 target gene, incorporation of a guide sequence targeting a secondary target influencing courtship behavior would likely prevent spread through wild type populations, due to the selective advantage of wild type males in mating success. This would permit rigorous evaluation of gene drive constructs prior to removal of the safeguard sequences targeting genes influencing mating success.

811 High-throughput cardiac in vivo platform to functionally validate genome-wide candidate genes for congenital heart disease. *G. Vogler*[1], Jeanne L Theis[3], Marco Tamayo[1], Bosco Trinh[1], Maria Missinato[1], Karen Ocorr[1], Alexandre Colas[1], Timothy M Olson[2,3], Timothy J Nelson[2], Rolf Bodmer[1] 1) Development, Aging and Regeneration, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA; 2) Division of Pediatric Cardiology, Department of Pediatric and Adolescent Medicine, Mayo Clinic, Rochester, MN; 3) Cardiovascular Genetics Research Laboratory, Mayo Clinic, Rochester, MN.

Patient-specific genomics has become a major diagnostic tool for the understanding of the underlying cause of congenital heart disease (CHD). It also has the potential to predict the outcome following medical intervention and poised to help clinicians chose a specific treatment plan or follow-up protocols depending on the affected gene(s) that may pose a causal risk for CHD in the patient. The wealth of specific genetic information available for each patient is in stark contrast to the available functional data on affected genes and gene-gene interactions and presents the major hurdle in leveraging patient-specific genomics to its fullest. To close this genotype-to-phenotype gap we adapted an in vivo Drosophila heart model to allow rapid functional characterization of cardiac gene function and genetic interaction at high spatio-temporal resolution. We built this platform based on a recently published fluorescent reporter (Klassen, 2017) that specifically marks the larval and adult cardiomyocytes in Drosophila. We combined this reporter with a variety of tools that allow targeted gene-knock down and straightforward genetic testing in sensitized backgrounds with known cardiac determinants such as NKX2-5/tinman or GATA4/pannier. Following high-speed image-acquisition, the subsequent phenotypic characterization of the cardiac parameters is fully automated with unbiased extraction of physiological and structural parameters, such as contractility and heart size. This high-throughput approach allows us functionally characterize genes by RNAi and to benchmark the relative contribution of potential candidate CHD genes. The simplicity of the Drosophila genome is a unique feature of this genetic model organism, which has the benefit of permitting the rapid construction of CHD gene networks. This platform is tailored to complement currently employed alternatives, such as patient-derived iPS-cardiomyocyte cultures and genetically more buffered vertebrate model systems. Here, we present our current ongoing approach to fully functionally characterize hundreds of candidate genes for hypoplastic-left heart syndrome (HLHS), with the aim to better understand the complex etiology of this devastating CHD.

812 All *Drosophila* RNA-Seq, Re-aligned and Available on GEO: Easy to use and ready for download. *Justin Fear*, Isabelle Berger, Brian Oliver National Institute of Diabetes and Kidney and Digestive Diseases, NIH, Bethesda, MD.

Expression data from modENCODE and FlyAtlas are used by thousands of researchers on a weekly basis, but the wider Drosophila community has generated far more publically available data. Unfortunately, these data are difficult to extract, are often mapped to obsolete versions of the genome, and have been produced with different pipelines. This makes them nearly impossible to incorporate in new experiments or use as references. To address this problem, we have remapped all 14K (and counting) *Drosophila* RNA-Seq expression data to release 6 and applied extensive quality control metrics. These 275 billion reads represent 42 times more data than produced by modENCODE and are now available via a web browser. This resource is user searchable by a range of

attributes including tissues type, PubMed ID, and quality control metric. Results are displayed as browser tracks using the newly deployed NCBI Genome Viewer or downloaded as gene level coverage counts. We have also amalgamated and analyzed tissue and developmental stage datasets, where we identified highly correlated expression datasets to merge into tissue and stage specific references. Together these are valuable tools for analysing new expression data. For example, joint and individual analysis of 109 representative testes and 308 ovary samples provides important data for our work on generating tissue-specific gene regulatory networks using NetRex and provides support for our identification of hundreds of unannotated transcripts in the testis that we have validated by full-length PacBio sequencing. This is a valuable resource for geneticist and genomicists alike, and we encourage the Drosophila community to embrace their data.

813 *In-silico* definition of the matrisome of *Drosophila melanogaster*. Martin N. Davis[1], *Sally Horne-Badovinac*[2], Alexandra Naba[1] 1) Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL; 2) Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL.

In recent years *Drosophila* has become an increasingly popular model to investigate the assembly and function of diverse extracellular matrices (ECMs), including basement membrane, cuticular and non-cuticular apical matrices, the egg shell, etc. To assist in this effort, we report here the definition of the matrisome - a detailed inventory of ECM and ECM-associated genes and proteins – of *Drosophila*. We previously defined the matrisomes of humans and mice by pairing bioinformatics with proteomic screening and validation (Naba et al, 2012). This work took advantage of the conserved-domain-based organization of ECM proteins to build a list of domains (e.g. Fibronectin type I-III, Laminin G, Collagen triple helix repeats) used to interrogate the proteomes of both species and identify their ECM components. Combined with proteomic analysis and knowledge-based curation, we identified 1027 human and 1110 mouse matrisome genes. More recently we sought to define the matrisomes of zebrafish (Nauroy et al, 2018), *C. elegans* (Teuscher et al, under review), and *Drosophila*. To define the *Drosophila* matrisome, we first identified *Drosophila* orthologs to human matrisome genes using orthology prediction tools from Flybase, DIOPT, and Ensembl. In a second step, common ECM-protein domains along with domains characteristic of *Drosophila* ECM proteins (e.g. Chitin-binding domain) were used to search for additional matrisome proteins encoded by genes not identified by orthology. We then utilized Gene Ontology annotations to both identify matrisome genes missed by the first two steps and remove known non-ECM genes. As for our previous studies, searching the literature and consulting with ECM experts from the *Drosophila* community were the last steps to ensure that the final list was comprehensive and well-curated. Using this pipeline, we report that the *Drosophila* matrisome consists of approximately 585 genes. The complete *Drosophila* matrisome and bioinformatic pipeline will be available at http://matrisome.org. We hope the matrisome list will provide a valuable tool for researchers in the *Drosophila* community studying the role of the ECM in development and disease.

814 Building bioinformatics bridges: New resources at the DRSC connect related information about genes, orthologs, proteins, modifications, diseases, researchers and expression. *C.Y. Hu*[1,2], Aram Comjean[2], Verena Chung[2], Jonathan Rodiger[2], Fangge Li[2], Jonathan Zirin[1,2], Stephanie Mohr[1,2], Norbert Perrimon[1,2,3] 1) Department of Genetics, Harvard Medical School; 2) Drosophila RNAi Screening Center, Harvard Medical School; 3) Howard Hughes Medical Institute.

Over the years, the Drosophila RNAi Screening Center (DRSC) bioinformatics group has implemented a suite of informatics tools that help Drosophila researchers design experiments and analyze experimental results. Examples include our DIOPT online resource for ortholog mapping; UP-TORR and RSVP, which facilitate finding of RNAi reagents and results; and FlyPrimerBank, a resource of precomputed qPCR primer designs. We have updated many of our online resources by updating underlying data, algorithms, and gene annotations, as well as by adding additional functionality. DIOPT, for example, is now based on 17 different algorithms and includes links to our Gene2Function integrated resource. One of our newest online resources, BioLitMine, allows scientists to search the published literature with ease and identify potential collaborators. SNP-CRISPR was developed to help design sgRNAs specifically targeting SNP alleles that are different from the FlyBase reference genome. In addition, we have developed a single cell RNA (scRNA) data portal that allows researchers to mine and visualize scRNA data recently generated by the Perrimon lab. These resources facilitate experimental design, reagent identification, and data analysis, helping to accelerate the pace of fundamental and translational research studies in *Drosophila* and other species.

