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Abstract Book

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6 The physics of cephalic furrow formation: From cellular forces to tissue flow

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Cephalic furrow formation is a major morphogenetic process in Drosophila gastrulation, and yet, it remains poorly understood. The furrow demarcates the head-trunk boundary, a conserved feature of bilaterians, and is established by a precise molecular patterning and highly stereotypic morphogenesis. While the furrow invagination initially appears as a straight line of cells along the dorsoventral axis, it is subsequently displaced as gastrulation progresses. The dorsal region is shifted towards the posterior end of the embryo, and the ventral region moves to the anterior end. Importantly, local cell divisions (in so-called mitotic domains) occur in the vicinity of the invagination site. Later, the furrow unfolds, raising questions about its developmental role. In this work, we extract quantitative parameters of tissue flow and cell geometry on the anterior and posterior side of the cephalic furrow from Single Plane Illumination Microscopy (SPIM) recordings of wild-type embryos and of different cephalic furrow mutants. By performing laser ablation experiments to investigate the tissue properties around the furrow, we find that the strain builds up as the furrow forms, and dissipates with distance both in the anterior and the posterior tissue. On the other hand, laser cauterization assays indicate that the tissue flow is altered in an asymmetric fashion. We are investigating if and how these differences are associated with the cell divisions in the mitotic domains. This work will allow us to obtain a complete picture of the highly dynamic but transient process of cephalic furrow formation, integrating molecular patterning data with the mechanical properties of the tissues surrounding the furrow across developmental time.

7 CLAMP and Zelda function together as pioneer transcription factors to promote *Drosophila* zygotic genome activation

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During the essential and conserved process of zygotic genome activation (ZGA), chromatin accessibility must be increased to promote zygotic transcription. *Drosophila* is a well-established model used to define mechanisms for ZGA. Zelda (ZLD) is one of pioneer transcription factors (TFs) which promote ZGA in *Drosophila* embryo. However, a large subset of ZLD binding sites (60%) are highly enriched for GA-rich motifs and have constitutively open chromatin even in the absence of ZLD (Schulz et al., 2015). Therefore, we hypothesized that other pioneer TFs that function with ZLD have not yet been identified in early embryos, especially those that capable to bind GA-rich motifs, such as CLAMP (Chromatin-linked adaptor for Male-specific lethal MSL proteins).

Here, we identify the GA-binding factor CLAMP as a new pioneer transcription factor in *Drosophila*. We first characterized the phenotypic changes caused by maternal depletion of *clamp* by single molecule fluorescence *in situ* hybridization (smFISH) and immunostaining critical zygotic genes, including cytoskeletal elements and pair-rule genes. Then, we combined genomic and biochemical approaches and demonstrated that: 1) CLAMP is a novel pioneer factor which binds to nucleosomal DNA that increases chromatin accessibility; 2) CLAMP and ZLD function cooperatively to regulate transcription, chromatin accessibility and facilitate each other's occupancy at a subset of promoters; 3) When ZLD is bound to a locus but does not increase chromatin accessibility, CLAMP can often function to open the chromatin; 4) CLAMP and ZLD functioning together in zygotic transcription activation via mediate chromatin accessibility. Thus, we identified a novel function for CLAMP as a pioneer transcription factor that plays an essential role in *Drosophila* zygotic genome activation. Taken together, our study suggests that regulating the chromatin landscape in early embryos to drive ZGA requires the cooperation of multiple transcription factors in a sequential manner.

8 A valine to leucine mutation in hypomorphic *Wolbachia* CidB yields reduced deubiquitylation and cytoplasmic incompatibility

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Maternally transmitted *Wolbachia* bacteria infect about half of all insect species and often manipulate host reproduction to spread. Many *Wolbachia* cause cytoplasmic incompatibility (CI) that reduces the viability of uninfected embryos fertilized by *Wolbachia*-infected males. Two WO prophage-associated genes (*cifA* and *cifB*) expressed in males cause CI, and one gene (*cifA*) expressed in females rescues CI. This rescue from CI provides infected females a relative fitness advantage that promotes *Wolbachia* spread. CI strength ranges from very weak to very strong and determines equilibrium *Wolbachia* frequencies in nature, making it crucial to identify causes of CI-strength variation. Here, we

test the functional consequences of naturally segregating *w*Mel-like *Wolbachia cif* variation using *in vivo* and *in vitro* approaches. *Wolbachia* that infect *Drosophila yakuba*-clade hosts (*w*Tei in *D. teissieri,* (*w*Yak in *D. yakuba*, *w*San in *D. santomea*)) are closely related to *w*Mel that infects *D. melanogaster* (0.11% genome divergence between *w*Yak-clade and *w*Mel *Wolbachia*), but *w*Yak-clade *Wolbachia* cause relatively weaker CI. Only a handful of mutations distinguish *cif* loci shared by *w*Mel and *w*Yak-clade *Wolbachia* (*cid* loci), but *w*Yak-clade *Wolbachia* have a second set of *cifs* (*cin* loci) acquired horizontally from a divergent *Wolbachia* strain. To determine the contributions of *cif* copy and sequence variation to CI strength, we introduced the *w*Yak-clade *cid* loci, *cin* loci, and several individual CidB mutations independently into flies and yeast. A single mutation in the deubiquitylase (DUB) domain of *w*Yak-clade CidB (Valine to Leucine) disrupts toxicity in yeast and reduces both CI strength and *in vitro* DUB activity by ~50%. This is the first described hypomorphic *cifB* mutation characterized to reduce, but not fully eliminate, CI. These data further implicate a role for deubiquitylating activity in *cid*-mediated CI. Maternal transgenic expression of *w*Yak *cid* loci fully rescues strong CI caused by a cytoplasmic *w*Mel infection. In contrast, paternal expression of *w*Yak-clade *cifs* individually does not cause CI, indicating that interactions between *cid* and *cin* loci and/or host background effects underlie differences in *w*Mel-like *Wolbachia* CI strength in nature. Our characterization of a naturally occurring mutation that reduces toxicity and CI strength *in vivo*, and DUB function *in vitro*, contributes to our understanding of how *Wolbachia* genomic variation influences its spread in nature.

9 Remodeling of oxygen-transporting tracheoles drives intestinal regeneration and tumorigenesis

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The *Drosophila* tracheal system, as the functional equivalent of mammalian blood vessels, responds to hypoxia and transports oxygen throughout the body. Although the signaling pathways involved in tracheal development and the hypoxic response are well studied, how adult tracheae interact with their target tissues is largely unknown. Here we show that the tracheae that serve the adult intestine are dynamic and respond to enteric infection, oxidative agents and the development of gut tumors with increased terminal branching. Increased tracheation is necessary for efficient damage-induced intestinal stem cell (ISC)-mediated midgut regeneration, and sufficient to drive ISC proliferation in the absence of damage. Gut damage or tumors induce Hif 1α/Sima, which in turn stimulates tracheole branching by inducing *FGF/bnl* in the intestinal epithelium and triggering *FGFR/btl* activation in the trachea. We show that chemical or *Pseudomonas* derived ROS directly affect the trachea and are necessary for branching and intestinal regeneration. Similarly, tracheole branching and the resulting increase in oxygen supply are essential for tumor growth in the midgut. Thus, we have identified a novel mechanism of visceral tracheal-intestinal tissue communication, whereby oxidative damage and tumors induce neo-tracheogenesis in adult *Drosophila.* This process is reminiscent of cancer-induced neo-angiogenesis in mammals.

10 Obesity and Oogenesis in *Drosophila*: Increased fat storage alone does not impair fertility

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Obesity shows a high correlation with infertility; however, the potential underlying mechanisms remain largely unknown. The *Drosophila* ovary is an excellent system for studying how physiology impacts oogenesis. Our previous research revealed complex roles of adipocytes at multiple steps of oogenesis. Proteins involved in lipid metabolism are highly evolutionarily conserved between *Drosophila* and mammals, including the *Drosophila* homolog of human Adipose Triglyceride Lipase (ATGL), Brummer, which is the lipase responsible for mobilizing triacylglycerols from lipid droplets. To investigate how excess fat accumulation in adipocytes (i.e. obesity) affects oogenesis, we manipulated fat metabolism specifically in adult adipocytes and analyzed various processes in the ovary. Specifically, we used an adipocyte-specific Gal4 in combination with Gal80^{ts} to induce RNAi against several genes involved in lipid metabolism in adult female adipocytes. Adipocyte-specific *brummer* knockdown resulted in a 3-fold increase in triacylglycerol content and a dramatic increase in lipid droplet size in adipocytes, which was the largest increase in adiposity we observed. Intriguingly, however, these highly obese females did not show any significant difference in any of the oogenesis stages/processes we tested, and their rates of egg production and egg quality were comparable to those of control females. We obtained similar results in obese females generated by adipocyte-specific RNAi against *adipose*, which negatively regulates lipogenic processes. These results indicate that increased fat storage is not sufficient to cause reduced fertility. Interestingly, global *brummer* mutants had an increased frequency of degenerating vitellogenic follicles and a small reduction in egg production, suggesting that loss of *brummer* function in other cell types besides adipocytes affects oogenesis. We also tested females in which obesity was induced by a high sugar diet, which had

been previously reported to reduce egg production. We found that females on a high sugar had a comparable increase in triacylglycerol content and lipid droplets relative to females with reduced *brummer* or *adipose* function. Strikingly, however, females on a high sugar diet had a drastic reduction in egg production and quality (in agreement with previously published studies), and we also found that these females had increased early germline cyst death and vitellogenic follicle degeneration. Our future studies will investigate the underlying mechanistic basis for the ovarian differences we observe in these different types of obese females.

11 The hybrid sterility gene *Overdrive* is a necessary component of the *Segregation Distorter* system in *D. melanogaster*

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The gene *Overdrive* functions as a hybrid incompatibility allele in *Drosophila pseudoobscura*, causing near-sterility of hybrids between *pseudoobscura* subspecies. In hybrids, it also behaves as a selfish element, preferentially destroying Y-bearing sperm. We show that *Overdrive* is dispensable for viability and fertility in *Drsophila melanogaster*, and its deletion has little apparent phenotype in a wild-type background. However, it is required for the function of the selfish *Segregation Distorter* (*SD*) system. When an *SD* allele is present, the vast majority of non-*SD* sperm typically undergo developmental arrest as a result of an unknown mechanism targeting the *Responder* repeat sequence, causing the overwhelming transmission of *SD* to the next generation. But we show that in *Overdrive* nulls, the transmission of *SD* is reduced to a near-Mendelian rate, and we observe a corresponding increase in total fertility as non-*SD* sperm survive. Examination of sperm development confirms that deletion of *Overdrive* rescues the elongation and condensation defects characteristic of *Responder* sperm in an *SD* background. Our results demonstrate that *Overdrive* plays a necessary role in the enigmatic *SD* system, establish it as a contributor to two independent meiotic drive systems separated by tens of millions of years of evolution, and raise the possibility that *Overdrive* represents a "high-value target" for co-option by sperm-killing selfish elements.

12 The nuclear to cytoplasmic ratio directly regulates zygotic transcription in *Drosophila* through multiple modalities

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Early embryos must rapidly generate large numbers of cells to form an organism. Many species accomplish this through a series of rapid, reductive, and transcriptionally silent cleavage divisions. Previous work has demonstrated that the number of divisions before both cell cycle elongation and zygotic genome activation (ZGA) is regulated by the ratio of nuclear content to cytoplasm (N/C). To understand how the N/C ratio affects the timing of ZGA, we directly assayed the behavior of several previously identified N/C-ratio-dependent genes using the MS2-MCP reporter system in living *Drosophila* embryos with altered ploidy and cell cycle durations. For every gene that we examined, we found that nascent RNA output per cycle is delayed in haploid embryos. Moreover, we found that the N/C ratio influences transcription through three separate modes of action. For some genes (*knirps* and *fushi tarazu*) the effect of ploidy can be entirely accounted for by changes in cell cycle duration. However, for other genes (*giant*, *bottleneck*, *frühstart*, and *kruppel*) the N/C ratio directly affects ZGA. For *giant* and *bottleneck*, the N/C ratio regulates the kinetics of transcription activation, while for *frühstart* and *kruppel*, it controls the probability of transcription initiation. Our data demonstrate that the regulatory elements of N/C-ratio-dependent genes respond directly to the N/C ratio, through multiple modes of regulation, independent of interphase length.

13 Emergence of a smooth interface from growth of a dendritic network against a mechanosensitive contractile material

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Smoothening of raw materials allows assembly of structures and machines. Self-organized smoothening is relevant to cell and tissue morphogenesis, as well as fabricating devices with smart materials. Contraction between fixed points can straighten a material, but smooth structures also form without high contractility or specific anchorage sites. In the early Drosophila embryo, a smooth interface forms between an Arp2/3-based actin cap and a surrounding actomyosin network, demarcating the circumference of a subsequent dome-like cortical compartment. We found that interface smoothening requires Arp2/3 in vivo. To dissect the physical basis of this requirement, we generated node-based models of the networks alone and together. Simulated actomyosin networks with holes instead of Arp2/3 actin caps displayed persistent rough boundaries when actomyosin contractility was low. With addition of expanding Arp2/3 networks, network-network interfaces failed to smoothen, but accumulated myosin nodes and tension. Incorporating mechanosensitivity, by elevating myosin node activity in

response to aggregation, allowed Arp2/3 network growth to induce local contractility and smoothening of the interface. In this way, structural order can emerge from the lateral interaction of irregular starting materials.

14 Shaping the extracellular matrix through kinesin-3 and kinesin-1 driven polarized secretion

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Basement membranes (BMs) are sheet-like extracellular matrices (ECMs) found in nearly every organ in the body. In addition to providing attachment sites for cells, these structures act as a reservoir for growth factors, provide polarity information to cells, and provide mechanical support to guide tissue morphogenesis. In epithelial cells, polarized secretion of BM proteins ensures that the BM matrix assembles exclusively along the basal surface. Working in the follicular epithelium of the *Drosophila* egg chamber, our lab previously identified the GTPase Rab10 as a key regulator of sorting BM proteins into the basolateral secretory pathway. In general, basolateral cargos are thought to exit the cell near adherens junctions where Par-3 acts as a receptor for the exocyst vesicle tethering complex. Since BM proteins are designed to form networks upon exposure to the extracellular environment, we hypothesized that additional control over the site of their secretion within the large basolateral plasma membrane may be important. We have now used a combination of genetics and *in vivo* live imaging to infer the site of BM secretion and determine how this site is selected. Rab10-based BM secretion is biased to the basal-most region of the lateral plasma membrane and the basal surface. We think this bias is achieved via the activity of two kinesins, kinesin-1 and the kinesin-3 Khc-73, which transport Rab10+ vesicles along the polarized microtubule (MT) network towards the basal surface prior to secretion. Comparison of BM secretion defects and Rab10+ vesicle location and motility in different motor mutant backgrounds shows that both kinesins are needed to fine-tune the site of Rab10 localization, and likely BM secretion. When this kinesin-based transport is lost, a network of BM proteins forms in between cells, interfering with normal cell organization and movement. These findings highlight the importance of controlling the secretion site for BM proteins through the use of kinesin-driven transport, as secretion site influences the final structure of the basement membrane. A better understanding of the role of MT motor-driven transport for other cargos will enhance our understanding of how diverse epithelial tissues use polarized secretion to establish, maintain, and alter their polarized domains during development.

15 Relative contributions of Bicoid and Zelda binding sites to enhancer activity in the developing *Drosophila melanogaster* embryo

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Anterior-posterior patterning of the *Drosophila melanogaster* 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 genome architecture. Bcd is distributed as a long-range maternal gradient and activates the transcription of a large number of target genes, many of which are precisely expressed as an anterior stripe in the embryo. This spatiotemporal regulation of early developmental genes is largely controlled by the direct binding of Bcd to specific sequences in target enhancers. Instructions for the correct deployment of target gene expression are therefore dependent on variations within an enhancer sequence as well as on combinations of proteins bound to the enhancer. Perturbations in either enhancers or trans-factors (or both) affect function, disease, and evolution. The molecular mechanisms governing context-specific developmental regulation are not well understood.

All Bcd target enhancers contain different combinations and sequences of binding sites, and the arrangement of these sites does not follow an easily discernible pattern. 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 its cofactors. We modified the affinity, spacing, and number of Bcd binding sites in several enhancers. Our results define specific patterning contributions of optimal and suboptimal binding sites for Bcd, as well as contributions from the early maternal pioneer factor Zelda (Zld), that coordinate the precise timing of gene expression patterns during embryonic development.