815 FlyBase: a valuable source of molecular interaction data. *J. Agapite*[1], G. dos Santos[1], C. Tabone[1], P. Baker[2], L. Crosby[1], K. Falls[1], J. Goodman[3], A. Schroeder[1], V. Strelets[3], D. Bean[4], the FlyBase Consortium 1) FlyBase, Harvard University, Cambridge, MA; 2) FlyBase, University of New Mexico, Albuquerque, NM; 3) FlyBase, Indiana University, Bloomington, IN; 4) EzyN, University of Cambridge, Cambridge, UK.

The knowledge of a molecule's binding partners can provide insights into that molecule's function and/or its involvement in a particular biological process. FlyBase curation of molecular interactions is primarily focused on capturing protein-protein, RNA-protein and miRNA-mRNA interactions from low-throughput studies, in which interactions are typically supported by multiple independent forms of evidence. The FlyBase molecular interaction dataset consists of a total of 42,548 interactions which represent 28,969 distinct pairwise interactions and involve the products of 5,817 genes. Importantly, the vast majority of the low-throughput studies curated by FlyBase are not curated by other interaction databases. Only 7.6% and 5.5% of publications with FlyBase curated interactions have also been curated by BioGRID and the IMEx Consortium, respectively, making FlyBase an essential source of these well-supported interactions.

816 Finding GAL4 drivers and other transgenic tools in FlyBase. *S. Gramates*[1], G. Millburn[2], J. Goodman[3], K. Falls[1], V. Strelets[3], J. Thurmond[3], D. Emmert[1], The FlyBase Consortium 1) FlyBase/MCB, Harvard University, Cambridge, MA; 2) FlyBase, University of Cambridge, Cambridge UK; 3) FlyBase, Indiana University, Bloomington, IN.

The rich genetic tool-kit that is available for Drosophila melanogaster helps to make it an ideal model organism to answer a wide range of biological questions, but also creates a potential problem - how to find the most appropriate fly line for a particular experiment. FlyBase has introduced a number of improvements to help address this question. These include the 'GAL4 etc' QuickSearch tab which allows searches for GAL4 and other binary drivers and non-binary reporters by temporal-spatial expression pattern, and dedicated 'Experimental Tool' reports for commonly used tools such as GAL4 and EGFP, which gather information on related transgenic constructs in a single webpage. Together with enhancements to hit lists, a specialized Integrated Table view hit list, and the 'Frequently Used GAL4 Driver' table, this has improved the access of these important transgenic tools to FlyBase users.

817 Finding human disease models in FlyBase: You can get there from here. *S. Gramates*[1], M. Crosby[1], J. Goodman[2], S. Marygold[3], V. Strelets[2], J. Thurmond[2], The FlyBase Consortium 1) FlyBase/MCB, Harvard University, Cambridge, MA; 2) FlyBase, Indiana University, Bloomington, IN; 3) FlyBase, University of Cambridge, Cambridge UK.

Many human diseases have been modeled in Drosophila melanogaster, and a large body of literature has accumulated in recent years. FlyBase has been curating disease models using Disease Ontology (DO) annotation and Human Disease Model reports. We provide multiple methods to access disease model data in FlyBase, including a dedicated 'Human Disease' QuickSearch tab, DO term reports, and disease model information embedded in gene and allele reports. We have organized disease model information in a highly interconnected way, so that a user who has landed on any such information can easily navigate to other related information.

818 iProteinDB: an integrative database of Drosophila post-translational modifications. *C.Y. Hu*[1,2], Richelle Sopko[1], Verena Chung[1,2], Marianna Foos[1], Romain Studer[3], Sean Landry[4], Daniel Liu[2], Leonard Rabinow[1], Florian Gnad[4], Pedro Beltrao[3], Norbert Perrimon[1,2,5] 1) Department of Genetics, Harvard

Medical School; 2) Drosophila RNAi Screening Center, Harvard Medical School,; 3) European Molecular Biology Laboratory (EMBL), European Bioinformatics Institute, Wellcome Genome Campus; 4) Department of Bioinformatics, Cell Signaling Technology Inc; 5) Howard Hughes Medical Institute.

Post-translational modification (PTM) serves as a regulatory mechanism for protein function and is critical in many signaling pathways. The best characterized PTM is phosphorylation and proteins are often phosphorylated at multiple sites. Identifying those sites that are important for function is a challenging problem, as any given phosphorylation site may or may not have functional relevance. One way to prioritize further study is to identify evolutionarily conserved phosphosites, with the idea that these are more likely to be functionally relevant sites. To facilitate such an approach, we generated a large-scale phosphoproteomics dataset from *Drosophila* embryos collected from six closely-related *Drosophila* species and built iProteinDB, a resource integrating these data with literature annotations and results from other large-scale analyses of phosphorylation in *Drosophila* and other organisms. At iProteinDB, scientists can view the PTM landscape for any *Drosophila* protein and identify predicted functional phosphosites based on a comparative analysis of data from closely-related *Drosophila* species, as well as compare results for orthologous proteins from other model organisms, including human, mouse, rat, *Xenopus tropicalis*, *Danio rerio,* and *Caenorhabditis elegans proteins. Altogether, the iProteinDB online resource can be used in a variety of ways to identify known or putative PTM sites in proteins of interest and to prioritize PTMs for further study.*

819 Analyses of the Drosophila 4th chromosome. S.L. Goldsmith[1,2], S.M. Daly[1,2], M.J. Stinchfield[1,2], *S.J. Newfeld*[1] 1) Sch Life Sci, Arizona State Univ, Tempe, AZ; 2) Contributed equally.

Our lab has taken two approaches to the 4th chromosome. The first is focused on the genetic analysis of individual genes with an emphasis on dCORL. The second results from frustrations in the first and is designed to create reagents that align the genetics of the 4th with that of the other chromosomes. We will describe one project from each approach. For dCORL, we conducted a transgenic clonal analysis of mCORL1 and mCORL2 in the third instar larval mushroom body to test the hypothesis that one of them retains all the functions of dCORL and the other has acquired neofunctionalization since the duplication. For reagents we have employed two methods, including recombination in a Bloom Syndrome Helicase mutant background, to generate 4th chromosomes that contain an FRT and Gal80. We are now testing these chromosomes for the ability to support MARCM.

820 Development and characterisation of the split-QF system for *Drosophila*. *O. Riabinina*[1], S Vernon[1], B Dickson[2], R Baines[1] 1) FBMH, University of Manchester, Manchester, GB; 2) Janelia Research Campus, HHMI, 19700 Helix Drive, Ashburn VA, 21407, USA.

Binary expression systems GAL4/UAS, LexA/LexAop and the Q-system enable labelling and functional manipulations of genetically defined subsets of cells in *Drosophila*. To decipher the neuronal basis of behaviour, it is often necessary to genetically activate or silence a single neuron. This goal is very hard to achieve, and several methods have been developed to limit expression of effectors to small specific subsets of cells. One of these methods, the split-GAL4 system, directs expression of GAL4 DNA-binding domain independently of GAL4 or another activation domain. Only where the expression patterns of two subsets overlap, the fully functional GAL4 or chimeric transactivator will be reconstituted.

Here we report the development of a split-QF system that drives strong expression, is repressible by QS and inducible by quinic acid. We qualitatively and quantitatively characterise split-QF expression in the nervous system of larval and adult *Drosophila* by GFP expression, luciferase assays, electrophysiology and behavioural experiments. We also demonstrate that the split-QF system is fully compatible with existing split-GAL4 and split-LexA lines, thus allowing for advanced intersectional experiments and greatly expanding the range of possible anatomical, physiological and behavioural assays in *Drosophila*.

821 *microPublication Biology*: publish your single experimental findings. *K.J. Yook*[1], D. Raciti[1], T.W. Harris[2], T. Schedl[3], P.W. Sternberg[1] 1) Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, USA; 2) Informatics and Bio-computing Platform, Ontario Institute for Cancer Research, Toronto, ON, Canada; 3) Department of Genetics, Washington University School of Medicine, St Louis, MO, USA.

microPublication Biology is an online, peer-reviewed, open access journal that publishes single experimental results. Our journal presents a welcome alternative in scholarly communication, expanding both the nature of published data as well as engaging more members of the science community in the publication process. Starting with articles focused on nematode biology, *microPublication Biology* has expanded to other model organism communities such as Drosophila and Xenopus, giving authors the opportunity to publish experimental observations that would otherwise remain invisible to the public – stand-alone results, negative results, experimental replications and so on. When accepted after peer-review, manuscripts are published online, and atomized data is delivered directly to the authoritative database for each community (i.e., WormBase, Flybase, Xenbase), ensuring fast integration with biological knowledgebases for deep data integration and public discoverability. Publishing in *microPublication Biology* provides a bona-fide citation (currently a DOI), that will eventually be indexed and findable in major citation indexers such as PubMed. We will present our latest publishing metrics and progress in engaging the Drosophila science community as well as showcase our first Drosophila micropublications. We have a lot to show you!

822 PhotoGal4: a new multi-purpose light-dependent switch for spatiotemporal control of gene expression. *L. de Mena*, P. Rizk, C. Cruz, P. Trejo, D.E. Rincon-Limas Neurology, University of Florida, Gainesville, FL.

Tools that enable manipulation or perturbation of gene function in a spatiotemporal manner are critical to define its contribution to normal development and disease. Unfortunately, current inducible expression systems in flies preclude accurate spatiotemporal control of gene expression and do not allow for sub-territorial manipulations within a given tissue. What if transgene expression could be manipulated a la carte with a switch triggered by light? To address this question, we developed a new and powerful photoactivable gene expression system in Drosophila referred to as PhotoGal4. The light "switch" itself is a sensitive and reversible photosensor called phytochrome B (PhyB), a cytoplasmic chromoprotein that controls growth and development in plants. In response to red light, PhyB is activated and moves to the nucleus, but it returns to the inactive state under far-red light. Thus, we assembled a single protein device consisting of several unrelated modules, based on the heterodimerization of PhyB with its cofactor Pif6. To test the system, we capitalized on the well-characterized GMR enhancer to drive specific expression to the Drosophila eye territory. Thus, we engineered flies containing all the elements required to induce transcription of genes by light, and crossed them with a UAS-GFP reporter line to test PhotoGal4 functionality. We found that upon red light stimulation, PhotoGal4 efficiently triggers gene expression in long-term ex vivo cultures of eye discs at different developmental stages. We also found that manipulation of light intensity and duration of the stimuli gives control over reporter dose response. Then, we used a 2-photon microscope and a digital micromirror device (DMD) to specifically illuminate a defined group of cells within the GMR expression domain, while keeping the rest of the GMR territory in the dark. Strikingly, we found robust GFP expression only within the restricted area of illumination.To our knowledge, this is the first time that a targeted personalized sub-pattern of gene expression is induced in a light–dependent manner within time and space dimensions. Thus, we anticipate that that PhotoGal4 will be a valuable resource for the Drosophila community to investigate complex and multistage biological, developmental and pathological processes with unprecedented resolution. This work was supported by the NIH grant NS088866 to DERL and by an HHMI-LSRF postdoctoral fellowship to LDM.

823 National BioResource Project "*Drosophila*". *K. Saito*[1], T. Takano[2], M. Watada[3], T. Awasaki[4] 1) National Institute of Genetics, Mishima, JP; 2) Kyoto Institute of Technology, Kyoto, JP; 3) Ehime University, Ehime, JP; 4) Kyorin University, Tokyo, JP.

The purposes of this program are to comprehensively maintain, manage, and widely distribute to research communities the genetic resources of *Drosophila*,

such as (1) mutant strains of *Drosophila melanogaster*, which are useful as a basis or platform for life science studies, and (2) mutant strains of the wild species of *Drosophila* or related species of *Drosophila melanogaste*r, which are important for evolution and biodiversity studies. To this end, four organizations –the National Institute of Genetics, Kyoto Institute of Technology, Ehime University, and Kyorin University –are to constitute a consortium for the joint project. The consortium aims to assume international responsibility as the fully developed, world's largest stock center by collecting the resources and improving the quality according to the needs of the times; thus we will contribute to the acceleration of leading-edge research activities in user communities.

824 A large library of UAS-human cDNA constructs and transgenic *Drosophila* stocks to facilitate translational research. *S. Yamamoto*[1,2,3], K.H. Wan[4], T. Johnson[5], H.K. Andrews[1], V.H. Bhavana[1], H. Pan[6], D. Bei[1], K. Oguz[1], S. Jangam[1], S.I. Park[4], W.W. Fisher[4], Y. Takashima[4], S. Ravani[4], M. Tomaru[7], T. Ohsako[7], M.F. Wangler[1,3,8], C. Warr[9], T. Takano-Shimizu[7], H.J. Bellen[1,2,3,6], S.E. Celniker[4] 1) Department of Molecular and Human Genetics, Baylor College of Medicine (BCM), Houston, TX; 2) Department of Neuroscience, BCM, Houston, TX; 3) Jan and Dan Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX; 4) Berkeley Drosophila Genome Project, Lawrence Berkeley National Laboratory, Berkeley, CA; 5) School of Biological Sciences, Monash University, Melbourne, Australia; 6) Howard Hughes Medical Institute, Houston, TX; 7) Drosophila Genetic Resource Center, Kyoto Institute of Technology, Kyoto, Japan; 8) Department of Pediatrics, BCM, Houston, TX; 9) School of Medicine, University of Tasmania, Hobart, Australia.

Whole-exome and whole-genome sequencing technologies have revolutionized human genomics research and clinical diagnosis, leading to identification of hundreds of new disease causing genes and pathogenic variants. At the same time, these assays are revealing thousands of variants of uncertain significance (VUS) in known disease genes that require functional assessment. Moreover, variants in genes of unknown significance (GUS) are uninterpretable due to lack of gene function information in human.

To facilitate functional annotation of human genes and variants, we are generating a large collection of *UAS* constructs and transgenic flies based on sequence validated full length human cDNAs using the *ΦC31* transgenesis system. So far, we have generated >3,000 constructs and established >1,000 transgenic lines that are available from the *Drosophila* Genomics Resource Center (DGRC) and Bloomington (BDSC)/Kyoto (DGGR) Stock Centers, respectively. These reagents can be used to study the function of a genetic variant linked to a human disease by "humanizing" the fly ortholog through the *T2A-GAL4* system (see abstract by Andrews *et al.,* Kanca *et al.* and Wangler *et al.*), or through tissue specific over-expression of reference and variant human cDNAs (see abstract by Harnish *et al.*). By further probing the *in vivo* function of these genes in flies and translating these findings to mammalian systems, *Drosophila* researchers can facilitate the understanding of biological mechanisms underlying genetic disorders in human to develop effective therapies.