16 Genetic analysis of hybrid male sterility between *Drosophila simulans* and *D. mauritiana*

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Two widespread patterns point to a special role for X chromosomes in speciation: Haldane's rule, the observation that hybrids of the heterogametic sex preferentially suffer sterility and inviability; and the large X-effect, the observation that the X

chromosome has a disproportionately large effect on hybrid sterility. Both of these patterns are observed in crosses between the sibling fruit fly species *Drosophila simulans* and *D. mauritiana.* High-resolution introgression analysis indicates that at least six independent X-linked regions cause male sterility when introgressed from *D. mauritiana* into a *D. simulans* background. In principle, these X-linked *D. mauritiana* alleles could cause sterility through interactions with *D. simulans* alleles located on the autosomes, the X chromosome outside of the introgressed region, or the Y chromosome. Our genetic analyses find evidence for all three types of interactions. First, we find evidence that two separate X-linked regions interact to enhance sterility. Second, we find that some, but not all, introgressed X-linked regions recover male fertility when crossed to different strains of *D. simulans*, implicating segregating genetic variation at incompatible loci that interact with X-linked *D. mauritiana* sterility factors. Third, we crossed the *D. mauritiana* Y chromosome into the *D. simulans* w^{XD1} background and discovered, contrary to previous reports, that *D. simulans* males carrying the Y^{mau} chromosome are robustly fertile. However, while w^{XD1}/Y^{mau} males are fertile, no introgressed X-linked interval recovers fertility when paired with the Y^{mau} chromosome, suggesting the incompatible *D. simulans* alleles are autosomal. However, some introgressed X-linked intervals contain multiple sterility factors, and in particular one interval includes both unknown sterility factors and the *OdysseusH* locus, previously identified as a hybrid male sterility factor in this cross. We find that $OdsH^{mau}/Y^{mau}$ males are fertile, while $OdsH^{mau}/Y^{sim}$ males are completely sterile, indicating that $OdsH^{mau}$ causes sterility through interactions with the *D. simulans* Y chromosome, which provides complementary genetic support for previously reported cytological observations.

17 Functional recovery of central nervous system in *Drosophila* adult

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Mammalian central nervous systems (CNS) exhibit limited regenerative capacity. The mechanisms of regeneration are not fully understood. We present a novel experimental design to damage the central nervous system by contusion that reproduces faithfully neural trauma in humans, while sparing other tissues. The design allows the study of cellular responses in the short and long terms. For the first time, we demonstrate that *Drosophila* undergoes spontaneous functional recovery after CNS injury. The contusion injury, however, leads to an intermediate level of functional recovery, which is ideal to screen for genes that facilitate or prevent the regeneration process. Here we focus in the immune responses of glial cells as they are key regulators of nervous system function and homeostasis. We demonstrate that glial cells and macrophages jointly contribute to neural repair through mechanisms involving JNK pathway and Draper. We show that macrophages are recruited to the injury site and are required for recovery at later stages of response. We found that in glial cells JNK activation requires Grindelwald and Draper and that Draper expression also requires JNK activation. Finally we linked neuron-glia communication to functional recovery by demonstrating the requirement of neuronal vesicular transportation for JNK pathway regulation and recovery.

18 A protease-initiated model of epithelial wound detection

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Wounds trigger surrounding cells to initiate repair, but it is unclear how cells detect wounds. The first known wound response of epithelial cells is a dramatic increase in cytosolic calcium, which occurs within seconds, but it is not known what initiates this calcium response. Specifically, what is the instructive signal created after damage that is detected by surrounding cells, causing them to increase their cytosolic calcium? To identify the mechanisms driving wound-induced calcium signaling, we monitored the calcium response after wounding *in vivo* in genetically modified *Drosophila* pupae. Using the Gal4/*UAS* system, we wounded on the border between tissue expressing a UAS-RNAi for potential genes of interest and an internal control tissue lacking knockdown, and compared the symmetry of the calcium response between the two sides. By doing this, we identified a G-protein coupled receptor acting through the Gq-signaling pathway to release calcium from the endoplasmic reticulum in epithelial cells. We also identified the pathway-initiating ligands, which are secreted into the extracellular space in an inactive/pro-peptide form and proteolytically cleaved into their active forms. Both the ligand and receptor are necessary for the calcium signal to be detected many cell diameters away from the wound margin. Furthermore, we discovered this same mechanism drives calcium responses of wing imaginal discs cultured *in vitro* when exposed to whole animal lysate. Interestingly, multiple classes of proteases are sufficient to activate the ligand and initiate this signaling cascade. We suggest that proteases released from cell lysis following damage are an instructive signal, cleaving the latent pro-ligand into its active from that diffuses extracellularly and binds to its receptor on distal cells to inform them of the presence of a nearby wound.

19 Dnmt1a is required for the maternal-zygotic transition in the wasp *Nasonia*

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DNA methylation at cytosine residues is a critical While DNA methylation is present in most insects the toolkit has been lost in the lineage leading to Drosophila. Previous experiments in the wasp Nasonia vitripennis showed that the methylation toolkit component DNA methyltransferase 1 (Nv-dnmt1a) plays an important role in early embryogenesis, and we are using this model to understand the ancestral role of DNA methylation in insect embryos, and how it was replaced in species where the toolkit has been lost. We found that embryonic lethality of Nv-dnmt1a knockdown is preceded by scattered failures of blastoderm cellularization, and subsequent failures of morphogenetic movements. Such phenotypes are typical of defects in the maternal-zygotic transition (MZT), indicating that DNA methylation may have a role in regulating this process in the wasp. Using whole genome bisulfite sequencing, we show that knockdown of Nv-dnmt1a leads to strong reduction of gene body methylation throughout the genome. Using RNAseq, we show that ~90% of genes downregulated after Nv-dnmt1a RNAi are methylated (in wild-type embryos). This is not unexpected, as it has been previously shown that insect DNA methylation is associated with efficient transcription (and not repression or imprinting). Reciprocally, ~90% of the significantly upregulated genes are unmethylated. This was a surprising result. Further investigatioe found that a significant subset of the upregulated non-methylated genes are those that are normally absent (or nearly so) in control embryos in the middle and late blastoderm stages. Since most of these nominally “upregulated” genes are expressed at high levels at earlier time points, our evidence indicates DNA methylation is important for proper clearing transcripts as development proceeds. We propose that the combined effects of subtle dysregulation of many genes after Nv-dnmt1a knockdown leads to disruption of the MZT, developmental instability, and embryonic lethality.

20 Evolution of a testis-specific centrosome gene duplication in *Drosophila willistoni*

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The centrosome is the major microtubule-organizing center in eukaryotic cells, playing important roles in cell division, polarity, and motility. The function of the centrosome changes depending on the specific collection of components it recruits. Thus, to acquire new functions during evolution, the centrosome must alter or gain components. One possible mechanism for the evolution of new centrosome components is via gene duplication. After a gene duplicates, both copies can accumulate mutations independently; thus, each copy could become specialized to perform different functions. To understand whether gene duplication played a role in centrosome evolution, we searched the genomes of 12 *Drosophila* species for duplications of 10 core centrosome genes. We found four gene duplication events in the *Drosophila* genus, including a duplication of *spindle-defective 2* (*spd-2*) in *D. willistoni*. In *D. melanogaster*, *spd-2* is expressed in both somatic and male germline cells, where it function in centrosome duplication and in the recruitment of Pericentriolar Material (PCM) required for microtubule (MT) nucleation. Given these, and likely other, Spd-2 functions, gene duplication might allow for the evolution of specialized variants of Spd-2. Our sequence analyses shows that the ancestral spd-2 gene (we named spd-2a in *D. willistoni*) is conserved across the *Drosophila* genus while the duplicate in *D. willistoni* (we named spd-2b) is rapidly evolving, suggesting functional divergence. We generated *D. melanogaster* flies expressing endogenously regulated spd-2a and spd-2b transgenes and characterized their expression patterns. We found that, similar to D*. melanogaster* Spd-2, Spd-2a, is ubiquitously expressed while Spd-2b showed testes specific expression. We hypothesized that spd2a likely maintains the ancestral function, whereas the fast evolving and testis-restricted expression of spd2b performs novel functions. We tested this hypothesis by through rescue experiments and showed that Spd-2a, but not Spd2b, rescues PCM recruitment in Neuroblasts. Conversely, Spd-2b, but not Spd2a, rescued spermatocyte meiosis defects and male fertility, indicating that spd2b is specialized for testes. These results suggest the possibility that Spd-2a has retained the somatic function of the ancestral Spd-2, while Spd-2b has specialized for germline functions. Interestingly, both spd-2a and spd-2b are expressed in testes, suggesting that the failure of spd2a to rescue testes function is a result of protein divergence . We are now working to identify the protein differences that convey this neofunctionalization using protein chimeras and mutagenesis. Altogether, our work shows how evolution by gene duplication can adapt and specialize the function of a major molecular machine to different cell type-specific contexts.

21 Defining the Mechanisms by which Canoe/Afadin Links Adherens Junction with the Actin Cytoskeleton during Morphogenesis

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Establishing tissue architecture requires apical-basal polarity establishment, cell-cell and cell-matrix adhesion, and linkage of adhesions to the actin cytoskeleton. We use Drosophila embryos to define how cells change shape and move while maintaining epithelial integrity. This requires a robust connection between cadherin-based adherens junctions (AJs) and the actomyosin cytoskeleton. This linkage is intricate, with multiple players acting in parallel. One important protein is the multi-domain linker Canoe (Cno)/Afadin. Cno is essential for many fundamental cell behaviors, including polarity establishment,

apical constriction, cell intercalation, and collective cell migration. To define Cno's mechanism of action, we are defining the role of its protein domains. Our initial hypothesis was that the small GTPase Rap1 regulates Cno localization and activity, and that Cno's PDZ and F-actin-binding (FAB) domains respectively bind AJ proteins and actin, mediating the linkage. Using CRISPR/Cas9, I engineered *cno*'s locus to reintroduce a series of mutants, deleting Cno's PDZ and FAB domains, to define how they contribute to Cno localization and function. I also created a mutant deleting the RA-domains, which bind Rap1. To our surprise, neither the PDZ nor the FAB domains are essential for adult viability. However, maternal/zygotic (M/Z) mutants expressing CnoΔFAB or CnoΔRA at reduced levels suggest both domains contribute to function, with the FAB domain playing a more critical role. *CnoΔFAB* mutants have defects in ventral furrow invagination. AJs are destabilized at tricellular (TCJs) and multicellular junctions during germband extension, and embryos have reduced epithelial integrity along the ventral midline. In contrast, *CnoΔPDZ* mutants only have subtle defects, with occasional gaps at TCJs and multicellular junctions; and delays in dividing cell's return to columnar architecture. The most striking phenotype was observed in embryos lacking the RA domains, where active Rap1 binds. Maternal/zygotic *cnoΔ*RA mutants are near null in phenotype, revealing an essential role for this domain. Surprisingly, CnoΔRA remains localized to AJs after apical-basal polarity establishment, but Cno's planar polarization to anterior/posterior borders is abolished; instead, it localizes to the dorsal/ventral edge. This research reveals that Cno is a surprisingly robust machine, linking AJs with the actin cytoskeleton during key conserved developmental processes.

22 Cell migration and alternating myosin polarity during *Drosophila* heart development

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Heart development begins with the formation of a primitive tube, as cardiac progenitors migrate from opposite sides of the embryo and merge medially. Defective cell movement results in improper heart tube formation and congenital heart defects. However, the mechanisms of progenitor movement and coordination thereof remain largely unknown. In *Drosophila*, the embryonic heart is a linear structure composed of 52 pairs of bilateral cardiac precursors (cardioblasts, CB) that migrate dorsally and medially to form a tube. We investigated the dynamics of CB migration during heart development. We developed machine learning tools to segment and track individual CB nuclei from confocal microscopy movies of embryos expressing a fluorescent nuclear reporter. We found that CB velocities oscillated between positive (forward) and negative (backward) values. The forward steps were larger in both amplitude and duration, resulting in net forward movement of the cells. In eukaryotic cells, force generation by the actin-based molecular motor non-muscle myosin II is critical for cell movements. Using quantitative time-lapse microscopy of embryos expressing a fluorescent myosin reporter exclusively in cardiac precursors, we found that myosin displayed an alternating pattern of localization between the leading and trailing ends of migrating CBs. The periodic pattern of myosin localization produced oscillatory waves that traversed the cells. Live imaging of embryos co-expressing fluorescent myosin and nuclear markers revealed that cyclic myosin flows were anti-correlated with oscillations in the position of the CB nucleus. Changes in nuclear position were preceded by changes in myosin polarity by approximately 30 seconds, suggesting that myosin-based contraction drives CB migration. Notably, the alternating pattern of myosin polarity in CBs was associated with alternating contraction of the leading and trailing edges of the cell, respectively. Furthermore, we found that myosin flows were predominantly anti-correlated between neighbouring cells. Reducing myosin motor activity by expressing a motor-deficient form of the myosin regulatory light chain in CBs resulted in increased misplacement of migrating CBs. We propose that alternating contractile forces generated by oscillatory myosin waves generate peristaltic cell shape changes that facilitate coordinated CB movement during heart development.

23 Profiling chromatin dynamics behind the replication fork

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Chromatin proteins displaced during DNA replication must rebind DNA and undergo maturation to restore steady-state chromatin structure. Old and new histones must be deposited onto nascent DNA, and histone-modifying enzymes must be recruited to modify new histones. RNA polymerases and transcription factors must also bind to reestablish the transcriptional landscape of the parental cell. Previous work from the Henikoff lab and others has demonstrated that the recovery of nucleosome depleted regions (NDRs) following replication fork passage differs among distinct species. Additionally, distinct cell types in *Drosophila* display cell type specific patterns of NDR recovery. However, it is unclear how chromatin maturation proceeds during differentiation when large-scale changes in transcription are required to enact cell-type specific regulatory programs.
To better understand the causal relationship between chromatin structure and gene regulation, we have developed Nascent

CUT&Tag, a novel technique that combines 5-ethynyl-2'-deoxyuridine (EdU) labeling of nascent DNA with antibody targeting of the Tn5 transposome to quantitatively profile chromatin features behind the replication fork. Using Kc167 cells, we profiled chromatin dynamics during steady state growth as well as during differentiation induced by the steroid hormone ecdysone. During steady-state growth, we observed enrichment of replication proteins like PCNA immediately behind the fork, depletion of RNA Polymerase II and associated histone post-translational modifications, and disruption of promoter chromatin organization. RNA Pol II and H3K4me2 became reestablished within thirty minutes, while H3K4me3 and H3K36me3 did not fully mature until six hours later. The delayed maturation of H3K4me3 and H3K36me3 suggests that iterative rounds of transcription and/or extended RNA pol II occupancy are necessary for mark maturation. We are now testing if disruption of chromatin structure by DNA replication is required to establish novel transcription programs during cellular differentiation.

24 A gap junction-mediated calcium signaling network controls stem cell fate decisions in hematopoiesis

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Stem cell homeostasis requires coordinated fate decisions amongst stem cells that are often widely distributed within a tissue. While stem cell niches orchestrate the signaling environment around stem cells to regulate self-renewal and differentiation, these signals are unlikely to be present at identical levels across an entire tissue. This raises the question of what mechanisms ensure robust fate decision within a population of stem cells. Here we show that in the *Drosophila* hematopoietic organ, the lymph gland, gap junctions form a network that coordinates fate decisions between the prohemocytes. Using live imaging of calcium signaling in intact lymph glands, we find stochastic calcium spikes in the prohemocytes that are repeatedly propagated in waves between prohemocytes. Cross correlation analysis of calcium signals reveals a network that links prohemocytes. Blocking gap junction function using chemical or genetic approaches disrupts this network, alters the pattern of encoded calcium signals, and leads to loss of prohemocytes and precocious blood cell differentiation. Ectopic and uniform activation of the calcium signaling mediator CaMKII restores prohemocyte homeostasis when gap junctions are disrupted. Overall, this data shows that gap junctions equilibrate cell signals between prohemocytes to ensure coordinated fate decisions and maintain hematopoietic homeostasis. This work suggests that repeated exchange of spontaneous signals through a gap junction-mediated network allows stem cells to form a self-organized system that drives their collective fate outcomes.