825 Introducing the fruit fly as a powerful teaching tool for Nigerian high school Biology- A pilot study in Zaria. *R. Abdulazeez*[1], M Gbadamosi[2], H Badmos[3], D Shehu[4], N Danjuma[5] 1) Zoology, Ahmadu Bello University, Zaria, Kaduna, NG; 2) Neurobiology, International Centre for Genetic Engineering and Biotechnology, Trieste,IT ; 3) Biochemistry, University of Liverpool, UK; 4) Zoology, Ahmadu Bello University, Zaria, Kaduna, NG; 5) Pharmacology, Ahmadu Bello University, Zaria, Kaduna, NG.

Biology is at the forefront of our understanding of existence and environments ranging from the smallest molecules to macromolecules. It improves students' attitude towards life as they develop a sense of wonder and curiosity in the discovery of scientific processes. The mode of teaching biology in Nigeria largely remains Teacher-centered where students do not participate actively in the learning process. We report for the first time in the country to the best of our knowledge, the introduction of *Drosophila,* a successful biomedical model as a teaching tool for high school biology. Our goal was not only to introduce the use of the fruit fly but to also stimulate the teachers' and students' minds alike, exposing them to the opportunities involved in using this model to proffer a lasting solution to the persistent decline in students' biology performance. Five modules and materials taught to the authors by TReNDinAfrica, DrosAfrica and Manchester Fly Facility were used for the outreach involving 30 students and 6 biology teachers from 3 schools and 8 volunteers. A short quiz was conducted after the theoretical session and questionnaires were distributed at the conclusion of the program. Participants had a relatable experience with the flies and their reactions were astonishing and encouraging. Although *Drosophila* cannot proffer solution to all the problems of Biology education in Nigeria, we have been able to demonstrate that appropriate utilization of this model in the classroom and laboratory provides an enabling environment that is interactive and vivacious as evident from this study whose outcome indicates an openness to more innovative ways of teaching and learning as we have schools seeking further training and implementation. With the necessary support, we plan to lay this foundation in as many high schools as possible in Nigeria.

826 Leveraging a CRISPR-Cas9 undergraduate lab course to generate knock-in alleles for the research community. *A. D'Brot* Biological Sciences Department, Southern Methodist University, Dallas, TX.

CRISPR-Cas9 has become a staple in research labs, but has yet to be widely adopted in undergraduate lab courses. Here I present a rigorous CRISPR-Cas9 undergraduate lab course format adapted from Gratz et. al., 2015 that aims to crowd-source the generation of attP-DsRed knock-in alleles for the *Drosophila* research community. Students learn to design and clone their own sgRNA and attP-DsRed donor constructs, set up fly crosses and screen F1 progeny for RFP expression. Because of the modular nature of the CRISPR-Cas9 strategy used in the lab, students can be divided into several projects, each of which targets a novel gene for knock-in with an attP-DsRed cassette. This course has been designed such that it can be easily adopted into other colleges and universities or adapted as an undergraduate training program for research labs.

827 Flies across the curriculum: Engaging students in molecular biology and biochemistry lab courses in authentic research. *R.L. Kurzhals*[1], C.M. Ragain[2] 1) Department of Biology, Southeast Missouri State University, Cape Girardeau, MO; 2) Department of Chemistry and Physics, Southeast Missouri State University, Cape Girardeau, MO.

Through an interdepartmental collaboration, we have developed a two-semester authentic research experience primarily for upper-level undergraduate biology and chemistry students. The students in BI450: Investigative Molecular Biology and CH533: Biochemistry Laboratory work on an overarching research project characterizing components of the terminin complex, which is required for telomere protection. The project is broken into two individual research goals allowing students to take either individual course or both courses. In BI450, students receive cDNA of a telomere associated protein and an expression vector. The students are given the goal of inserting the cDNA into the desired expression vector. In CH533, the students start with an expression vector containing the gene that expresses one of the telomere associated proteins. The CH533 students are asked to express and purify the protein in order to set-up crystal screens by the end of the semester. Our goals in creating this collaboration were 1) increase the number of students taking part in molecular biology and biochemistry research experiences and 2) to introduce students to the close tie between molecular biology and biochemistry. In fall semesters, both courses meet once a week for a combined "research lab meeting." During our meeting, students give presentations about the progress on their individual research project as well as to take part in "journal club." Students learn how to analyze their data and prepare figures that contribute to their semester-long final research paper. We will present course creation details, system of interest, and early analysis of the course.

828 From cytogenetics to gene expression. *C. Ting* Dept Life Sci, National Taiwan Univ, Taipei, TW.

Classic genetics laboratory modules enable student ability in practice hands-on techniques. One of the traditional modules is to perform polytene chromosome of *Drosophila*. To extend this traditional modules to gene expression, we designed a module on *Drosophila* polytene chromosome. In addition to

standard polytene chromosome squashes, we included a heat shock treatment into the experimental design and asked students to compare the differences between treatments. In combination of literature reading on heat shock responses and gene expression, students can observed gene expression in action.

829 The National *Drosophila* species stock center at Cornell university. *Lidane Noronha*, Patrick O'Grady Entomology, Cornell University, Ithaca, NY.

The National *Drosophila* Species Stock Center (NDSSC) maintains a living collection of nearly 250 *Drosophila* species represented by over 1400 individual stocks. Together, these are representative of the diversity present in the genus *Drosophila*, a large group that contains about 1500 formally described species (Bächli, TaxoDros v1.04). The NDSSC is an internationally recognized biodiversity collection that serves researchers focusing on questions in evolution, ecology, developmental biology, physiology, neurobiology, comparative genomics, and immunology. Each species in the NDSSC varies with respect to the number of strains maintained, reflecting factors such as close phylogenetic relationship to species with sequenced genomes, the presence of genetically marked or transgenic stocks, the availability of stocks from diverse collection localities, and the ease with which stocks can be obtained and reared.

Late Abstracts

830 Functional analysis of *de novo* evolved genes in male *Drosophila* reproduction. *G. Mascha*, B. Kelly, P. Rumde, P. Patel, G. Findlay Department of Biology, College of the Holy Cross, Worcester, MA.

De novo evolved genes arise from previously non-coding genomic material and have potential to develop integral functions within a relatively short evolutionary time-frame. Many *de novo* genes in *Drosophila melanogaster* are expressed predominantly in male reproductive organs, suggesting roles in improving male fertility. Our lab is performing an RNAi screen to identify testis-expressed *de novo* genes that impact male fertility. To date, five such candidates have been identified. One gene, *saturn,* originated early in the evolutionary history of the *Drosophila* genus and has since been duplicated and lost in various species. Fertility assays using both RNAi-mediated knockdown (KD) and CRISPR-mediated knockout flies showed that *saturn* is required for full male fertility. Western blots confirmed that *saturn* encodes a protein, and preliminary immunofluorescence experiments suggest that the protein may be enriched in the post-meiotic testis. We also identified two distinct roles for *saturn*, as its loss both reduces sperm production in males and prevents efficient localization of sperm to the storage organs in females. *Redstone* is another newly evolved gene with enriched expression in the testis. *Redstone* KD and null males exhibit normal sperm production. However, females mated with *redstone* mutant males show deficits in both egg-laying and egg-to-adult viability. Interestingly, CRISPR-induced deletions of *redstone* failed to replicate the observed phenotypes, underscoring the importance of using multiple genetic techniques to investigate novel gene function. Nonetheless, the overall results of our screen suggest that *de novo* genes have evolved to influence multiple steps of *Drosophila* spermatogenesis. As we continue to construct and screen RNAi lines for over 50 additional putative *de novo* genes, we expect to identify more novel regulators of male fertility.

831 Unraveling the gradual evolution of Bicoid's DNA binding activity. *Y. Umezawa*, S. Small, P. Onal New York University, New York, NY.

The transcription factor Bicoid (Bcd) evolved its novel role in anterior patterning after a gene duplication event that also gave rise to its sister protein Zen. Post gene duplication, Bcd's developmental role quickly evolved, and acquired the ability to regulate more than fifty mostly conserved target genes. Our previous results suggested that most of the critical changes of the Bcd protein occurred within its homeodomain (HD), which binds directly to DNA. Specifically, the substitution of a lysine (K) into position 50 (K50) completely changed the ancestral protein's DNA-binding preference *in vitro*. However, when tested in a *bcd* gene replacement assay *in vivo*, the K50 substitution resulted in the activation of only a small subset of Bcd's target genes. In subsequent experiments, we identified additional substitutions that were required for the activation of Bcd's full complement of target genes. These results suggest that Bcd's evolution occurred via a series of single substitutions that gradually increased its ability to activate more and more target genes. How this occurred at the molecular level is not clear. Here we hypothesize that each substitution caused an increase in the number of places in the genome where Bcd can bind. To test this, we crossed flies containing partially active Bcd proteins with transgenic lines containing reporter genes driven by Bcd-dependent enhancers. Each enhancer was activated by some partially active proteins, but not others, which supports the DNA-binding hypothesis. We are currently testing this hypothesis directly using ChIP-PCR to these target enhancers, and propose to perform ChIP-Seq in the future to characterize the global binding differences between these proteins. These experiments will tell us whether the binding preference differences between differentially active Bcd variants can explain the functional divergence.

832 Amitosis of Polyploid Cells Regenerates Functional Stem Cells in the *Drosophila* Intestine. *Elena Lucchetta*, Benjamin Ohlstein Genetics and Development, Columbia University Medical Center, New York, NY.

Organ fitness depends on appropriate maintenance of stem cell populations, and aberrations in functional stem cell numbers are associated with malignancies and aging. Symmetrical division is the best characterized mechanism of stem cell replacement, but other mechanisms could also be deployed, particularly in situations of high stress. Here, we show that after severe depletion, intestinal stem cells (ISCs) in the *Drosophila* midgut are replaced by spindle-independent ploidy reduction of cells in the enterocyte-lineage through a process known as amitosis. Amitosis is also induced by the functional loss of ISCs coupled with tissue demand and in aging flies, underscoring the generality of this mechanism. However, we also found that random homologous chromosome segregation during ploidy reduction can expose deleterious mutations through loss of heterozygosity. As such, amitosis may bear ramifications, with such errors carrying profound implications on tissue homeostasis.

Although described across phyla from primitive ciliates to humans, the genetics and cell biology underlying amitosis remain largely unknown. Current work aims to determine molecular players involved in the initiation and progression of amitosis in the fly midgut, providing a foundation for future studies in other tissues and organisms containing polyploid cells, in which amitosis may bear functional significance.

833 A comparative proteomics study of the Me31B interactome reveals its dynamics during *Drosophila* germline development. A. McCambridge[1], D. Solanki[1], N. Olchawa[1], N. Govani[1], J. Trinidad[2], *M. Gao*[1] 1) Biology Department, Indiana University Northwest, Gary, IN; 2) Department of Chemistry, Indiana University, Bloomington, IN, USA.

Me31B is an essential component of *Drosophila* germ granules and plays an important role in germline development by interacting with other proteins and RNAs. To understand the dynamic changes the Me31B interactome goes through from oogenesis to early embryogenesis, we cross-linked Me31B complexes to stabilize low-affinity components and compared complexes immunopurified from ovaries and 0-1 hour embryos. We observed that Me31B interacts with RNA regulation proteins, glycolytic enzymes, cytoskeleton/motor proteins, and conserved germ plasm proteins to a different extent in the two tissues. We further show that two RNA regulation proteins, Cup and Tral, extensively colocalize with Me31B in different types of germ granules in both ovaries and early embryos. Moreover, we present Cup and Tral proteins' roles in regulating *me31B* mRNA stability and suggest that Cup may regulate the stability of a wide range of germline mRNAs. Finally, we show that Me31B-Vas interaction occurs in different types of germ granules, but the established Osk-Vas-Tud-Aub germ granule assembly pathway is not likely responsible for recruiting Me31B to the granules.

834 Investigating the requirements for the binding of the seminal "sex peptide" to sperm. *Snigdha Misra*, Akanksha Singh, Mariana Wolfner Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Statement of purpose: The Drosophila seminal fluid protein sex peptide (SP) induces post mating behavioral and physiological responses in females, contributing to reproductive success. SP's effect on females is prolonged by its retention in females due to its binding to (and gradual release from) sperm inside females. SP's sperm-binding is mediated by eight other "network" seminal proteins. It is not known, however, whether SP binding to sperm is entirely mediated by male proteins. We therefore examined the binding of SP (and the network proteins) during entire course of the sperm's journey from male to female storage, focusing on three stages- sperm in the male ejaculate, sperm in the female bursa after mating, and sperm stored in seminal receptacles of mated females.

Methods: We obtained sperm from ejaculates by subjecting Fru-Gal4/UAS dTRPa1 males to 29°. We also dissected sperm from the bursa of mated females at 35min after the start of mating (ASM) or from the seminal receptacles of mated females at 2hr ASM. We carried out immunofluorescence on the sperm

following staining with antibodies against SP or network proteins.
Results: SP binding to sperm in ejaculates was visible, though weak and patchy. The binding was stronger and smoother in the bursa, and strongest and smoothest in the SR. Some network proteins were seen bound to sperm in these stages; studies of others are ongoing.
Conclusion: Our data suggest that female molecules may promote, or affect the character of, SP binding to sperm. Our data to date do not completely rule out that time, or reproductive tract features like pH play a role. These will be tested in future experiments.

835 Developmental control of the subapical domain by the mid-blastula transition. *A. Schmidt*, J. Großhans Developmental Biochemistry, University Goettingen, Göttingen, Niedersachsen, DE.