25 A conserved *trans* regulatory loop involving an odorant binding protein controls male mating behavior in flies

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A major goal in evolutionary biology has been to understand how natural variation is maintained in sexually selected and sexually dimorphic traits. The house fly, *Musca domestica*, is a promising system to understand how genotype-by-environment interactions affect polymorphism in sexually selected traits. Two common Y chromosomes segregate as stable polymorphisms in natural house fly populations, appear to be locally adapted to different thermal habitats, and differentially affect male mating success. Here, we perform a meta-analysis of RNA-seq data which identifies genes encoding odorant binding proteins (in the *Obp56*h family) as differentially expressed between the heads of males carrying the two different Y chromosomes. Differential expression of *Obp56h* has been associated with variation in male mating behavior in *Drosophila melanogaster*. We similarly find differences in male mating behavior between house flies carrying the Y chromosomes that are consistent with the relationship between male mating behavior and differential expression of *Obp56h* genes in *D. melanogaster*. We also find that male mating behaviors in house fly are affected by temperature, and the same temperature differentials further affect the expression of *Obp56*h genes. Using a network analysis, we find evidence for a sex-specific trans regulatory loop between *Obp56h* and one of the house fly Y chromosomes that is conserved between *D. melanogaster* and house fly. Our results provide a functional mechanism by which the regulatory architecture controlling gene expression affects male mating behavior, which could explain how variation in a sexually selected trait is maintained.

26 Intracellular hydrogen peroxide in hemocytes modulate JAK/STAT signaling during a systemic wound response

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Tissue injury is one of the most severe environmental perturbations for a living organism. When damage occurs in adult Drosophila, not only is there a local response of the injured tissue, but a coordinated action across different tissues is elicited to help the organism overcome the deleterious effect of an injury. This is called a systemic wound response (SWR) and is dependent on hemocyte activation. It remains unknown how SWR is induced and what the mediators are that elicit responses

in distant tissues. On injury, in different model organisms there is a rapid production of H2O2 in response to wounding. Here, we show that hydrogen peroxide is required for the activation of Toll signaling and induction of the evolutionarily conserved cytokine, upd3, from hemocytes in Drosophila. We show that the NADPH oxidase Duox is required in hemocytes to increase levels of H2O2 and in the absence of hemocyte activation to this danger signal, flies show increased lethality on injury. We provide evidence that production of the cytokine Upd3 involves the accumulation of intracellular ROS in hemocytes, which is facilitated by the diffusion of hydrogen peroxide through a newly identified channel protein, Prip. Thus, levels of ROS inside hemocytes appear as a critical mechanism that regulates the inflammatory response of hemocytes.

27 A rapidly evolving actin mediates fertility and developmental tradeoffs in *Drosophila*

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Most actin-related proteins (Arps) are highly conserved in eukaryotes, where they carry out well-defined cellular functions. *Drosophila* and mammals also encode divergent non-canonical Arps in their male germline whose roles remain unknown. Here, we show that Arp53D, a rapidly-evolving *Drosophila* Arp, localizes to fusomes and actin cones, two male germline-specific actin structures critical for sperm maturation, via its non-canonical N-terminal tail. Although we expected *Arp53D* loss to reduce male fertility, we instead find that *Arp53D*-KO males have increased fertility, both in isolation and in competition with wildtype males. In contrast, under heat stress, *Arp53D*-KO females lay embryos with reduced viability, which is exacerbated in KO embryos. Multi-generation evolutionary experiments reveal that *Arp53D* is required for optimal fitness in *D. melanogaster*. We conclude that 'testis-specific' *Arp53D* is detrimental to male fertility, but required for embryonic development, leading to its long-term retention in *Drosophila.*

28 The *Drosophila* hnRNP M homolog Rumpelstiltskin promotes barrier activity of the Homie chromatin insulator

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The 3D organization of the genome is critical for maintaining proper gene expression, but how genome organization is established during development and maintained over time is not well understood. Chromatin insulators play an integral role in controlling gene expression through the partitioning of the genome into distinctly regulated transcriptional domains. *Drosophila melanogaster* harbors several classes of insulator complexes as well as a number of associated proteins that regulate insulator activity. Homie (Homing insulator at *eve*) is a unique insulator sequence that mediates long-range interactions between the promoter of the Polycomb Group (PcG)-regulated gene *even-skipped* (*eve*) and distal regulatory elements. Previous studies have shown that Homie acts as a barrier to Polycomb spreading at *eve* and the neighboring, ubiquitously expressed gene *TER94*. However, the mechanistic basis of Homie insulator activity and particularly whether *trans*-acting factors are required are unknown.

Here we develop a novel *in vivo* reporter of Homie insulator activity and demonstrate that Homie can function as a barrier to Polycomb spreading in a genomic context separate from *eve* and *TER94.* Consistent with previous studies, we find that the orientation of Homie affects the efficiency of its barrier activity. Importantly, we show at both embryonic and larval stages that Homie insulator activity depends on Rumpelstilskin (Rump), the *Drosophila* hnRNP M homolog and an antagonist of the well-characterized *gypsy* insulator. Rump associates with the Homie insulator on chromatin, and *TER94* levels are reduced in Rump-depleted cells as expected with loss of Homie barrier function. Consistent with higher PcG repression and chromatin compaction due to loss of Homie barrier activity, we observe by 3C that *cis* looping is increased near Homie in *rump* mutants. More broadly, by ChIP-seq of H3K27me3 we find that loss of Rump alters the spread of numerous Polycomb domains across the genome, including at Homie. We demonstrate using Oligopaint DNA FISH that Polycomb domain foci are more clustered after Rump depletion, supporting a regulatory role for Rump in the 3D organization of Polycomb domains. Ongoing efforts focus on the mechanism by which Rump regulates insulators genome-wide to affect H3K27me3 accumulation, and identification of other factors influencing Homie activity. Overall, our findings identify Rump as the first known *trans*-acting factor required for Homie insulator activity and an important regulator of genome organization.

29 Epithelial cell division opens the door for macrophage tissue invasion in the *Drosophila* embryo

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Tissue-resident macrophages are indispensable in embryo- and organogenesis, and also influence the progression of major

types of tumors in both invertebrates and vertebrates [1,2]. These functions rely on their ability to invade into interfaces formed by tissues, yet how surrounding tissues influence these capabilities *in vivo* has been the focus of little study.
We study such invasive migration in the early *Drosophila* embryo, where macrophages migrate along the inner basal side of an epithelia and eventually separate this ectodermal layer from the underlying mesoderm, thus invading between these tissues [3,4]. Ectoderm-mesoderm attachment is mediated by a thin extracellular matrix (ECM). Previous experiments imply that the time for macrophage entry is influenced by the mechanical resistance of the surrounding cells [4]; however, what determines the choice of when macrophages start to invade remained mechanistically unclear.
Here we show that breaching of the ectoderm-mesoderm barrier by the first macrophage always correlates with the mitotic rounding or division of the ectodermal cell at the entry site. This correlation holds even when the timing of division is altered genetically or pharmacologically: increasing the division rate fosters invasion, and decreasing division frequency impedes it. If ectodermal divisions are completely blocked, macrophages cannot invade the tissue. It is known from *in vitro* work that cells gradually lose focal adhesions during mitotic rounding until only weak reticular contacts remain [6]. In our *in vivo* context, the facilitation of invasion by division appears to act through the dissolution of these same focal adhesions in the basally-dividing ectoderm cells flanking macrophage entry; we observe the disappearance of Vinculin-mCherry-marked ECM attachments facing the mesoderm just prior to macrophage advancement. Loosening the ecto-meso attachment by knocking down focal adhesion components specifically in the ectoderm through RNAi of Vinculin, Talin, or beta-PS Integrin facilitates macrophage invasion. Thus, we show that focal adhesions at the tissue edge prevent cells separation by a migrating cell, and that adhesion loss caused by division is required for initial breaching of this barrier.
Our study demonstrates how cell division at a tissue edge influences cell invasion into a confined environment *in vivo.* These results may also be relevant for immune cell infiltration of solid tumors and cancer cell invasion into confluent tissues.
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30 Non-canonical Hh signaling directs germ cell migration through regulating PI(4,5)P_2 and actin dynamics

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Directional cell migration is a complex process requiring coordinated changes in signal transduction, cytoskeletal structures and membrane organization. *Drosophila* embryonic germ cell (GC) migration has served as an ideal model for studying directional cell migration. Whereas two distinct guidance mechanisms have been proposed in GC migration (the attractant model with Hedgehog (Hh) signaling vs. the repulsion model with lipid phosphatases), how these signals regulate cytoskeletal changes that power germ cells to navigate their migration is not understood well. Here, we report a set of findings that support a role for Hh signaling to guide GC migration by stabilizing polarized actin assembly through the GPCR Tre1 and its downstream effectors. GCs null for Tre1 are fully capable of migrating but cannot navigate. Tre1 induces local PI(4,5)P_2 synthesis by recruiting dPIP5K, a *Drosophila* PI4P 5-kinase. WASP, an actin nucleation promoting factor activated by PI(4,5)P_2, is required for directional GC migration and localizes at the F-actin enriched protrusions in GCs. F-actin protrusions were diminished in Tre1 mutant GCs, together with loss of local concentrations of dPIP5K and PI(4,5)P_2. Smo, the signal transducing GPCR in Hh signaling, is enriched at the F-actin protrusions in GCs and increases Tre1 localization on the plasma membrane and Tre1 binding to dPIP5K. Taken together, these results indicate that Hh signaling steers GC migration by regulating Tre1 localization and PI(4,5)P_2 synthesis to generate the F-actin protrusions that “pull” migrating GCs. These findings have potential clinical implications for Hh-related diseases, including cancer metastasis.

31 Host chromatin environment shapes the evolutionary dynamics of transposable elements

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Transposable elements (TEs) are genomic parasites that can increase their copy number at the expense of host fitness. To prevent the selfish increase of TEs, hosts can epigenetically silence them by depositing repressive heterochromatic marks at euchromatic TE sequences. However, these repressive epigenetic marks at TEs were shown to spread to adjacent functional sequences, changing the chromatin states of genes and impairing host fitness. How to strike a balance between the benefits of TE silencing and minimizing the inadvertent spreading of repressive marks is an important, but still largely unexplored, question for the evolution of TEs and their host genomes. We investigated the evolution of TE-mediated spreading of repressive marks in six closely related *Drosophila* species. Despite their close relatedness (diverged ~ 10 million years ago),

these species have highly variable TE content (from ~3% to ~12%), offering a powerful system to address our question. We identified euchromatic TE insertions from PacBio assemblies and studied the deleterious epigenetic effects of TEs using epigenomics targeting H3K9me2, a repressive heterochromatic mark. We found that the tendency and extent of H3K9me2 spreading from euchromatic TEs significantly vary within and between species. The magnitude of H3K9me2 enrichment around euchromatic TEs negatively correlates with the number of TEs across genomes, even after controlling for the clade effect. This observation suggests a model that more extensive spreading of silencing marks from TEs would result in stronger selection removing TEs and, accordingly, a lower genomic TE content. To test this hypothesis, we are inferring the strength of selection against TEs from TE frequency spectra. Interestingly, between-species variation in the spreading of repressive marks from TEs significantly correlates with the expression levels of genes known to enhance heterochromatin spreading (Su(var)s). In particular, the expression of genes in the nucleosome remodeling and deacetylase (NuRD) complex, which deacetylates H3K9ac and enables H3K9 methylation, is strongly associated with the levels of H3K9me2 enrichment around TEs. We are experimentally investigating how changes in expression levels of these genes may impact TE-mediated spreading of repressive marks. Our investigations point to the importance of the host epigenetic environment in shaping TE evolutionary dynamics, which ultimately impacts the evolution of host genomes.

32 Intestinal progenitor P-bodies maintain stem cell identity by suppressing pro-differentiation factors

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Post-transcriptional gene regulatory mechanisms allow cells to rapidly respond to extracellular fluctuations independent of nascent transcription. However, the role of these mechanisms in adult stem cell population maintenance and lineage commitment remain poorly characterized. In this study, we address this question by investigating the role of processing bodies (P-bodies), a key site of post-transcriptional regulation, in intestinal stem cells of adult Drosophila. We report that, in contrast to neighboring differentiated cells, these progenitor cells possess P-bodies that contain Drosophila orthologs of canonical mammalian P-body components DDX6, EDC3, EDC4 and LSM14A/B and are ultrastructurally organized in a "core-shell structure". Using a targeted RNAi screen, we identified >100 genes that affect normal P-body morphology including *Patr-1*, a gene that we found to be necessary for mature P-body assembly. Using both verified *Patr-1* RNAi strains and newly generated *Patr-1* loss-of-function alleles, we show that P-body assembly defects correlated with loss of intestinal progenitor cells. Additional experiments validated that the precocious progenitor-to-EC differentiation was the leading cause of progenitor cell loss, and that this phenotype could be rescued by overexpressing *escargot*, the well-known transcriptional repressor of pro-differentiation genes in intestinal progenitor cells. Both transcriptomic and single molecule *in-situ* hybridization analyses showed that pro-differentiation transcripts were significantly upregulated in *Patr-1* mutant progenitor cells and that this upregulation was likely independent of *escargot*. Interestingly, these experiments revealed that rather than being completely absent, pro-differentiation transcripts were weakly present in wild type intestinal progenitors suggesting that the *escargot*-mediated transcriptional repression of these genes was not complete. Although transcripts were present in both genotypes, protein products of these pro-differentiation genes were observed only in *Patr-1* mutant progenitor cells indicating that these transcripts were post-transcriptionally regulated in wild type cells. Therefore, we propose that weakly transcribed pro-differentiation genes are targeted to P-bodies for translational repression and/or degradation, and that the absence of mature P-bodies in *Patr-1* deficient progenitor cells derepresses these transcripts to undergo translation and drive premature differentiation. Taken together, this work delineates a novel P-body-dependent post-transcriptional regulatory mechanism of pro-differentiation genes to ensure proper adult progenitor cell maintenance.

33 Genetic variation in female control of mating plug ejection in *D. melanogaster*

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Polyandrous females have the opportunity to select the paternity of their offspring by exerting sperm-precedence for and against particular mates. One avenue through which females can do this is by controlling how soon after mating she ejects the mating plug that contains her mate's sperm and seminal proteins. Mating plugs form in most female insects soon after mating begins. In *D. melanogaster*, the mating plug is ejected from the female reproductive tract within five hours of mating. The timing of this ejection affects the male's reproductive success: earlier ejection gives fewer of his sperm the chance to be stored, for example, and the effect is magnified in a multiply mated female. Using the DGRP, we tested for genetic variation in the timing of mating plug ejection by females. We assessed the time of mating plug ejection by females from 30 DGRP

lines after mating to a standard male. We saw significant variation in mating plug ejection time across the lines. A genome-wide association study reveals, among others, candidate genes with neural functions. This is consistent with previous studies that reported a role for the female nervous system in mating plug ejection. We are validating these results by knocking down each candidate gene with RNAi and assessing their effect on mating plug ejection. Our long term goal is to understand the evolutionary histories of the genes and neural circuits that control mating plug ejection, and thereby sperm precedence mechanisms by the female.

34 Image Award Presentation

Image Award Presentation

35 Zooming in on gonadogenesis

Brian Oliver[1] 1) National Institutes of Health, Bethesda MD.

The most significant differences in the genome (XX or XY) and in gene expression amoung members of a population relate to sex. We explored these differences is sex-biased expression in whole animals, tissues, and more recently using single cell methods. Gene expression in the testis is particularly remarkable for the inactivation of the X and 4th chromosomes in primary spermatocytes and activation of Y-linked gene expression. In developing gonads, both the germline and somatic line cells need to know their sex in order to ultimately produce sex-specfic gametes. The soma provides sexual cues to the germline and the germline autonomously reads the sex chromosome karyotype. Mismatches in sex due to dsirupting somatic sexual identiy results in massive disregulation of gene expression. Morpholgically, cell contacts amoung germline and somatic cells is disrupted.