A central feature of cell polarity is the segregation of polarity proteins and patterning of the plasma membrane into cortical domains. During early *Drosophila* embryogenesis the number of distinct cortical domains increases from one in the preblastoderm to two, cap and intercap region, in the syncytial blastoderm to finally four domains in the cellular blastoderm. Here we focus on the formation of the subapical domain, which emerges next to the apical/cap domain at the onset of cellularization. The subapical domain provides the positional information for adherens junctions, that form in the second half of cellularization and further matures later on.
Here we analyze the temporal control for the formation of the subapical domain. We found that accumulation of subapical markers depended on zygotic gene expression. This suggests that a yet unknown zygotic gene is involved in subapical domain formation. Consistently the subapical markers emerge precociously in mutants with an earlier onset of zygotic gene expression. In contrast, the subapical domain does not respond to the nucleo-cytoplasmic ratio. In haploid embryos, subapical markers are detected in interphase 14 similar to wild type, although the embryo undergoes one more nuclear division. In addition, we analyzed the position of Bazooka within the signaling pathway controlling the subapical domain. We and others have previously shown, that the GEF complex ELMO/Sponge and Rap1 act upstream of Canoe for early subapical domain localization and subapical Bazooka and Armadillo restriction during the course of cellularization.
The functional relationship of Bazooka on Canoe is not fully understood. Canoe is required for subapical restriction of Bazooka and marks the subapical domain earlier than Bazooka. However, Bazooka is also required for subapical Canoe localization at least later in cellularization, suggesting a feedback loop. Here we investigated whether the feedback loop also affects the subapical restriction of ELMO/Sponge. We tested an alternative model that Bazooka acts in parallel to Canoe with a mutual interaction. Data from the ongoing experiments will be presented.

836 Degradation mechanism of labile transcriptional repressor Blimp-1 whose degradation speed affects prepupal period. *Koichi Miyagawa Miyagawa*[1], Hamdy Aly[1], Kazutaka Akagi[2], Yuji Kageyama[3,4], Hitoshi Ueda[1,5] 1) The Graduate School of Natural Science and Technology, Okayama University, Japan; 2) Aging Homeostasis Research Project Team, National Center for Geriatrics and Gerontology, Obu, Japan; 3) Research Center for Environmental Genomics, Organization of Advanced Science and Technology, Kobe University, Japan; 4) Department of Biology, Graduate School of Science, Kobe University, Japan; 5) Department of Biology, Faculty of Science, Okayama University, Japan.

Many developmental timings of living organisms are somehow determined, but the molecular mechanism to measure a specific period during the development have not understood well. We have shown that prepupal period is determined as about 11 hours in rearing condition at 25 °C by the time measuring system which is composed of two ecdysone inducible transcription factors, Blimp-1 and βFTZ-F1 in *Drosophila melanogaster*. Blimp-1 is a transcriptional repressor and the disappearance timing of Blimp-1 determines the expression timing of the *βftz-f1* gene. Then, the expression timing of βFTZ-F1 determines pupation timing by inducing gene encoding an ecdysone-20-monooxygenase Shade, which produces active ecdysteroid 20-hydroxyecdysone from ecdysone. Consequently, degradation speed of Blimp-1 is critical factor for this timer system. Thus, we investigated the degradation mechanisms of Blimp-1. We observed that the prepupal period is elongated in some of 26S proteasome subunit mutants, and Blimp-1 degradation is inhibited by proteasome inhibitor MG132. Furthermore, prepupal period is elongated in the mutants of *pri* whose product is known as a 26S proteasome mediator for protein processing. These results suggest that Blimp-1 degrades by 26S proteasome and Pri contributes to this degradation process by controlling the degradation activity.

837 *Syd/Mapk8ip3* regulates Hippo signaling to control tissue growth. *V. Ahmad*[1], Yves C Chabu[1,2] 1) Division of Biological Sciences, University of Missouri, Columbia , MO; 2) School of Medicine, University of Missouri, Columbia , MO.

Tissue size control is fundamental to organ function and the evolution of organisms. Secreted factors globally influence organ size. However, tissue intrinsic mechanisms can override these systemic signals to ultimately control organ size. The underlying molecular details are not well-understood. We used a candidate genetic screen approach in *Drosophila* and found that the Jnk-interacting protein *Sunday driver* (Syd/Mapk8ip3) controls wing size. Knocking down Syd/Mapk8ip3 in the developing wing caused smaller adult wings. Interestingly, mechanistic studies revealed that Syd/Mapk8ip3 regulates wing size via a novel Jnk-independent mechanism: Syd/Mapk8ip3 regulates vesicle trafficking to modulate Hippo signaling, a central organ size regulator. In addition, Syd/Mapk8ip3 controls oncogenic Ras-mediated tumor growth and metastasis. Taken together, Syd/Mapk8ip3 acts independent of Jnk to ultimately control tissue size during development and tumorigenesis.

838 DNA replication stalls during S-phase in the longitudinal flight muscle of *Drosophila* spp. *C.E. Hjelmen*[1], M.E. Novak[3], E.R. Czaikowski[4], V. R. Holmes[2], J. S. Johnston[2] 1) Department of Biology, Texas A&M University, College Station, TX; 2) Department of Entomology, Texas A&M University, College Station, TX; 3) Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX; 4) IBis Northwestern University, Evanston, Illinois.

We discovered that *Drosophila* underreplicate the DNA of thoracic nuclei, stalling during S-phase at a point that is proportional to the total genome size in each species (see Johnston et al. Poster195310857). In *Drosophila* salivary glands, all of the nuclei are highly polytene and all underreplicate the heterochromatin. In contrast, half of the thoracic nuclei stall before completing one round of replication; the other half do not initiate S-phase. Our question was, "why half?" To address this question, we used microscopy and flow cytometry to compare phenotypes with underreplication percentages when two genes, Aret & Mef 2IR are knocked down. These comparisons suggest that longitudinal flight muscle accounts for the majority of the nuclei that underreplicate. With this in mind, we dissected out the different thoracic tissues and report the proportion of stalled DNA synthesis in each. Our take home is that stalled S-phase replication in the thorax is a property of the longitudinal flight muscles. We discuss how and why this tissue is a unique and powerful new tool for the study of heterochromatin formation, underreplication and DNA replication control.

839 Developmental defects in *white* mutants. *T. Lopes*, S. Redhai, C. Pilgrim, O. Riabinina, B. Chanana, I. Miguel-Aliaga MRC London Institute of Medical Sciences, Imperial College London, Hammersmith Hospital Campus, London, United Kingdom.

The ability to sense and respond to nutritional information is key to the survival of many organisms, and the intestinal tract plays an increasingly recognised role in this context. We sought to explore a possible contribution of an intestinal cell type – the digestive/absorptive enterocytes – to nutrient sensing. To this end, we knocked down putative nutrient sensors from enterocytes and used time to pupariation as a phenotypic readout, given the known regulation of developmental time by nutrition. During the course of these experiments, we became aware that larvae mutant for the *white* (*w*) gene develop slightly but significantly slower than larvae with a functional *w* gene in an otherwise comparable genetic background. Indeed, w^{1118} mutant larvae took longer to pupariate than w^{+} larvae generated by backcrossing the functional *white* gene of OrR flies into the w^{1118} background. We then investigated whether the *mini-white* (mw^{+}) gene commonly found in transgenic stocks (which lacks the majority of the first *w* gene intron and some of its regulatory region) was able to rescue the developmental delay of *w* mutants. We did so by comparing the pupariation time of w^{1118} mutants to that of several w^{1118} transgenic lines harbouring insertions with a mw^{+} gene (non-active UAS and Gal4 lines). We found that all *w* lines carrying at least one mw^{+} transgene developed faster than *w* only lines. This observation extends the number of phenotypes resulting from *w* mutation such as low copulation success or male-male courtship behaviour. Whilst the use of *w* stocks and mw^{+} transgenes will continue to provide a powerful and convenient way to generate and monitor transgenic *Drosophila*, our work underscores

the need to use relevant controls to rule out genetic background contributions, which should include exploration of whether (and how many) *mw*$^{+}$ transgenes rescue any relevant phenotypes affected by *w* mutation.

840 Muscle Atrophy in Drosophila Model of Tumor induced Wasting- Protein Homeostasis. *R.L. Silimon*[1], M. Baylies[1,2] 1) Weill Cornell Medical College , New York, NY ; 2) Memorial Sloan Kettering, New York, NY.

Cancer Cachexia is a severe muscle wasting syndrome, occurring in ~80% of cancer patients with advanced cancers. To date, there is no treatment for cachexia, and the mechanisms of muscle atrophy are poorly understood. To address these gaps in our knowledge, we are using a Drosophila model of tumor-induced organ wasting (YkiACT)[1] to investigate muscle in an environment that mimics cachexia in humans. In this model, adult flies conditionally expressing activated Yorkie (Yki) in intestinal stem cells rapidly develop intestinal tumors, and systemic wasting subsequently ensues. Additionally, YkiACT flies develop functional and physiological changes consistent with muscle wasting accompanied by the accumulation of protein aggregates. I hypothesize that perturbations in pathways regulating protein homeostasis are responsible for muscle dysfunction in YKiACT flies. I will determine temporal changes to YkiACT muscle as well as how the transcriptome differs between YkiACT and age matched control flies. Finally, I will genetically manipulate the muscle to understand how different signaling pathways contribute to muscle atrophy in this model.

841 Conserved functions of Wnk kinases in axon branch patterning and maintenance. *A. Izadifar*[1,2], J. Courchet[3], S. Sachse[1,2], A. Misbaer[1,2], J. Yan[1], D. Ayaz[2], B. Yan[4], M. Erfurth[5], D. Dascenco[2], T. Lewis [6], F. Polleux[6], D. Schmucker[1,2] 1) Neuronal Wiring Laboratory, Research Group Molecular Neurobiology (VIB-KU Leuven Center for Brain & Disease Research), Leuven, Belgium; 2) University of Leuven (KUL), Department of Neurosciences, Leuven, Belgium; 3) Institute NeuroMyoGène , Faculty of Medicine and Pharmacy, Lyon, France; 4) University of Leuven , Department of Development and Regeneration, Leuven, Belgium; 5) University of Antwerp, Center for Molecular Neurology, Antwerp, Belgium; 6) Columbia University, Department of Neuroscience, New York City, United State.

In a reverse genetic screen, we discovered that loss of *Drosophila* Wnk kinase function results in developmental axon growth and branching defects of adult sensory neurons. This neurodevelopmental function of Wnk appears to be conserved in mammals. *In vivo* knockdown of Wnk1 as well as Wnk2 in mouse cortical neurons severely affects axon extension and branching. Wnk is an essential kinase, found to be misregulated in different diseases such as hypertension, cancers and a rare type of neuropathy. Yet a neurodevelopmental role of mammalian Wnk has not been described.

We further discovered that a lack of Wnk function also leads to early onset axon degeneration in mature *Drosophila* sensory neurons as well as mouse cortical neurons. Moreover, we found that loss of Nmnat, a key regulator of axon survival, as well as overexpression of Axundead (Axed), an effector in axon degeneration, have axon branching defects that are remarkably similar to Wnk mutants. Both, axon branching defects and neurodegeneration (of Wnk mutant neurons) can be suppressed by gain of Nmnat and loss of Axed function. Conversely, overexpression of Wnk can suppress loss of Nmnat and gain of Axed. This epistasis analysis suggests an intriguing interrelation between Wnk, Nmnat and Axed, where Wnk enhances Nmnat function but also represses Axed activity. It further suggests an intriguing functional overlap between some developmental regulators of axon branching and factors involved in axon maintenance or degeneration. We will discuss this novel hypothesis and conserved roles of Wnk kinases in axon branch patterning as well as protection against degeneration.

842 A non-nuclear isoform of the Dif NFκB gene influences both circadian rhythmicity and sleep. *Nicole P. Stephens*, Thilini P. Wijeskera , Nigel S. Atkinson Department of Neuroscience, The University of Texas at Austin, Austin TX.

The body's immediate defense system, the innate immune system, is conserved across many species including *Drosophila melanogaster*. The innate immune system of *Drosophila* involves both the Toll and IMD pathways. For the Toll pathway, the terminal NFκB protein is either Dorsal or Dif (Dorsal-like immunity factor) while for the IMD pathway the terminal NFκB protein is Relish. The Dif gene, which has been shown to be important in innate immunity, is expressed in two splice isoforms, Dif A and Dif B. This study explores the functioning of each Dif protein isoform in both circadian rhythmicity and sleep. Previously, it has been shown that there is a strong bidirectional relationship between immunity and circadian rhythmicity. Age matched flies of Dif A and Dif B mutants were tested under free-running conditions and 12:12 light-dark conditions using the Drosophila Activity Monitor System (Trikinetics) to assay for locomotion. We observed that the absence of the Dif A isoform results in a significantly longer period length under free-running conditions. However, Dif B mutant flies were shown to be arrhythmic under free-running conditions and rhythmic only under light-dark conditions. Furthermore, the absence of either Dif A or Dif B differentially changed the amount and pattern of daily sleep when compared to the parental line or to a wild type Canton S line. We have demonstrated that, in adult brains, Dif B is non-nuclear (previously hypothesized based on the absence of a nuclear localization signal in the encoded protein). We have also observed that Dif B is specifically expressed in the mushroom bodies and antennal lobes of the adult brain. The mushroom bodies are known regulators of circadian rhythm and sleep in fruit flies, which suggests a role for Dif in these processes. Collectively, this study demonstrates that both the non-nuclear and the nuclear isoforms of Dif influence circadian rhythmicity and sleep phenotypes.

843 Identification of microRNAs that block TDP-43 toxicity in transgenic flies. *Alfonso Martin-Pena*, Deepak Chhangani, Diego Rincon-Limas Dept. of Neurology, McKnight Brain Institute, University of Florida, Gainesville, FL.

Abnormal distribution and phosphorylation of TAR DNA-binding protein 43 (TDP-43) are hallmarks of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). Although the mechanisms underlying TDP-43 proteinopathies are largely unknown, recent evidence suggest that aberrant microRNA (miRNA) biogenesis or function might be linked to TDP-43 neurotoxicity. Indeed, FTLD/ALS patients display abnormal expression of at least 23 miRNAs. Since the mechanisms controling these miRNAs and the identification of their bona fide targets remain to be elucidated, the overall impact of miRNAs on TDP-43 proteinopathies is largely unknown.

To shed light on this issue, we recently screened a library of 107 *Drosophila* miRNAs in a fly model expressing human TDP-43^{M337V}. This library contains miRNAs that are highly conserved throughout evolution and was recently created with an attP-based UAS vector to ensure identical miRNA expression levels from all constructs. We found that most miRNAs do not modify the TDP-43^{M337V}-induced eye phenotype or lead to severe developmental eye phenotypes when misexpressed on their own. However, we found that over-expression of mir-33 dramatically suppressed TDP-43 toxicity in the *Drosophila*eye. This is relevant because human mir-33 is downregulated in FTD patients with TDP-43 pathology. Importantly, mir-33 does not rescue eye phenotypes in fly models of other proteinopathies, suggesting that the robust ability of mir-33 to block TDP-43 toxicity is highly specific. Interestingly, we tested several mir-33 targets for their potential to mimick the rescue of TDP-43 phenotype when knocked down by expression of their corresponding RNAi contruct. These results led us to identify a potential mir-33 target with protective activity against TDP-43 insults. Remarkably, this target is a component of the extracellular matrix (ECM) glycoproteins involved in cell adhesion, signaling, and neurite outgrowth. Thus, we hypothesize that TDP-43 pathology triggers mir-33 dysfunction, resulting in ECM alterations. We are currently assessing the role of mir-33 in the fly CNS to better understand its involvement in TDP-43 pathogenesis. This work is supported by NIH grant R01AG059871 to DERL.