36 The evolution of novelty by small steps and giant leaps: a tale of two toxins

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Evolutionary biology finds itself in a golden age. The genomics revolution and new genome editing technology are providing answers to major questions in evolution that were out of reach even a few years ago. This profusion of information is difficult to integrate with existing theory. On one hand, the Modern Synthesis posits that adaptation proceeds through small, gradual steps up adaptive peaks, a view largely supported by these new data. On the other, horizontal gene transfer, hybridization, and symbioses result in large, sudden leaps to previously inaccessible fitness peaks, processes largely ignored by the Modern Synthesis. I will share two empirical studies from my laboratory that suggest these seemingly disparate adaptive processes are two sides of the same coin. Diverse insects have co-opted two different toxins—heart poisons from plants and DNAses from bacteria—as defenses against natural enemies. I will show how these remarkable adaptations require both small steps and giant leaps to explain their origin and elaboration.

37 The evolution of coloration and color vision in butterflies

Adriana Briscoe[University of California, Irvine] 1) University of California, Irvine.

Among terrestrial animals that use color as a social signal (unlike aquatic animals), there is surprisingly little evidence for the correlated evolution of color vision and coloration. Here I describe the exceptional color vision system of *Heliconius* butterflies, a genus of neotropical butterflies that evolved a unique ultraviolet opsin via gene duplication and adaptive evolution. At the same time this new photopigment gene emerged in *Heliconius*, these butterflies evolved a new 3-hydroxy kynurenine-based yellow coloration on the wing. Intracellular recordings demonstrate that *Heliconius* eyes contain at least five spectrally-distinct classes of photoreceptor, including ultraviolet-, violet-, blue-. green- and red-sensitive photoreceptors. Behavioral experiments further demonstrate the evolution of two ultraviolet opsins in *Heliconius* has paved the way for the evolution of enhanced ultraviolet color vision in these butterflies in females, perhaps associated with feeding behavior, while visual modeling suggests the evolution of the new violet receptor, has enhanced male vision for yellow color discrimination. Taken together, these results suggest the evolution of a new color receptor in the eye of *Heliconius* together with new yellow coloration on the wing evolved rapidly as the result of both natural and sexual selection.

38 The Bloom syndrome helicase trilogy

Jeff Sekelsky[1], Melissa Adams[1], Colette Anikwue[1], Michaelyn Hartmann[1], Talia Hatkevich[1], Corbin Jones[1], Kathryn Kohl[1], Susan McMahan[1], Mitch McVey[1] 1) University of North Carolina.

Crossovers are essential in female meiosis to promote proper chromosome segregation, but are avoided in mitotic cells to preserve genome stability. I will describe roles of Blm helicase in both processes, using the Dark Knight trilogy as a model.

1: Blm Begins. Bloom syndrome is associated with high rates of cancer. Cells lacking Blm helicase have elevated spontaneous mitotic crossover, suggesting an anti-crossover function in DNA repair. Synthesis-dependent strand annealing (SDSA), a model developed in Drosophila, is a major non-crossover pathway for repairing DNA double-strand breaks (DSBs). Using a P element excision assay, we showed that Blm promotes SDSA, presumably by dismantling recombination intermediates. Thus, Blm is a crossover-fighting hero.

2. "You either die a hero or you live long enough so see yourself become the villain." Meiotic recombination also uses DSBs. Most DSBs are repaired as noncrossovers (NCOs) through Blm-dependent SDSA. At sites selected to become crossovers (COs), Blm is a villain that must be thwarted. In other organisms, crossover-designated recombination intermediates are stabilized by Msh4-5…but higher flies lack these proteins. We showed that the meiMCM complex (Rec, Mei-217, and Mei-218) evolved under positive selection to promote meiotic crossovers; we hypothesized it does this by stabilizing recombination intermediates to block Blm from making NCOs.

3. Blm Rises. In somatic and male germline cells, Blm absence leads to elevated COs; however, in female meiosis, COs are reduced in Blm mutants. We showed that these COs do not use the meiotic machinery and they lack all hallmarks of meiotic COs, including loss of both interference and centromere reduction…we even get COs on chromosome 4! We conclude that Blm is necessary to make meiotic recombination meiotic, and that these mutants are really using mitotic repair pathways: Blm was never really the villain, just misunderstood.

Sequel: A tale of the role of mei-MCM in meiotic crossover designation and crossover interference is in production. The mechanism of crossover interference has been mysterious since Sturtevant described it more than 100 years ago. Recent research in the lab of Abby Dernburg suggested that the synaptonemal complex that forms between paired meiotic chromosomes has liquid crystalline properties, and that recombination proteins diffusing within the SC and be ejected through dewetting. We are testing a model in which an anchored kinase phosphorylates mei-MCM to produce a biomolecular condensate that Adelaide Carpenter termed the recombination nodule. This process results in crossover-designated sites being widely separated, thus explaining crossover interference.

39 Tdrd5l defines a novel germline granule that regulates distinct aspects of germline differentiation

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Tudor-domain containing proteins are conserved across the animal kingdom for their necessary functions in germline development. Recent work in our lab identified Tudor domain containing protein 5-like (Tdrd5l), which promotes male germline identity and spermatogenesis in the testis, but is repressed by the RNA binding protein Sex lethal (SXL) in female GSCs. Tdrd5l is one of the fly homologs of mouse TDRD5, which is also required for spermatogenesis. Tdrd5l localization is unique in that it forms hollow spheres that can be either perinuclear or cytoplasmic. Our hypothesis is that Tdrd5l coats the outside of a novel membrane-less organelle/RNA granule. Tdrd5l granule are embedded in Vasa-positive nuage, but nuage components are excluded from the body, suggesting an independent function. Consistent with this, *Tdrd5l* mutants have no change in transposon expression which is regulated by nuage-dependent piRNAs. Tdrd5l bodies can also associate with Decapping protein-1 positive P-bodies, supporting a role in posttranscriptional regulation of RNA.
Tdrd5l is also expressed in the later germline in both males and females, indicating that it may act to control germline differentiation in both sexes. Consistent with this, Tdrd5l bodies are found in nurse cells and oocytes in developing egg chambers. As this is when maternal RNAs are being transported from nurse cells to oocytes, and are subjected to strict translational control, we examined the key maternal determinants. Strikingly, we find that in *tdrd5l*-mutants the dorsal determinant Gurken (Grk) accumulates in nurse cells where it is normally repressed. However, the distribution of *grk* mRNA remains unchanged. We also see a strong decrease in hatching rate of embryos from *tdrd5l*-mutant females, and these eggs display dorsal appendage defects characteristic of *grk* mutants. *grk* translation in the oocyte is activated by Orb (oo18 RNA-binding protein), and the absence of Orb in nurse cells is important for the lack of *grk* translation there. In *tdrd5l* mutants, we see ectopic Orb expression in nurse cell suggesting that this is the basis for the ectopic Grk expression. *tdrd5l* mutants also exhibit premature Oskar translation in the oocyte that is mis-localized to the middle of the oocyte. This could mean that *osk* is also a target for regulation by Tdrd5l, or that the defects in Osk are due to anterior-posterior defects in the oocyte that can be caused by loss of *grk*. Lastly, we see no change in Nanos protein in the oocyte, but see an increase in Nos expression earlier in developing germline cysts of the germarium, suggesting that the Tdrd5l body can also regulate gene expression in this context. Overall, we propose that the Tdrd5l body is a novel RNA granule regulating post-transcriptional gene regulation in the germline, including male identity in undifferentiated germ cells and the progression of both spermatogenesis and oogenesis

during germline differentiation.

40 A coordinated cellular program to boost mitochondrial energy production powers pioneer immune cell tissue invasion

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Adaptation is a critical feature of cells migrating through physically challenging contexts in development, inflammatory responses and tumor metastasis. However, how cells can produce the increase in energy required during such *in vivo* cell invasion is still unanswered. Here, we identify a novel conserved metabolic switch in *Drosophila melanogaster* immune cells or macrophages that by enhancing their bioenergetic potential enables developmentally programmed tissue invasion. We show that this regulation requires a novel conserved nuclear protein, named Atossa. Atossa enhances the transcription of a set of proteins, including two metabolic enzymes and an RNA helicase Porthos, each of which increases the tissue invasion of leading *Drosophila* macrophages and can rescue the *atossa* mutant phenotype. Porthos selectively regulates the translational efficiency of a subset of mRNAs containing a 5'-UTR cis-regulatory TOP-like sequence. These 5'TOPL mRNA targets encode metabolic enzymes and mitochondrial-related proteins, including subunits of mitochondrial oxidation phosphorylation (OXPHOS) components III and VI. We show that Porthos powers up mitochondrial OXPHOS to engender a sufficient ATP supply, which is required for the tissue invasion of leading macrophages. We find that Atossa's mammalian orthologs, known to be enriched in vertebrate immune cells, maintain its regulatory capacity. Our elucidation of a conserved program that increases the energy state underlying immune cell tissue infiltration may allow modulation of immune responses involved in disease.

41 Specific mutation patterns shape Y chromosome evolution in the *Drosophila simulans* clade

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Y chromosomes across diverse species groups share features in that they are gene poor, rich in repetitive elements, and heterochromatin structure. Despite this degenerate structure, Y-linked variation often impacts male fertility, aging, and fitness. Sexual antagonism and a loss of recombination play major roles in the degeneration of young Y chromosomes. However, the processes shaping the evolution of mature, already degenerated Y chromosomes are largely unexplored. Recent studies demonstrate that Y chromosomes across diverse species evolve a similar organization that includes an enrichment of duplicated genes, LTR transposable elements, and satellite DNA. The mechanisms driving this convergent structure are not well understood. Because Y chromosomes evolve rapidly, it is challenging to study their evolutionary history without comparing closely related species. Here, we generated *de novo* long read assemblies complemented with cytological validation to reveal Y chromosome organization in three closely related species of the *Drosophila simulans* complex, which only diverged 250,000 years ago. Although these species share >98% sequence identities, we found that their Y chromosomes are divergent, harboring different repeat units and transposable elements. We also discovered the recent birth of high-copy number (40–150) gene families specific to the Y chromosomes of the *D. simulans* complex. Our phylogenic analyses detected signatures of positive selection and rampant gene conversion on Y-linked ampliconic gene families. In addition to high duplication and gene conversion rates, we found that Y chromosomes are also enriched for large deletions. These mutation patterns suggest that the repair of double-strand breaks on Y chromosomes may be biased toward microhomology-mediated end joining over canonical non-homologous end-joining. We propose that these repair-related properties are general mechanisms contributing to the parallel evolution of Y chromosome organization across animals.

42 Positioning a stem cell niche during organogenesis

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Stem cells are required for tissue homeostasis, and for regeneration after damage or due to aging. Accomplishing such tasks often requires intimate association between stem cells and their niche. Unfortunately, we know little about how niches are positioned within tissues during development. We study the testis, a paradigm in niche-stem cell biology, and have succeeded in live-imaging niche development, which occurs when the gonad first forms (Anllo et al., 2019). Just before niche formation, the gonad is a sphere containing an interwoven mix of somatic cells encysting neighboring germ cells. We found that niche progenitor cells extend protrusions to pull themselves out to the gonad periphery, where they migrate anteriorly along extracellular matrix toward the anterior. Imaging also revealed that the niche assembles with a stereotypic inward tilt with

respect to the body axis, suggesting that some tissue outside the gonad directs niche position during development. Learning these dynamics has motivated us to seek the extrinsic cues and intrinsic factors necessary for niche morphogenesis. To identify a source for the extrinsic cue, we genetically ablated various tissues adjacent to the gonad. We found that niche assembly was disrupted when the visceral mesoderm (Vm) was removed, and in particular the pro-niche cells that are specified furthest from the gonad anterior were unable to reach the proper location. Without Vm, disrupted niches also failed to properly polarize F-actin, and the adhesion protein E-cadherin. Interestingly, well before niche assembly we found that pro-niche cells were in direct contact with a subset of Vm cells, suggesting that the extrinsic cue could be contact-dependent. We have evidence that *slit* and FGF ligands, both expressed in Vm, are each important for niche assembly. While exploring extrinsic cues, we have been identifying factors required intrinsically for niche formation. The transcription factor *islet* regulates cell guidance response in neurons, and our evidence of *slit* involvement in niche formation motivated us to test a similar role for *islet* in pro-niche cells. We found that *islet* was expressed in niche cells, its expression was dependent on the Vm and the ligands *slit* and FGF, and the niche did not assemble properly in *islet* mutants. This research is among the first describing how the niche is positioned correctly during its development, and bridges an important gap in our knowledge of stem cell-niche biology.

43 Dissecting cell mechanisms of tissue fluidity using optogenetic manipulation of Rho activity

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Epithelial tissue sheets can be shaped into complex forms through series of stretching, flowing, and folding events, orchestrated by sequences of myosin-generated forces that are patterned in space and time. In one dramatic example, the rapid tissue flow that elongates the *Drosophila* embryonic body axis requires a planar polarized pattern of myosin II regulated by the Rho/Rho-kinase signaling pathway. Changes in cell tension and cell shape have been predicted to influence the ability of a tissue to remodel and flow during specific developmental events. However, is not well understood how different patterns of actomyosin contractility and tension influence distinct aspects of cell behaviors that contribute to tissue fluidity. Here we use powerful optogenetic tools to manipulate Rho1 signaling in the germband epithelium of the *Drosophila* embryo to achieve rapid changes in patterns of actomyosin contractility across the tissue. The ability to flexibly induce these changes in the germband allows us to analyze the effects of distinct myosin patterns and dynamics on contractile tensions, cell shapes, and tissue fluidity during convergent extension. We find that optogenetic activation of Rho1 at the apical cell membrane is sufficient to transform a planar polarized myosin pattern into one reminiscent of the radial patterns observed in some epithelia prior to invagination. This change is associated with altered force distributions in the tissue, characterized by increased isotropic tension. In contrast, optogenetic deactivation of Rho1 decreases junctional and medial myosin accumulation and reduces tension. Both perturbations (activation/deactivation) are sufficient to override endogenous myosin planar polarity in the tissue, reducing tension anisotropy, cell rearrangements, and tissue flow during convergent extension. However, these two perturbations have distinct effects on cell shapes and active fluctuations, revealing distinct pathways to disrupting tissue fluidity that are consistent with recent theoretical predictions for solid-fluid transitions in anisotropic tissues. These studies directly link Rho1 activity to myosin-generated force patterns and tissue flows during epithelial morphogenesis, revealing how actomyosin contractility and mechanical tension control the onset of rapid cell rearrangements and tissue flow during convergent extension.

44 Maternal transcripts and their regulation are highly conserved across *Drosophila.*

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Across multicellular animals, development originates from gene products that mothers deposit into eggs. These maternally provided mRNAs and proteins direct the critical processes of early development and set up trajectories that can profoundly affect the organism throughout its lifetime. Previously, our lab determined that maternal transcripts are highly conserved across *Drosophila*, and more highly conserved than zygotic transcripts following activation of the zygotic genome. This was surprising as the maternal transcriptome is produced during oogenesis by polyploid nurse cells rapidly transcribing a large amount of RNA which mechanistically does not seem to lend itself to precision. Additionally, maternal transcripts are subject to considerable post-transcriptional regulation, thus the transcript level does not need to be the target of selection to achieve conservation. To investigate this apparent contradiction, we needed to understand more about the mechanistic basis of regulation of the maternal genome across species. We used a large comparative RNA-Seq dataset across 14 Drosophila species and crosses between closely related species to find multiple lines of evidence that maternal transcription across species is largely regulated at the level of chromatin. We found enrichment across species of motifs for factors demonstrated to associate with topologically associated domains, an enrichment of these factors in ovaries, and evidence that co-transcribed maternal genes are highly co-localized in groups on chromosomes. In general, we find that maternal regulation itself is highly conserved, with one notable exception – in two species of the *D. pseudoobscura* species subgroup we also find motifs for novel

regulators, which are associated with transposable elements specific to this species group. While most maternal transcripts are conserved, for those maternal transcripts that change in representation between species we show that this is due to changes in *trans* regulation, and that the important *trans* regulators are those involved in chromatin state regulation. Armed with this evidence, we propose that the maternal contribution to the egg may be highly conserved at the transcript level because of the limitation of the low precision regulation at the level of chromatin. If evolutionary novelty is to be produced for maternal protein representation, selection on the post-transcriptional processes, rather than transcription, may be the ideal way to target individual genes.

45 Regulating the fusion pore of giant exocrine vesicles

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A distinct class of exocrine tissues utilize exceptionally large, actin-coated vesicles- reaching up to ten microns in diameter- for secretion. These secretory systems are faced with unique mechanistic challenges that do not apply to exocytosis via the far smaller secretory vesicles (<100 nm) employed in more conventional settings (e.g. synaptic neurotransmission). We used mucin ("glue" protein) secretion by *Drosophila* larval salivary glands, to elucidate the mechanisms underlying fusion pore formation and maintenance in large secretory vesicles. By using super resolution live imaging of secreting salivary glands, we observed that- unlike for smaller vesicles- the fusion pore of large vesicles initially expands up to 3 µm to provide a stable opening during content release, and subsequently constricts back to less than 1 micron within 3-4 minutes. Since constricting a membrane tube requires considerable energy input, we hypothesized that a dedicated protein machinery mediates this phenomenon. To identify this machinery, we performed a candidate gene-based knockdown screen, and identified several conserved proteins from the BAR-domain superfamily, that act as key regulators of pore dynamics. Three major knockdown phenotypes were observed, highlighting distinct stages in pore formation: A "kiss and run" phenotype indicated that stabilization of the pore immediately after fusion is required; Conversely, a "full collapse" phenotype, that does not require actomyosin for content release, demonstrated that pore stabilization during secretion is essential; Finally, a dramatic compound fusion phenotype suggested that pore components normally limit the diffusion of fusogenic proteins from the apical surface to the vesicle membrane. The machinery that builds and maintains the fusion pore insulates the vesicle membrane from the apical membrane, thus providing a novel mechanism for membrane homeostasis during extended secretion.

46 Exploring the role of dynein in transporting *cen* mRNA to the centrosome

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The centrosome functions in diverse roles such as nucleating and organizing microtubules, facilitating ciliogenesis, and anchoring the bipolar mitotic spindles during cell division. These centrosomal functions are regulated by the pericentriolar material (PCM), which is composed of a protein matrix and mRNAs. The functional significance of mRNA at the centrosome is of emerging importance. Within *Drosophila* syncytial blastoderm embryos, *centrocotin* (*cen*) mRNA is one component of the PCM that forms micron-scale RNA granules near the centrosome in a cell cycle dependent manner. *cen* mRNA granules also contain Cen protein and a translational repressor, Fragile-X mental retardation protein (FMRP). Recently, we showed localization of *cen* mRNA to centrosomes facilitates error-free mitosis. However, how *cen* mRNA localizes to the centrosome is unknown. Preliminary data from immunofluorescent imaging combined with smFISH reveals *cen* mRNA decorates astral microtubules. Further, biochemical studies show that Cen protein interacts with the dynein motor complex. Taken together, these data suggest that *cen* mRNA is transported to the centrosome via dynein-directed trafficking along microtubules. To test this hypothesis, *cen* mRNA localization was quantified in mutants and CRISPR-edited embryos designed to disrupt dynein interactions with *cen* mRNA. Inhibiting the interaction of *cen* mRNA with dynein results in a decrease in *cen* mRNA granules localized to the centrosome, consistent with a role for dynein in RNA trafficking to the centrosome. This work provides insight into the dynamic localization of *cen* mRNA and an aspect of centrosome regulation through mRNA components of the PCM.

47 Aubergine and piRNAs are key regulators of energy metabolism in germline stem cells

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An important aspect of stem cell biology is the preferential utilization of glycolysis as major metabolic pathway instead of oxidative phosphorylation (Oxphos). The molecular mechanisms of stem cell metabolic reprogramming are poorly understood and we are investigating the implication of PIWI-interacting RNAs (piRNAs). piRNAs are a specific class of small non-coding

RNAs involved in the repression of transposable elements in germ cells. In addition, a key role of piRNAs in mRNA regulation is now emerging for multiple biological processes, including stem cell biology. We have previously shown that the PIWI protein Aubergine (Aub) is essential for self-renewal of germline stem cells (GSCs) in the *Drosophila* ovary, through the regulation of *Cbl* mRNA (Rojas-Rios et al. *EMBO J.* 2017). Aub iCLIP in embryos and GSCs have revealed that many glycolytic and Oxphos mRNAs are directly bound by Aub, suggesting the regulation of energy metabolism by Aub. A role for Aub in translation activation was recently described (Ramat et al. *Cell Res*. 2020). Here, we propose that Aub and piRNAs act in GSC self-renewal by increasing glycolysis through translational activation of glycolytic mRNAs in GSCs.
First, we have used mutants or RNAi of the glycolytic enzymes aldolase, enolase and pyruvate kinase to show that high glycolysis is required for GSC self-renewal, but not differentiation. Then, we confirmed Aub interaction with glycolytic mRNAs in early ovarian stages using RNA-IPs. We also showed that Pyruvate kinase levels are higher in GSCs that in differentiating cyst cells. Importantly, this high expression of Pyruvate kinase is lost in *aub* mutant GSCs, consistent with the positive regulation of *pyruvate kinase* mRNA by Aub. We have set up the expression of FRET metabolic sensors in GSCs, and the utilization of the lactate sensor has revealed the essential role of Aub in maintaining high glycolytic rates in GSCs. Finally, genetic interaction experiments have shown that double heterozygous mutants for *aub* and glycolytic genes display GSC loss, in agreement with Aub regulating glycolytic mRNAs for GSC self-renewal.
Together, our data support the role of Aub/piRNAs as key regulators of the metabolic reprogramming in GSCs. Analysis of the precise requirement of piRNA targeting in glycolytic mRNAs for their regulation in GSCs is in progress.

48 Role for class II PI3-Kinase in T-tubule Remodeling

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Muscle cells are highly organized with specialized structures for contraction. Plasma membrane domains, called Transversal tubules (T-tubules), invaginate throughout the muscle cell to aid synchronized sarcomere contractions. While defects in the T-tubule membrane network are associated with human myopathies, little is known about how T-tubules are normally formed or maintained. We are leveraging the genetic and live imaging advantages of studying T-tubule requirements in Drosophila body wall muscles. We found an endogenous requirement for *shibire,* a Dynamin large GTPase, in the initiation of T-tubule disassembly within a wildtype developmental muscle remodeling program. Consistent with its role in disassembly, overexpression of *shibire* led to inappropriate T-tubule fragmentation and defects in muscle function for larval mobility. Our findings point to a regulated role for dynamin in T-tubule disassembly that may underlie how dominant mutations in the conserved human Dynamin, DNM2, result in centronuclear myopathy. Previously, we implicated class II PI3-kinase (PI3KC2/Pi3K68D), in abdominal muscle remodeling defects associated with loss of Mtm PI3-phosphatase that also has a human homolog, MTM1, linked to centronuclear myopathy. We now identified that *PI3KC2*, like dynamin, is necessary for T-tubule disassembly at initiation of muscle remodeling. Strikingly, disruption of *PI3KC2* function also blocked the ectopic T-tubule disassembly induced by *shibire* overexpression, raising the possibility of human PI3KC2A-targeted therapies for both dominant DNM2- and recessive MTM1-related myopathies. We asked if shared requirements for *PI3KC2* and *shibire* in T-tubule disassembly could reflect a similar pathway to clathrin-mediated endocytic vesicle formation, whereby PI3KC2A-generated PI(3,4)P2 recruits SNX9 and consequently dynamin membrane scission. Preliminary results indicate that the single Drosophila SNX9/SNX18 homolog, *SH3PX1*, is also required for T-tubule disassembly. Current studies are uncovering phosphoinositide dependence and sites of dynamin function for T-tubule membrane disassembly. Altogether, our results suggest that there are shared mechanisms for regulation of dynamin function in endocytosis and T-tubule disassembly.

49 Mode of epistatic interactions between deleterious transposable elements

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Transposable elements (TEs) are widespread genome parasites that increase their copy number at the expense of host fitness. Natural selection against TEs' deleterious fitness effects is a potent evolutionary mechanism for counterbalancing the selfish increase of TEs in host populations. Classic theoretical analyses suggest that the mode of epistatic interactions between deleterious TE insertions plays a crucial role in the evolutionary dynamics of TEs, especially whether TE copy number can be stably contained in host populations. There are many well-studied mechanisms by which TEs impair host fitness, including insertions of TEs into functional sequences, ectopic recombination between nonhomologous TE insertions, and TE-induced changes of local chromatin environment. Several of these deleterious effects have been hypothesized to lead to synergistic epistasis among TE insertions, which is theoretically required for maintaining a stable equilibrium of TE copy number. While this theoretical prediction has been widely discussed, we still lack empirical investigations for the presence of synergistic epistasis among TEs and the relative importance of various deleterious effects of TEs in contributing to such interaction. Purifying selection with synergistic epistasis generates repulsion linkage of deleterious alleles and, accordingly,

an underdispersion of mutational burden. We leveraged this population genetic signal to investigate the predicted synergistic epistasis among TEs in a large panmictic *Drosophila melanogaster* population. We found evidence supporting the presence of synergistic epistasis among TE insertions, especially TEs that likely exert large fitness impacts. By categorizing TEs according to their family identity, the likely unit in which synergism arises, we further identified that TE burden of more than half of the families likely underdisperse. Curiously, even though ectopic recombination among TEs was long predicted to result in nonlinear fitness decline with increased TE copy number (i.e., synergistic epistasis), TEs prone to be involved in this process are not more likely to interact synergistically. On the other hand, TE families exhibiting synergistic epistatic effects have a higher tendency to impose deleterious epigenetic effects. These TE families also have stronger ping-pong signals of piRNA amplification, which is one of the predicted sources from which synergism of TE-mediated epigenetic effects arises. Our discoveries provide a path forward for further investigating the role of epistatic interactions in the evolutionary dynamics of TEs.

50 Caspase regulate the onset of extrusion through downregulation of an apical microtubule mesh.

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Cell extrusion is a sequence of remodeling steps leading to the removal of a cell from an epithelium without impairing its sealing. In previous work, the lab has shown that caspases precede and is necessary for cell extrusion in the drosophila pupal notum. Thus we undertook to study how caspase regulates the remodeling steps of cell extrusion in that tissue.

So far, studies on cell extrusion mainly focused on the role of contractility and adhesion on the process. Indeed, these two factors are two main modulators of cell shape. We thus decided to convey a quantitative phenomenology of different factors during extrusion in the notum. Surprisingly, the onset of constriction is not associated with a drastic change in MyoII levels or in actin dynamics. Moreover, caspase inhibition in clones, which leads to inhibition of extrusion is associated with a significant increase in MyoII levels. This suggests that myosin accumulation is not limiting extrusion and that other regulators contribute to extrusion.

Indeed, using different markers, we observed that the downregulation of an apical microtubule mesh was correlating with the onset of constriction. Moreover, the modulation of microtubule polymerization led to a modulation of cell size. Furthermore, depleting microtubules through colcemid injection rescued the extrusion in caspases inhibited clones. This suggests that the downregulation of this apical microtubule mesh by caspase is a rate-limiting step for extrusion. In addition, we showed that Microtubules are modulating cell compressibility. Thus their depletion leads to increased compressibility associated to a shape-conservative constriction contrary to the rounding observe through the formation of an actomyosin ring. More generally, this underlies a so far neglected role of microtubule in cell shape regulation.

51 3D scaling during *Drosophila* retinal morphogenesis

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Bridging cellular behavior and tissue-scale deformation is crucial to understand how organ shape is determined, yet the inherent complexity on both scales presents challenges for 3D characterization. We approached this question using Drosophila compound eye morphogenesis, during which ommatidial units uniformly change their 3D geometry without cell rearrangement, while at tissue scale the epithelium transforms from a thin planar array into a hemispherical organ.
In order to characterize tissue-scale growth patterns, we used microCT to capture retinal 3D geometry at different pupal stages. A major advantage of this approach is that it preserves both tissue geometry and its physical relationship to the growth environment. 3D reconstruction and quantitative analysis revealed that retinal morphogenesis can be separated into two phases: first, the retina bends to establish a curved thin epithelium; and second, concomitant with attachment to the head cuticle, the retina undergoes significant tissue growth while maintaining constant curvature. Lateral elongation and basal contraction are coordinated during the second phase.
To understand the cell-based mechanisms that underlie this 3D coordination, we considered each ommatidium as a mesoscale multicellular unit. Each unit is centrally organized by a 3D cytoskeletal network contributed by the different retinal cell types. We developed a machine-learning based pipeline using Ilastik to recognize each ommatidial boundary and characterize their geometrical arrangement across the tissue. Using a genetic approach, we disrupted the ommatidial organization at an early stage, and found that the initial geometrical arrangement affects the subsequent morphogenetic events and thus the final tissue morphology. Further, by disrupting cytoskeletal organization at specific planes, we uncovered an unexpected feedback mechanism that connects concurrent morphogenetic events to coordinate tissue geometry.

Together our discoveries reveal a novel tissue-intrinsic property that spatiotemporally controls retinal morphogenesis to achieve a functional final form. We suggest such mechanisms will be at the core of even more complex morphogenetic programs.

52 Evolutionary conservation and divergence of 3D genome organization in Drosophila

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Topologically associating domains (TADs) are 3D organizational units of chromatin that regulate gene expression by constraining enhancer/promoter interactions. Several early studies found that TADs are highly conserved in both vertebrates and in Drosophila. However, more recent research suggests that TADs diverge rapidly and that their reorganization is not associated with widespread divergence in gene expression. In this study, we generated Hi-C chromosome conformation capture data for eleven Drosophila species diverging between 4-32 million years ago. Using these data, we applied two orthogonal methods, one TAD based and another TAD free, to measure the divergence of 3D genome architecture and associated gene expression profiles. Our results show that TADs enriched with broadly-expressed, transcriptionally-active genes are evolving rapidly and contain more differentially-expressed genes between species. On the other hand, TADs enriched with Polycomb-repressed and developmentally-regulated genes remain conserved and have fewer differentially-expressed genes between species, likely because reorganization would cause deleterious misregulation of developmental genes. These results help explain the apparently contradictory results of previous studies evaluating the evolution and role of 3D genome organization and gene regulation.

53 *Dystrophin* and *ensconsin* have opposing roles in regulating nuclear positioning

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Long-range nuclear movements during muscle development are evolutionarily conserved suggesting that these movements are critical for proper muscle development. Additionally, mispositioned nuclei are a hallmark of poor muscle function. Critically, the basic mechanisms of muscle development, the overall cellular structure of the muscle, and the movement and spacing of nuclei are conserved in *Drosophila,* making it an ideal organism to identify the mechanisms and functions of nuclear movement during muscle development. We have previously demonstrated that disruptions of nuclear spacing are a direct consequence of mutations linked to both Centronuclear myopathy and Emery-Dreifuss muscular dystrophy, but whether disrupted nuclear spacing is a direct consequence in the most prevalent muscular dystrophy, Duchenne muscular dystrophy (DMD), was not known. Using a variety of GAL4 drivers we found that *Dystrophin*, the gene mutated in DMD patients, is not critical to initially establish nuclear spacing in the embryo but is necessary to maintain nuclear spacing during larval development. To determine the genetic network for this regulation we screened animals that were doubly heterozygous for mutations in *Dystrophin* and mutations in previously identified regulators of nuclear spacing. Surprisingly, we found that animals heterozygous for mutations in either *Dystrophin* or the microtubule binding protein and kinesin-cofactor *ensconsin* had disrupted nuclear spacing, but animals that were doubly heterozygous displayed normal nuclear spacing. These data suggest that *Dystrophin* and *ensconsin* have counteracting roles in maintaining the spacing of nuclei. Because both *Dystrophin* and *ensconsin* have been shown to bind microtubules in mammals, we explored whether this rescue is based on changes in the organization of microtubules within the muscle cell. To resolve subtle differences in the complex nuclear microtubule network, we turned to a combination of AiryScan and structured illumination microscopy. These approaches revealed subtle, but different disruptions of microtubule organization in both *Dystrophin* and *ensconsin* heterozygotes, that was partially restored in the doubly heterozygous animals. To quantify this, we are developing a variety of computational methods that will determine the complexity of the microtubule network and measure variations in subtle changes. Together, these data suggest *Dystrophin* and *ensconsin* coordinate nuclear position via independent roles in regulating microtubule organization.