844 Modeling Nemaline Myopathy in *Drosophila* to identify causes and cures. *C. Zapater i Morales*[1,2], M. Balakrishnan[1,2], M. Baylies[1,2] 1) Developmental Biology, Sloan Kettering Institute, New York, NY; 2) BCMB Allied program, Weill Cornell Graduate School of Medical Sciences, New York, NY.

Nemaline myopathy (NM) is a hereditary muscle disorder that causes severe muscle weakness and can lead to death. There is currently no cure. One hallmark of NM is the presence of protein inclusions called nemaline bodies (NBs). NBs are composed of actin and Z-disc proteins, which are components of the sarcomere, the fundamental contractile unit of the muscle. Currently, there are 15 genes linked to NM and all appear associated with the sarcomere. How mutations in these genes contribute to the disease is not well understood. Our lab has shown that muscle specific knockdown of Twinstar (Tsr), the *Drosophila* homolog of the NM disease gene *CFL2*, leads to NM in *Drosophila*. Importantly, data from our lab show that a loss in muscle function correlates with specific alterations in muscle structure; we also found that enhancing proteasome function delayed disease progression in this model. To address

whether these observations are generalizable to all forms of NM, we performed a selective RNAi screen in which we targeted the putative homologous genes known to lead to NM in patients. We have focused thus far on *tropomodulin (tmod),* the homolog of Tmod and Lmod, which are, respectively, actin capping and nucleator proteins. Muscle-specific knockdown of *tmod* in larvae resulted in muscle weakness and abnormal muscle structure: specifically, we detected disarrayed myofibrils, aberrant non-sarcomeric actin filaments and internalized nuclei. Since Tmod's vertebrate homolog interacts with an E3 ubiquitin ligase complex, we investigated whether tmod is involved in the Ubiquitin-Proteasome System (UPS), and specifically, whether there are changes in ubiquitin marks in *tmod* RNAi muscle. We find a significant increase in FK2 staining, which detects single and poly-ubiquitin, compared to controls. We hypothesize that there is improper protein homeostasis as a result of tmod RNAi, which in turn, leads to decreased muscle function. Our long-term goal is to use these different *Drosophila* models to identify general mechanisms underlying NM disease formation and new therapeutic targets.

845 Temperature response properties of new thermogenetic tools in Drosophila melanogaster. *Aditi Mishra*, Benton Berigan, Marzie Amirshenava, Abbey Robinson, Mirela Milescu, Lorin Milescu, Troy Zars Biological Sciences, University of Missouri- Columbia, Columbia, MO.

Extrinsic control of neuronal activity is necessary to understand the neural processes underlying behaviour. Usually, light and temperature based tools are used to influence neuronal activity. Thermogenetics, which relies on activation of temperature sensitive proteins to alter neuronal activity is limited by the highly conserved Transient Receptor Channel proteins. Recently, we showed that the temperature sensitive gustatory receptor Gr28bD in *D. melanogaster* can be used as a thermogenetic tool. In order to increase the thermogenetic toolkit, we tested the temperature response properties of orthologs of Gr28bD from 5 other *Drosophila* species that had 80-98% sequence identity to Gr28bD. We overexpressed them pan-neuronally with *nSyb-Gal4* and assayed for temperature dependent paralysis of flies in the heat-box. The flies were subjected to temperatures of 24-40^{o}C in steps of 2^{o}C in the heat box, a high throughput machine with a resolution of 1^{o}C. They were exposed to each temperature step for 90s. Our results showed that the majority of flies overexpressing orthologs from *D. simulans*, *D. yakuba*, or *D. pseudoobscura*, which are 98, 96 and 85% identical to GR28bD were paralysed between 30 and 32^{o}C. Flies with mis-expression of *D. willistoni* ortholog, which is 81% identical to GR28bD were paralysed between 36 and 38^{o}C. Finally, flies with overexpression of *D. mojavensis* ortholog, which has 80% identity to Gr28bD were not paralyzed in our experiments, suggesting that the ortholog is not temperature sensitive within 40 ^{o}C. To test the thermosensitivity of the orthologs in a smaller neural network, we assayed for the orthologs' ability to rescue Gr28b mutant flies that have no Gr28b proteins and show deficits in avoiding noxious temperatures. We overexpressed Gr28bD or orthologs in Gr28b mutant Hot cells and observed their avoidance behaviour. In this assay, one half of a chamber in the heat box was maintained at a reference temperature of 24^{o}C while the temperature of the other half increased from 24-39^{o}C in steps of 3^{o}C. In contrast to mutant genetic controls, flies rescued with Gr28bD or orthologs in the Hot cells had significantly high positive preference indices, suggesting that they could avoid high temperatures. Additionally, we are testing the thermosensitive properties of the orthologs in *D. melanogaster* motor neurons and in *Xenopus* oocytes, a heterologous system, to explore their possibilities of being used as thermogenetic tools in other systems.

846 Development of caspase-sensitive reporters to understand macrophage-apoptotic cell interactions during *Drosophila* embryogenesis. *O.R. Tardy*, E. Armitage, H. Roddie, L. Prince, I Evans Infection, Immunity & Cardiovascular Disease - IICD, The University of Sheffield, Sheffield, Yorkshire, GB.

Apoptosis, a form of programmed Cell Death (PCD), is known to play a key role in the termination of inflammatory responses and as a modulator of immune cell activity, including that of fly macrophages. However, the limitations of fixation-based apoptotic imaging methods (e.g. TUNEL and immunostaining) restricts the dynamic-imaging based approaches necessary to study apoptotic cell clearance. Other genetically-encoded reporters, such as Apoliner (Bardet et al., 2008), require imaging in two fluorescent channels. UAS-Caspase-ON constructs (GC3ai; Schott et al., 2017) expand the repertoire of tools available to track caspase activation in real time, within an intact organism. We have characterised these constructs within the developing embryo and are using these reporters to understand how macrophages find and engulf apoptotic cells in this system. Additionally, we are generating GAL4-independent versions of these reporters.

The GAL4-UAS system has been used to drive UAS-GC3ai ubiquitously within the developing embryo; the use of GAL4-independent macrophage reporters (Gyoergy et al., 2018) enables imaging of apoptotic cell death and macrophage behaviour simultaneously. Standard molecular biology approaches were used to generate GAL4-independent versions of GC3ai, with PhiC integrase-mediated transgenesis used for site-specific integration. Genetic induction of apoptosis was achieved via heat shock of *hsp70-hid* containing embryos to test lines and assess effects on apoptotic mechanisms and macrophage behaviour. Spinning disk and Airyscan microscopy was used to image immune cell behaviours in developing embryos.

GAL4-independent and GAL4-dependent GC3Ai is functional and effectively reports caspase activation within developing embryos. Caspase activation during apoptosis can be accurately measured in real time, corresponding to morphological events such as blebbing and cellular fragmentation. The response of individual immune cells to apoptotic particles observed supports the idea that apoptotic cell engulfment requires caspase activation. We plan to combine these live imaging techniques with modelling approaches to understand how dying cells regulate macrophage behaviours in vivo.