54 Y chromosome encodes evolutionarily young piRNAs to regulate a SUMO protease gene during spermatogenesis

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Evolutionarily old Y chromosome is often repeat-rich but gene-poor, like that of *D. melanogaster*. Dispensable for sex determination and viability, *D. melanogaster* Y appears to only encode functionalities required for spermatogenesis and male fertility. These include a dozen protein-coding genes, two loci that encode *Suppressor of Stellate (Su(Ste))* piRNAs, and some ribosomal DNAs. While the search for not-yet-known protein-coding genes on Y continues, it remains elusive whether Y chromosome encodes other functions. Here, we identified a new piRNA-producing locus, proximal to all protein-coding

genes and *Su(Ste)* loci, at the peri-centromeric region of Y. This locus produces abundant piRNAs that are complementary to mRNAs of the *CG12717/pirate* gene, which encodes a SUMO protease. Disruption of piRNA pathway led to derepression of *pirate* expression. Similarly, loss of the broad vicinity of Y centromere, but not any other part of Y, relieved the silencing of *pirate* expression. Interestingly, *pirate*-silencing piRNAs are encoded in an evolutionarily young locus on Y that is unique to *D. melanogaster* and absent in closely related sibling species. Accordingly, there is no evidence of *pirate*-targeting piRNAs in either *D. simulans* or *D. mauritiana* testis, suggesting a rather recent evolution of piRNA-mediated gene regulation, after the split of *D. melanogaster* and *D. simulans* species complex within the last 3 million years. These findings uncover a previously unknown function of *D. melanogaster* Y and highlight an intriguing evolutionary history of gene-regulating piRNAs, whose ancestral role is thought to be transposon control rather than gene regulation.

55 Tissue-specific stop codon readthrough in *Drosophila*

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Translational stop codon readthrough occurs in many organisms including mammals and is especially prevalent in decoding *Drosophila* and viral mRNAs. Recoding of UGA, UAG or UAA to specify an amino acid allows a proportion of the protein encoded by a single gene to be C-terminally extended. The extended product from *Drosophila kelch* mRNA is 160 kDa whereas unextended Kelch protein, a subunit of a Cullin3-RING ubiquitin ligase, is 76 kDa. Previously we reported tissue-specific regulation of readthrough of the first *kelch* stop codon. Here, we characterize major efficiency differences in a variety of cell types. Immunoblotting revealed low levels in malpighian tubules, ovary and testis but abundant readthrough product in lysates of larval and adult central nervous system tissue. Reporters of readthrough demonstrated greater than 30% readthrough in adult brains, and imaging in larval and adult brains showed that readthrough occurred in neurons but not glia. The extent of readthrough stimulatory sequences flanking the readthrough stop codon was assessed in transgenic *Drosophila* and in human tissue culture cells where inefficient readthrough occurs. A 99-nucleotide sequence with potential to form an mRNA stem-loop 3' of the readthrough stop codon stimulated readthrough efficiency. However, even with just six nucleotides of *kelch* mRNA sequence 3' of the stop codon, readthrough efficiency only dropped to 6% in adult neurons. Finally, we show that high-efficiency readthrough in the *Drosophila* central nervous system is common; for many neuronal proteins, C-terminal extended forms of individual proteins are likely relatively abundant.

56 Importin-α2 regulates cytoplasmic histone dynamics in Drosophila

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The rapid nuclear divisions of early embryos demand a continuous, regulated histone supply. For the histone variant H2Av in Drosophila, this is achieved by lipid-droplet–mediated buffering. Lipid droplets (LDs), cytoplasmic fat storage organelles, transiently sequester H2Av via the anchoring protein Jabba. Before the maternal-to-zygotic transition (MZT), H2Av continuously exchanges between LDs through the cytoplasm, thereby providing a steady, yet regulated H2Av pool for nuclear import. At the MZT, H2Av exchange ceases abruptly, and H2Av behaves like a resident LD protein. To identify the mechanism behind this developmental switch, we purified LDs from pre- and post-MZT embryos and compared them by proteomics. We find that the nuclear import factor Importin-α2 is significantly enriched on LDs post-MZT and confirm this pattern by immunostaining. Embryos from *Imp-α2* null females fail to reach the MZT; thus, the pattern of H2Av exchange cannot be monitored across the MZT. However, H2Av is already present on LDs in nurse cells and, using Fluorescence Recovery After Photobleaching (FRAP), we find that here H2Av also exchanges between LDs. Reduced Imp-α2 dosage slows exchange, and in *Imp-α2* null mutants, H2Av acts like a resident LD protein. This behavior could be explained if Imp-α2 shuttles H2Av through the cytosol, but, using luciferase complementation in cell culture, we find no evidence of Imp-α2 interacting with H2Av. Instead, it interacts with the anchor Jabba, and without Jabba, Imp-α2 fails to accumulate on LDs post-MZT. In cultured cells, a 21 aa stretch in Jabba is sufficient for the interaction with Imp-α2. Deletion of a 4-amino acid motif (KRPR) abolishes the interaction while LD localization and H2Av binding are unaffected. We are now testing the effect of this deletion in flies. When expressed in a *Jabba* null background, Jabba ΔKRPR localizes to LDs and recruits H2Av to them. However, H2Av exchange is abolished. Thus, Imp-α2 regulates H2Av dynamics via its binding to Jabba. The binding sites for Imp-α2 and H2Av are adjacent to each other on Jabba, and preliminary evidence suggests that Imp-α2, H2Av, and Jabba can form a tripartite complex. We hypothesize that Imp-α2 binding to Jabba leads to a conformational change that reduces Jabba-H2Av affinity, thus promoting

H2Av exchange. We are now investigating whether a change in post-translational modification of Imp-α2 that accompanies the MZT turns Imp-α2 from a promoter to a repressor of exchange.

57 Neo-sex chromosome shapes introgression in a hybrid swarm

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58 JAK/STAT signaling regulates Defective proventriculus (Dve) to determine dorso-ventral patterning in *Drosophila* eye

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Long range signaling plays an important role in patterning and growth. During organogenesis, axial patterning is involved in delineation of Antero-Posterior (AP), Dorso-Ventral (DV) and Proximo-Distal (PD) axes. We employ *Drosophila* eye model to study the mechanisms behind DV patterning, which marks the first lineage restriction event. We have identified a new dorsal eye selector gene, *defective proventriculus* (*dve*, a Homeobox gene), an ortholog of SATB homeobox 1 (special AT-rich sequence binding protein 1), which controls expression of *wingless* (*wg*), a negative regulator of the eye development, to determine the head fate. Loss-of-function of *dve* results in dorsal eye enlargement by downregulating Wg, which is similar to the gain-of-function of JAK/STAT signaling in the eye. Here we present that Unpaired (Upd), a long-range secreted ligand for JAK/STAT pathway, plays an important role in DV patterning by regulating Dve expression in the dorsal eye. Gain-of-function of JAK/STAT pathway in the eye disc exhibits dorsal eye enlargement by downregulating Dve and its downstream Wg. Conversely, inactivation of JAK/STAT pathway causes dorsalization of the entire developing eye field due to ectopic induction of Dve and Wg in the ventral eye domain resulting in no-eye phenotype. Our data strongly imply that JAK/STAT signaling plays a central role in DV axis determination by limiting the functional domain of the dorsal fate selector and thereby determine the boundary of eye versus the head field in the developing eye.

59 Hippo pathway and Bonus control eye vs. epidermis cell fate decisions

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The Hippo pathway is a conserved growth control system whose dysregulation leads to organ overgrowth and human cancers. Although the Hippo pathway has been intensively studied for its role in the regulation of cell proliferation and organ growth, its function beyond growth control remains elusive. Through affinity purification-mass spectrometry, we have identified Bonus (Bon), the only fly homolog of mammalian TIF1 family proteins, as one of the top interactors of Yorkie (Yki), which is the transcriptional coactivator of the Hippo pathway. Bon-Yki interaction 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, which suggests a cell fate switch from eye to epidermis. This trichome formation requires Yki, Scalloped (Sd), Bon-Yki interaction, Shavenbaby (Svb/ovo), as well as transcriptional regulators that bind with Bon and Yki. Similarly, overexpression of Yki or knockdown of Warts (Wts) also results in formation of trichomes on the adult eyes, which requires Bon and joint interactors of Bon and Yki. Through pupal eye RNAseq, we have identified multiple genes that are jointly regulated by Bon and Yki and direct the cell fate decision of epidermis vs. eye, where Bon and Yki are mutually dependent. Interestingly, upregulation of Yki early in

eye development results in a complete homeotic transformation of eye to antenna, which is Bon dependent. Overall, this work has identified a novel role of Yki and Bon in suppression of eye fate and activation of epidermal and antennal fate. This work broadens our understanding of the developmental functions of the Hippo pathway in controlling cell fate and differentiation, beyond its "classical" role in regulating organ growth.

60 Defective satellite DNA clustering into chromocenters underlies hybrid incompatibility in *Drosophila*

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In eukaryotes, the multiple chromosomes that comprise the genome are invariably encapsulated in a single nucleus. Recently, we demonstrated that sequence-specific satellite DNA-binding proteins have a critical role in maintaining the full complement of chromosomes in a single nucleus. These satellite DNA-binding proteins cluster pericentromeric satellite DNA from multiple chromosomes into nuclear foci known as chromocenters. Chromocenter disruption due to loss of satellite DNA-binding proteins and the resultant chromosome de-clustering led to micronuclei formation and cell death, highlighting an important role for satellite DNA in genome encapsulation.
It has been often postulated that the rapid divergence of satellite DNA between species may underlie hybrid incompatibility (HI), although the underlying cellular mechanisms remained elusive. Here we show that cells from affected tissues in interspecies *Drosophila* hybrids exhibit phenotypes consistent with chromocenter disruption. We demonstrate that the sterile gonads and atrophied somatic tissues in incompatible *Drosophila melanogaster – Drosophila simulans* hybrids exhibit chromocenter disruption and micronuclei formation. Two of the previously identified HI genes in these species are *D. melanogaster Hmr* and *D.simulans Lhr*, which gain a dominant negative function in the hybrid context. Strikingly, we observed that both chromocenter formation and genome encapsulation are restored when hybrid sterility/lethality is rescued by mutating these HI genes. Moreover, an extra copy of *D.melanogaster Hmr,* which induces lethality in the normally viable female hybrids, resulted in chromocenter disruption and micronuclei formation, indicating that these phenotypes are the direct consequence of genetic incompatibilities between these species. Finally, sterile male hybrids between the more closely related species, *Drosophila simulans* and *Drosophila mauritiana*, also exhibited chromocenter disruption, micronuclei formation and cell death, suggesting that these cellular phenotypes are a general feature of hybrid incompatibility. Therefore, for the first time, we have characterized the cellular defects that explain how the rapid divergence of satellite DNA repeats between closely related species can cause reproductive isolation.

61 The RNA-binding protein Orb2 regulates the activity of interphase centrosomes in neural stem cells to promote neurodevelopment

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Centrosomes are the microtubule-organizing centers (MTOCs) that assemble the bipolar spindle required for error-free mitosis. Although errant centrosome regulation is the leading cause of heritable microcephaly and associated with other neurodevelopmental disorders, precisely how centrosome activity is dynamically regulated is poorly understood. The essential determinant of MTOC activity is the pericentriolar material (PCM), a protein matrix that encircles the central pair of centrioles residing at the centrosome core. While all centrosomes are inherently asymmetric due to the differing ages of their centrioles, the centrosomes of asymmetrically dividing cells, such as neural stem cells (NSCs), show striking asymmetries in PCM composition and abundance during interphase. How such asymmetries arise is an area of active study. We identified Orb2, a conserved RNA-binding protein implicated in RNA localization and translational control, as required for the asymmetric recruitment of PCM to interphase NSC centrosomes. Orb2 negatively regulates centrosome activity, as loss of *orb2* leads to the precocious activation of the normally inactive mother centrosome. Consequently, *orb2* mutant NSCs show two active interphase centrosomes showing impaired separation, bent and misaligned mitotic spindles, and the accumulation of supernumerary centrosomes. Further, *orb2* mutants show a dramatic reduction in NSCs and produce microcephalic brains. Our data implicate Orb2 in NSC centrosome asymmetry and neurodevelopment.

62 A neural m^{6}A/YTHDF pathway is required for learning and memory in *Drosophila*

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The roles of epitranscriptomic modifications have received substantial attention, with appreciation growing for their phenotypically selective impacts within the animal. We adopted *Drosophila melanogaster* as a model to study *N*6-methyladenosine (m^6A), the most abundant internal modification of mRNA. Here, we report proteomic and functional analyses of fly m^6A-binding proteins, confirming nuclear (YTHDC1) and cytoplasmic (YTHDF) YTH domain proteins as the major m^6A binders. Since all core m^6A pathway mutants are viable, we assessed *in vivo* requirements of the m^6A pathway in cognitive processes. Assays of short term memory (STM) revealed neural requirements of m^6A writers working via YTHDF, but not YTHDC1, comprising the first phenotypes assigned to *Drosophila* mutants of the cytoplasmic m^6A reader. We then mapped m^6A from wild-type and *mettl3* mutant heads, allowing robust discrimination of Mettl3-dependent m6A sites. In contrast to mammalian m^6A, which is predominant in 3' UTRs, *Drosophila* m^6A is highly enriched in 5' UTRs. Genomic analyses demonstrate that *Drosophila* m^6A does not have directional effects on RNA stability, but it is preferentially deposited on genes with low translational efficiency. However, functional tests indicate a role for the m^6A/YTHDF pathway in translational activation. Finally, we show that Mettl3/YTHDF regulate STM specifically via the mushroom body, the center for associative learning. Altogether, we provide the first tissue-specific m^6A maps in this organism and reveal selective behavioral and regulatory defects for m^6A /YTHDF mutants.

63 Neuronal ribosomal protein function regulates *Drosophila* growth and development

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Stimulation of ribosome biogenesis is a conserved mechanism of growth control. While extensive studies have shown how ribosome synthesis controls cellular growth, less is known about how it contributes to the control of body growth and development. We have been studying this issue using *Minutes*, a class of dominant ribosomal protein mutants that show a characteristic delay in larval development. This phenotype is thought to be due to a reduction in overall 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. Instead, as discussed below, we found evidence of a cell type-specific function for one RP (rps13) in the control of development.
Termination of the larval period is controlled by a pulse of ecdysone secretion 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 reduced expression of the 'Halloween' genes that are needed for ecdysone synthesis, and we saw that ecdysone feeding partially reversed their delayed development. We postulated that these effects might reflect a cell- or tissue-specific role for *rps13* in regulating the CNS-PG neuroendocrine circuit. To test this, we used the GAL4/UAS system to test if tissue selective expression of *rps13* in *rps13/+* animals could rescue their delayed development. Expression of *rps13* in either the PG or imaginal tissues had no effect. In contrast, expression of *rps13*, either pan-neuronally or specifically in a subset of serotonergic (5-HT) neurons that innervate the PG, reversed the developmental delay by ~40%. Furthermore, we found that genetically enhancing protein synthesis in these neurons by expressing *Myc* or the TOR activator, *Rheb,* lead to a similar reversal of developmental delay. The *rps13/+* animals showed normal 5-HTergic innervation to the PG, and electric activation of these neurons did not rescue their delayed development. Instead, we found that over-expressing several synaptic vesicle (SNARE complex) proteins in the 5-HT neurons could rescue the delayed *rps13/+* development to the same extent as *rps13* expression. We will be further investigating these results by looking at whether translation of these SNARE complex proteins is dependent on RpS13. Our data suggests that RpS13 expression is needed for neuroendocrine control of development by maintaining protein synthesis and proper synaptic vesicle function in serotonergic neurons that trigger ecdysoen release from the prothoracic gland.

64 An unexpected contribution of Rab21 in mitochondrial dynamics

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Membrane trafficking defines the different routes used across the cell to deliver macromolecules from a compartment to another. This process is mediated by diverse class of regulators among which is the RAB GTPase protein family. RABs are small GTPases that cycle between active and inactive forms. Upon activation, RABs associate to membranes where they recruit effectors to modulate different processes (cargo selection, movement or fusion). Therefore, identification of RAB interactors is crucial to further the understanding of RAB functions. To date, such investigations have been mainly conducted *in vitro* or in cell culture models, thus ignoring cell specific effectors.

Rab21 is a small GTPase involved in early endosomal trafficking. We recently showed that Rab21 contributes to the survival of fly intestinal differentiated cells, the enterocytes (EC). In order to get insights into Rab21 function(s) in this context,

we characterized its associated proteome *in vivo* using the MiniTurbo proximity labeling approach. Using this method, we identified Rab21 specific interactors in EC, and revealed new unexpected interactors for Rab21, among which were mitochondrial proteins. Importantly, when comparing the Rab21 interactome to Rab5, another early endosomal Rab, we noticed that identified mitochondrial interactors were specific to Rab21. These data led us to investigate putative roles for Rab21 at mitochondria.
Using a cell specific inducible system combined with mitochondrial reporters, we evaluated Rab21 loss- and gain-of-functions on mitochondria, in adult EC. Rab21 deregulation resulted in defects in mitochondrial morphology and dynamics. Moreover, electronic microscopy revealed accumulation of smaller mitochondria in Rab21 depleted EC. In order to evaluate whether Rab21 mitochondrial function was conserved among species, we analyzed these organelles upon Rab21 knockout or knockdown in two different human cells lines. As observed in Drosophila, mitochondrial shape was strikingly affected upon loss of Rab21. Furthermore, we observed that Rab21 partially localized to mitochondria in human cells. Interestingly, such colocalization was greatly enhanced with a constitutively active form of Rab21.

Altogether, these data shed light on an unexpected contribution for Rab21 into mitochondrial dynamics. In a larger extend this study highlight the importance of investigating RAB partners *in vivo* to unravel unappreciated functions.

65 Evolutionary changes in a fatty acyl-*CoA* elongase gene underlie high levels of desiccation resistance in a desert *Drosophila* species

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Adaptation to diverse and extreme environments is key to long-term species persistence. One of the largest challenges for organisms living in terrestrial environments is water loss. Insects use cuticular hydrocarbons (CHCs), a lipid layer on the body surface, to prevent water from evaporation and therefore, withstand desiccation stress. Previous studies have shown that a subset of these CHCs, the methyl-branched CHCs (mbCHCs), are involved in desiccation resistance in some *Drosophila* species. In addition, longer mbCHCs are positively associated with higher desiccation resistance. For example, the desert fruit fly, *Drosophila mojavensis*, has the longest mbCHCs and the highest desiccation resistance among *Drosophila* species, suggesting that these very-long-chained mbCHCs could be the basis of their high desiccation resistance. However, the molecular mechanisms underlying the evolution of long mbCHCs and high desiccation resistance on this species are not known. In our study, we investigated the genetic basis underlying the synthesis of these very-long-chained mbCHCs in *D. mojavensis*. Using a candidate gene approach and CRISPR/Cas9 gene editing, we identified a fatty acyl-*CoA* elongase gene in *D. melanogaster*, which we named *mElo*, responsible for the elongation of median-chained mbCHCs in this species. We hypothesized that *mElo* ortholog in *D. mojavensis* could be responsible for the elongation of very-long-chained mbCHCs. To test this hypothesis, we identified the *mElo* ortholog of *D. mojavensis*, *Dmoj/mElo*, and overexpressed this gene in the *mElo-knockout D. melanogaster* background. We showed that the transgenic overexpression of *Dmoj/mElo* led to longer chain mbCHCs and significantly higher desiccation resistance, suggesting that the function of *Dmoj/mElo* in elongating longer mbCHCs may be due to protein-coding differences in this gene between species. Our results suggest that adaptation to the desert and the evolution of high levels of desiccation resistance in *D. mojavensis* may be due to protein-coding evolution in a single gene.

66 Depletion of trans-acting factors reveals mechanisms of multi-enhancer competition at the *short gastrulation* locus

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Metazoan transcription is controlled by non-coding elements called enhancers. Enhancer activity is determined by the transcription factor binding motifs found in their sequence, allowing combinations of transcription factors to drive precise gene expression in space and time. Many developmentally regulated enhancers work together, often acting on the same gene simultaneously. These so-called “shadow enhancers” are thought to confer developmental robustness in response to environmental or genetic insult (Hong et al., 2008; Perry et al., 2010, Barolo, 2012). Shadow enhancers encode similar transcription factor binding sites and drive broadly overlapping regions of expression. However, little is known about how systems with multiple enhancers coordinate their activity mechanistically. Here we present a genetic strategy for understanding how a pair of shadow enhancers work together to drive the expression pattern seen in the developing Drosophila embryo. Using CRISPR-Cas9, we created multiple enhancer deletions for the gene *short gastrulation* (*sog*). These deletions also contain a unique sequence inserted intronically, which can be labeled using single molecule FISH probes. Using two distinct probe sets, we can specifically label wildtype and enhancer deletion alleles in heterozygous animals. This not only allows us to assess transcriptional output across the Dorsal morphogen gradient responsible for specifying the *sog* expression domain, but also lets us compare, in single nuclei, how enhancer deletions alter the output of *sog* transcriptional activity.

Using this information, we build a model of how two enhancers work together and in isolation, allowing critical insights into the phenomenon of enhancer competition. We find that the amount of competition observed is a function of not only the Dorsal gradient, but also the repressors that refine the domain of *sog* expression. Competition also appears to alter over time, with earlier timepoints sampled displaying a more additive relationship between the two enhancers, and later timepoints displaying greater competition. To extend our analysis, and uncover the biological basis behind enhancer competition, we systematically depleted the transcription factors Groucho, CTCF, and Zelda from the maternal environment using RNAi on the background of all of our enhancer deletions. Our results advance our understanding of how mutliple factors contribute to not just the cis-regulatory logic of a single enhancer, but how a system of enhancers operates to generate percise transcriptional outputs in developmental time.

67 Making the impossible possible through objective-driven, long-term initiatives

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Unlike medical research directly relevant to people's life quality, communicating basic *Drosophila* research is far more challenging, with (short-term) curiosity being the main driver of audience engagement. Remedial strategies are objective-driven, long-term, ideally multi-facetted initiatives (details: 10.1016/j.semcdb.2017.08.017). In 2011, we launched the Manchester Fly Facility initiative to raise awareness of the importance that fruit fly research has for discovery processes in the biomedical sciences (details: 10.1016/j.semcdb.2017.06.004). We use multi-facetted strategies: (A) targeting the public including clinicians and politicians (science fair organisation/exhibition; YouTube videos, media work, website, engaging clinicians, politics journal articles), (B) addressing fellow scientists (blogs, publications, genetics training, conference talks), (C) collaborating with teachers to introduce flies as curriculum-relevant teaching tools in biology school lessons (website, downloadable lesson resources, school visits).

Gradually, our initiative gained in momentum: our school lessons bring life back into classrooms as a way to raise fly awareness at young age; resources were translated into Spanish, Turkish and Indonesian, and are used in 20 nations on 6 continents with many teachers reporting achieved benefits (10.1016/j.semcdb.2017.07.025); follower initiatives spreading this strategy across the globe are 'Fly Indonesia', 'droso4Nigeria', 'droso4LatAm', 'droso4schools-Croatia', and 'droso4Turkey' – all but one running their own websites. We organised the 'Brain Box' science fair in Manchester (UK) in 2016, showcasing *Drosophila* neuroscience in a themed way alongside mammalian neurobiologists, neurosurgeons, and artists - reaching 5,400 visitors in a single day (mcrbrainbox.wordpress.com). Our ‹Small Fly: BIG impact› YouTube videos have 40,000 views and inspired translations into Spanish, Indonesian and Arabic (tinyurl.com/yyj8ttom). Our genetics training package (>40,000 downloads across 3 platforms) facilitates the introduction of students or non-fly researchers to the culture of fly research and inspires them early to engage in science communication and advocacy.

These examples illustrate how long-term, objective-driven, multi-facetted strategies can achieve impact even in science areas less attractive to audiences. They allow researchers to collaborate in global networks focussing on a common communication goal – and anybody is invited to join in!

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68 Remote research: A bioinformatics adventure for undergraduates

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Numerous studies have demonstrated the value of involving undergraduates in laboratory research. However, access to research labs is often limited by available space and time. To reach a larger population of students, many faculty have incorporated portions of their research into Course-based Undergraduate Research Experiences (CUREs). CUREs provide authentic research experiences to a much larger number of students than traditional apprenticeship models, and their inclusion in biology curricula is becoming much more widespread. Although the COVID-19 pandemic prevented our undergraduates from physically working in the lab, it provided us an opportunity to pilot a bioinformatics-based CURE based on our current work. The Rieder lab is interested in factors that regulate the *Drosophila* histone genes, and our undergraduates engaged in a search for new regulators using publicly available high-throughput datasets. Because the *Drosophila* histone locus is a repetitive array, any sequencing reads mapping to this region are normally discarded. To address this issue, we used a custom genome containing a single copy of the locus. We first engaged our four experienced undergraduate laboratory members in background discussions and literature searches, and they formed hypotheses and chose candidate proteins. Next,

we guided students to find high-throughput ChIP-seq datasets in NCBI GEO and taught them to map the reads to the histone gene array using Galaxy, a free web-based platform that integrates many bioinformatics tools. After mapping, the students visualized their data using the open source Integrative Genomics Viewer (IGV) software. Strikingly, all four students discovered proteins that are enriched at the *Drosophila* histone array. Some of the students will be performing follow-up bioinformatics experiments in the spring (for example, mapping available RNA-seq datasets). We are repeating this program with a group of naive students to expand our research and obtain more diverse feedback. Our approach solved the accessibility issue exacerbated by the COVID-19 pandemic, is completely free, and presents an opportunity for more accessible and inclusive access to undergraduate research.

69 Characterizing *Drosophila* mutagen sensitive alleles through a collaborative Course-based Undergraduate Research Experience (CURE)

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Course-based Undergraduate Research Experiences (CUREs) provide large-scale student training in answering original research questions that are of interest to the broad scientific community. By bringing research into the classroom, CUREs boost inclusivity within the sciences and provide authentic research experiences for students who may lack resources to engage in more intensive research fellowships. Further, CUREs are a sustainable mechanism for faculty at primarily undergraduate institutions (PUIs) to boost their research productivity and remain active in the research community.

Our collaborative project brings together experts in DNA repair and replication from multiple PUIs to map and characterize mutagen sensitive genes in *Drosophila* through a series of classroom research modules (only 14 of the 58 discovered mutagen sensitive genes have been genomically mapped and characterized). The collaborative nature of this project allows undergraduate students in multiple biological disciplines to contribute to collective research on the function of the *mus* genes. Students at Winthrop University map *mus* genes using complementation crossing and have so far successfully mapped the gene *mus109*. Students in genetics and developmental biology at our other collaborating institutions work on *mus109* mutant characterization including sensitivity to various DNA damaging reagents (Northeastern Illinois University), maternal effects (Lewis-Clark State College), and ovarian morphology (Arcadia University). In the past 3 years, our CURE has engaged nearly 200 undergraduate students, who have reported positive experiences and higher learning gains in these courses. Our future prospects include creating additional research modules for our CURE and expanding our network to involve additional researchers.

70 The Genomics Education Partnership (GEP; thegep.org) is a nationwide collaboration of faculty from 100+ institutions which aims to integrate Course-based Undergraduate Research Experiences (CUREs) centered in genomics and bioinformatics into the curriculum

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Participating institutions include community colleges, PUIs, MSIs, HBCUs, and R1 universities. The GEP web-based platform includes curriculum/training materials that can easily be incorporated into existing courses. For many faculty and their students, the accessible and immersive curriculum and custom bioinformatics tools provided by the GEP enable a unique opportunity to participate in research, even during the COVID-19 pandemic. GEP faculty benefit from a national network of like-minded colleagues and professional development opportunities. With NSF and NIH support, GEP is actively recruiting additional faculty members to use the GEP curriculum in their classrooms, particularly at MSIs and community colleges, and both science and science education partners to collaborate on additional projects. Over 1,300 students per year learn to annotate newly-sequenced eukaryotic genomes, creating defendable gene models. Students leverage evidence from related informant species, experimental data (e.g., RNA-Seq), automated gene prediction algorithms, evolutionary conservation, and basic molecular biology rules to develop their models. GEP partnered with Galaxy to develop G-OnRamp, an open-source platform for constructing UCSC Assembly Hubs and JBrowse/Apollo genome browsers, enabling more varied research projects, including our investigation of venom evolution in parasitoid wasps. Other GEP projects investigate the evolution of insulin pathway genes across 27 *Drosophila* genomes, and expansion of the F element in four Drosophila species. Student

gene models are reconciled and collated to generate a large dataset for evolutionary genomic studies, with student/faculty co-authors; GEP is also piloting student publication of gene models as *microPublication*s. We find 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 deeper insight into annotation and can occur fairly quickly within our inexpensive, online framework. GEP students show significant gains in scientific knowledge and attitudes toward science. Supported by NSF grants 1915544 and 1431407, and NIH R25GM130517.

71 Kids Conquering Cancer: Celebrating culture to reduce health disparities

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Everybody eats. For humans, eating is a central cultural focus, with celebrations, social engagement, and health interventions all centered around food. On the flip side, humans have largely sidestepped evolutionary adaptation to local food sources, with excess eating, fad diets, or lack of access to healthy food leading to the obesity and malnutrition (often both) that are driving health challenges worldwide. Our long term goal is to leverage cultural dietary practices to develop diets that optimize health and reduce disease, with a particular focus on cancer and diabetes in patients from backgrounds experiencing egregious health disparities. To do this, we developed a large scale citizen science approach. Participants ranging from middle school to retirement conduct reverse genetics nutrient screens in *Drosophila*, comparing the impact of diet on fertility and survival in wild-type flies vs strains bearing mutations in genes that drive human disease. Using this approach, we are mapping the genetic targets of individual nutrients to create a comprehensive map of dietary impacts on disease-driving signal transduction pathways. The program is infused with culture, with students choosing test nutrients from interventions used in their families, and high school science classes voting on a disease focus for maximal impact of the research on their own communities. Students have the opportunity to continue their projects through 4 iterative training levels, with a focus on collaboration and self-designed independent research. 70% of classroom participants report an interest in "doing more research", a striking number given that fewer than 6% of Philadelphia public school students pursue STEM degrees in college. Perhaps more importantly, 75% of participants who complete advanced programming levels gain paid employment in research labs in college, with nearly 20% (and growing!) continuing as Ph.D. candidates in biomedicine. The program has naturally evolved to a population that represents the City of Philadelphia, with 65% of participants from backgrounds under-represented in science. This inclusive, diverse, collaborative community has transformed what we think of as an important scientific question, with creative, intelligent students leveraging their cultural experiences to address the most pressing genetics-based health challenges today. We welcome all fly fans to join our efforts as we expand nationwide (https://ecloseinstitute.org).

72 Managerial Engagement to Promote DEI in STEM

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Many organizational strategies to promote inclusive climates and advance diversity goals produce few or no effects, and some are even counterproductive. Based on their analysis of what works and doesn't work to promote diversity in 800 U.S. corporations across four decades, Frank Dobbin and Alexandra Kalev recommend a "managerial engagement approach," which mobilizes organizational leaders as change agents. In my talk, I outline the logic and effects of three strategies to operationalize managerial engagement theory in STEM higher education contexts. First, I describe the NSF-funded ADVANCE at UNM program, which builds on managerial engagement theory to improve the recruitment, retention, and advancement of women and URM STEM faculty at the University of New Mexico. By engaging university leaders in the reform of faculty search process, ADVANCE has increased diversity hiring. Next, I analyze the Diversity and Inclusion Hackathon, held at the American Political Science Association annual meeting in 2018. At the Hackathon, hundreds of people organized into 13 teams collaborated on distinct projects in real time, and post-meeting surveys showed that the experience increased perceptions of an inclusive climate. Finally, I discuss my ongoing work to develop and engage engineering faculty in an educational curriculum on bystander intervention, which involves giving people in engineering workplaces the skills and confidence to speak out when they witness episodes of incivility, harassment, racialized microaggressions, or exclusion.

73 Two Decades of Diversity Recruiting: Lessons Learned

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74 NIH Efforts to Cultivate and Support a Diverse Research Workforce

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NIH's ability to help ensure that the nation remains a global leader in scientific discovery and innovation is dependent upon

a pool of highly talented scientists from diverse backgrounds who will help to further NIH's mission. The NIGMS Division of Training, Workforce Development, and Diversity (TWD) supports programs that foster research training and the development of a strong and diverse biomedical research workforce. The division funds research training, student development and career development activities through a variety of programs. This talk will provide an overview relevant reserach on the factors that contribute to persistent workforce underrepresentation, and NIH efforts to enhance workforce diversity. Examples include the: Maximizing Opportunities for Scientific and Academic Independent Careers (MOSAIC) program to promotoe faculty diversity (https://www.nigms.nih.gov/training/careerdev/Pages/MOSAIC.aspx), and the new Common Fund Faculty Institutional Recruitment for Sustainable Transformation (FIRST) initiative (https://commonfund.nih.gov/first). Attendees are encouraged to read this short viewpoint before the presentation: https://www.ascb.org/publications-columns/science-and-society/promoting-diversity-and-advancing-racial-equity-in-the-biomedical-sciences/

75 Age-related neuroprotection by dietary restriction requires *OXR1*-mediated retromer function

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Dietary restriction (DR) is the most robust method to delay aging and the onset of neurodegenerative disorders across multiple species, though the mechanisms behind this phenomenon remain unknown. To elucidate how DR mediates lifespan extension, we analyzed natural genetic variants that associate with increased longevity under DR conditions in the Drosophila Genetic Reference Panel (DGRP) strains. We found that neuronal expression of a gene called mustard (mtd) in Drosophila, whose ortholog is known as Oxidation resistance 1 (OXR1) in other organisms, is necessary for DR-mediated lifespan extension. Neuronal RNAi for OXR1 also prevented the DR-associated slowing of age-related visual decline but not physical activity decline, arguing for a specific role of OXR1 in specific forms of neurodegeneration. Further, overexpression of the TLDc domain in human OXR1 is sufficient for extension of lifespan in a diet-dependent manner. Studies from Accelerating Medicines Partnership – Alzheimer's Disease (AMP-AD) network show that OXR1 protein levels are reduced in brains of patients with Alzheimer's disease (AD), and we found that overexpression of human OXR1 is protective in AD fly models. In seeking the mechanism by which OXR1 protects age-related neuronal decline, we discovered that it provides a necessary function in regulating the neuronal retromer complex, which is essential for the recycling of transmembrane proteins. We discovered that OXR1 deficiency can be rescued by genetic or pharmacological enhancement of retromer protein expression. Understanding how OXR1 functions could help uncover novel mechanisms to slow neurodegeneration and extend healthspan across species.

76 Genetic determinants of cell fate plasticity during regeneration after radiation damage in *Drosophila*

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Ionizing radiation (IR) is used to treat approximately half of all cancer patients because of its ability to induce cell death. However, IR can also induce cancer stem cell-like properties in non-stem cancer cells, potentially promoting tumor regeneration and diminishing therapeutic success. Our published studies have shown that we can model the induction of stem cell-like properties by IR in the larval wing imaginal discs of *Drosophila melanogaster.* Specifically, after radiation damage, IR-resistant cells in the hinge region of the wing disc translocate to the pouch region of the wing disc where they change fate to help regenerate the pouch (Verghese et al., 2016, PMID: 27584613; Verghese et al., 2018, PMID: 30462636). Here, we report a genome-wide RNAseq analysis of IR-induced gene expression changes in the cells of the wing discs that were dissociated and sorted to represent the hinge or the rest of the disc. We observed hinge-specific down regulation of differentiation factors and up-regulation of genes needed for ribosome biogenesis, mitochondrial function, and cell competition. We functionally tested 37 genes by RNAi-mediated depletion or forced overexpression, specifically in the hinge. Our results suggest that IR-induced cell fate changes require down-regulation of signaling pathways that determine the hinge fate and up-regulation of translational capacity, presumably to synthesize new fate determinants. These results can explain our previous findings that reducing translational capacity with small molecule inhibitors enhanced the effect of radiation in both *Drosophila* larvae (Gladstone, et al., 2012, PMID: 22344740) and human Head and Neck Cancer models (Keysar et al., 2020, PMID: 31911553).

77 Dynamics and functional characterization of the pan-metazoan ultra-conserved smORFeome in Drosophila

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The translatome which refers to the entirety of mRNAs associated with ribosomes for protein synthesis, is dynamic and complex, with a significant proportion of proteins and peptides being functionally specific to certain cell-types, tissues, or developmental stage(s). Ribosome profiling, a method where ribosome-bound mRNA fragments are isolated and subjected to deep-coverage sequencing, is a useful tool for exploring genome-wide translation. We conducted extensive ribosome profiling designed to accurately characterize the spatial and developmental Drosophila translatome. Our experimental approach enabled the identification of translation start sites and actively translated open reading frames (ORFs). To avoid misclassification of noncoding elements as protein coding we developed and applied a novel machine learning software package to more accurately classify ORF translation status. We focus on a subset of ~150 ultra-conserved unannotated small ORFs ("smORFs"; ≤ 100 amino acids), with evidence for translation via ribosome profiling and proteomics, that are present in the genomes of fruit fly, honeybee, zebrafish, mosquito, mouse, chicken, and human. Despite their deep conservation, many of these protein-coding genes have never been functionally characterized in any organism. An analysis of peptide domains revealed a predicted enrichment of smORF peptides predicted to localize to mitochondria and extracellular space. We initiated the functional characterization of these smORFs using germline and somatic knockouts, UAS-RNAi knockdowns, CRISPR-enabled and UAS-cDNA overexpression, and embryonic mRNA in situ imaging. These studies revealed diverse expression patterns and phenotypes, and a surprising lack of lethality given their deep conservation.

78 Transcription factors drive opposite relationships between gene age and tissue specificity in male and female *Drosophila* gonads

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Evolutionarily young genes are usually preferentially expressed in the testis across species. While it is known that older genes are generally more broadly expressed than younger genes, the properties that shaped this pattern are unknown. Older genes may gain expression across other tissues uniformly, or faster in certain tissues than others. Using *Drosophila* gene expression data, we confirmed previous findings that younger genes are disproportionately testis-biased and older genes are disproportionately ovary-biased. We noted that the relationship between gene age and expression is stronger in the ovary than any other tissue, and weakest in testis. We performed ATAC-seq on *Drosophila* testis and found that while genes of all ages are more likely to have open promoter chromatin in testis than in ovary, promoter chromatin alone does not explain the ovary-bias of older genes. Instead, we found that upstream transcription factor (TF) expression is highly predictive of gene expression in ovary, but not in testis. In ovary, TF expression is more predictive of gene expression than open promoter chromatin, whereas testis gene expression is similarly influenced by both TF expression and open promoter chromatin. We propose that the testis is uniquely able to expresses younger genes controlled by relatively few TFs, while older genes with more TF partners are broadly expressed with peak expression most likely in ovary. The testis allows widespread baseline expression that is relatively unresponsive to regulatory changes, whereas the ovary transcriptome is more responsive to trans-regulation and has a higher ceiling for gene expression.

79 Dynamics of histone H3 availability coordinate the cell cycle and developmental progression in the early *Drosophila* embryo

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The DNA damage checkpoint is crucial to protect genome integrity. However, the many metazoan embryos sacrifice this safeguard to allow for rapid cleavage divisions that are required for speedy development. These rapid cell cycles end when the cell cycle is remodeled with the addition of gap-phases and acquisition of DNA damage checkpoints at the Mid-blastula transition (MBT). It is well established that the ratio of nuclei to cytoplasm (N/C) ratio is a critical regulator of the MBT. This ratio exponentially increases during cleavage divisions as cells divide without growth. Additionally, the activation of the checkpoint kinase, Chk1, is critical for cell cycle slowing. Yet, how Chk1 activity is coupled to the N/C ratio to allow for cell cycle remodeling at precisely the correct developmental time point has remained an open question. Here, we show that dynamic changes in histone H3 availability in response to the increasing N/C ratio directly control Chk1 activity and thus time the MBT in the *Drosophila* embryo. We show that the excess pool of non-DNA-bound histones becomes depleted as the embryo approaches the MBT leading to falling nuclear H3 concentrations. We find that excess H3 N-terminal tail in the early cycles interferes with cell cycle slowing independent of chromatin incorporation and acts as a competitive inhibitor of Chk1 *in vitro* and reduces Chk1 activity *in vivo*. Mutational analysis of the Chk1 phosphosite in the H3 tail (H3T11) and mathematical

modeling reveal that titration of available H3T11 during cleavage cycles regulates cell cycle slowing. These results define Chk1 regulation by H3 as a key mechanism that couples Chk1 activity directly to the N/C ratio. Thus, our model provides a simple molecular mechanism for the longstanding problem of N/C ratio sensing in early development.

80 The Drosophila Amyloid Precursor Protein homologue mediates neuronal survival and neuro-glial interactions

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The amyloid precursor protein (APP) is a structurally and functionally conserved transmembrane protein whose physiological role in adult brain function and health is still unclear. Because mutations in APP cause familial Alzheimer's disease, most research focuses on this aspect of APP biology. We investigated the physiological function of APP in the adult brain using the fruit fly Drosophila melanogaster, which harbors a single APP homologue called APP Like (APPL). Previous studies have provided evidence for the implication of APPL in neuronal wiring and axonal growth through the Wnt signaling pathway. However, like APP, APPL continues to be expressed in all neurons of the adult brain where its functions and their molecular and cellular underpinnings are unknown. We report that APPL loss of function results in the dysregulation of endolysosomal function in neurons, with a notable enlargement of early endosomal compartments followed by neuronal cell death and the accumulation of dead neurons in the brain during a critical period at a young age. These defects can be rescued by reduction in the levels of the early endosomal regulator Rab5, indicating a causal role of endosomal function for cell death. Finally, we show that the secreted extracellular domain of APPL interacts with glia, regulates the size of their endosomes, the expression of the Draper engulfment receptor, and the clearance of neuronal debris in an axotomy model. We propose that APP proteins represent a novel family of neuro-glial signaling factors required for adult brain homeostasis.

81 Tip60 HAT mediated histone acetylation restoration as a common therapeutic strategy for multiple neurodegenerative diseases

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Epigenetic mechanisms, such as histone acetylation, regulate dynamic gene expression that is critical for several neural functions including learning and memory. Consequently, loss of neural histone acetylation has been implicated in multiple neurodegenerative conditions, yet the mechanisms underlying such alterations remain unclear. Our lab has previously shown that imbalance in Tip60 histone acetyltransferase (HAT) and histone deacetylase 2 (HDAC2) in a well characterized *Drosophila* AD model results in epigenetic repression of critical synaptic plasticity genes and functional cognitive deficits. Interestingly, these phenotypes are restored by increasing Tip60 HAT levels in the AD brain, supporting a neuroprotective role for Tip60 in AD-linked neurodegeneration. Here we show that similar to AD, disruption of Tip60 HAT/HDAC2 balance and repression of synaptic plasticity genes is a shared early event in Parkinson's Disease (PD), Huntington's Disease (HD) and Amyotrophic Lateral Sclerosis (ALS). Further, chromatin immunoprecipitation (ChIP) studies reveal that repressed neuroplasticity genes show reduced Tip60 enrichment and reduced histone acetylation at all gene loci examined with certain genes also showing inappropriate HDAC2 enrichment. Functional neuronal consequences of each of these disease conditions are reminiscent of human pathology and include locomotion, synapse morphology, and short-term memory deficits. Since Tip60 overexpression is neuroprotective in AD-linked neurodegeneration, we wanted to further explore if Tip60 plays a general neuroprotective role in multiple neurodegenerative conditions. Remarkably, increasing Tip60 HAT levels specifically in the learning and memory center of the *Drosophila* brain protects against locomotion and short-term memory function deficits. Together, our results support a model by which Tip60 protects against neurological impairments in different neurodegenerative diseases *via* similar modes of action, giving hope for a unified therapeutic approach.

82 Delineating the pathway that leads to aneuploidy-induced-cell senescence

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Chromosomal instability (CIN) – the continuous gain and loss of chromosomes – and the resulting aneuploidy - an unbalanced number of chromosomes - are hallmarks of most solid tumors and contribute to the gain of oncogene-carrying chromosomes and to the loss of tumor-suppressor-carrying chromosomes in cancer cells [reviewed in (Ben-David and Amon, 2020)]. However, work in yeast, flies and human cell has demonstrated that aneuploidy is highly deleterious at the cellular level and can be a source of stress due to the altered protein stoichiometry and impaired functioning of large protein complexes involved in replication, mitosis and metabolism [reviewed in (Zhu et al., 2018)]. More recently, aneuploidy has also been

reported to induce senescence - a cellular state characterized by permanent cell cycle arrest and an enhanced secretory phenotype (Macedo et al., 2018; Santaguida et al., 2017). How aneuploidy karyotypes lead to cellular senescence and what biological role senescence plays in a growing tissue are two fascinating questions that have not been addressed so far. To address these two questions, we have used a *Drosophila* epithelial model of CIN (Benhra et al., 2018; Clemente-Ruiz et al., 2014, 2016; Dekanty et al., 2012; Muzzopappa et al., 2017) that recapitulates most emerging cellular behaviors observed in mammalian epithelial tissues upon CIN, such as extrusion of aneuploid cells, invasive behavior, expression of pro-inflammatory signals and tumorigenesis. In our epithelial model, upon CIN induction, major protein quality control mechanisms- namely the ubiquitin-proteasome system, autophagy and the unfolded protein response (UPR) - are activated as a consequence of gene dosage imbalances in CIN-induced aneuploid cells. Our data indicate that near-saturation functioning of autophagy leads to impaired mitophagy, mitochondrial dysfunction and the production of radical oxygen species (ROS). We also found that dysfunctional mitochondria drive senescence by activating JNK signaling. Our studies show that all aspects of aneuploidy-induced senescence - including a cell cycle arrest in G2, changes in cellular and nuclear size, enlargement of the lysosomal compartment and the secretory phenotype- rely on the activity of the JNK signaling pathway. In our epithelial model mitochondria play an important role as both signaling and sensing organelles in activating JNK signaling through the production of ROS and the activity of the mitochondrial UPR. Moreover, we have experimental evidence that aneuploidy-induced senescence contributes to cellular proteostasis and tissue repair. Senescence exerts these actions by inducing autophagy and promoting proliferation. All these results will certainly open new avenues towards the use of chemical therapy to dampen aneuploidy-associated cellular stresses and target most solid tumors of epithelial origin.

83 Modeling gene expression evolution with EvoGeneX uncovers differences in evolution of species, organs and sexes

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While the evolution of DNA sequence is well-studied, an equally important factor in the evolution of species -- the evolution of gene expression -- is yet to be fully understood. In recent years, new tissue/organ-specific gene expression datasets spanning several organisms across the tree of life, have become available providing the opportunity to study expression evolution in more detail.

We introduce EvoGeneX, a computational method that models the evolution of gene expression as a stochastic process. Given such a stochastic model, we use maximum likelihood estimation and hypothesis testing to differentiate among three modes of evolution: 1) neutral (modelled as Brownian Motion), 2) constrained: when expression evolution is assumed to be biased toward an optimum value (modelled as Ornstein-Uhlenbeck process), and 3) adaptive: where the expression in different branches of the evolutionary tree is biased toward different optimum values. Importantly, in addition to modelling expression variations between species, EvoGeneX models within-species variation by formally incorporating the data from biological replicates in the mathematical model for the stochastic process. We show that EvoGeneX significantly outperforms the currently available computational method. In addition, to facilitate comparative analysis of gene expression evolution across tissues and sexes, we introduce a new approach to measure the dynamics of evolutionary divergence of a group of genes by using the Michalis-Menten (MM) curves.

We applied EvoGeneX to analyze expression evolution across different organs, species and sexes of the Drosophila genus, though our method is generic to be used for other species too. Our analysis demonstrated that, in Drosophila, neutral evolution cannot be rejected for a large fraction of the genes showing the evidence of many constrained genes. We also found that many constrained genes are common to all organs and both sexes. Our MM based approach revealed striking differences in evolutionary dynamics in male and female gonads. Finally, EvoGeneX revealed compelling examples of adaptive evolution.

While working with different species, tissues, sexes and other parameters, we developed our own workflow manager named JUDI which is generic to be used for building and efficiently executing bioinformatics pipelines with many parameters.

84 Rapid turn-over of centromere sequences in *D. melanogaster* and the *simulans* clade.

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During cell division, the genome is transmitted by faithfully segregating chromosomes to each daughter cell. Centromeres are structures essential for proper chromosome segregation and thus cell division. Despite their functional conservation,

centromeres evolve rapidly. Centromeres are typically defined epigenetically by the presence of the centromere-specific histone H3 variant, CENP-A. Centromeres form in repeat-rich regions of the genome but the roles of DNA sequences in centromere function are unclear. The highly repetitive nature of centromeres presents a major challenge for genome assembly and thus for understanding centromeric DNA organization.
We recently revealed that all centromeres in *D. melanogaster* correspond to islands of complex DNA enriched in retroelements and flanked by tandem repeats. Each centromere is unique—the only sequence they have in common is the *G2/Jockey-3* retroelement. We also find evidence for *G2/Jockey-3* at *D. simulans* centromeres. It is unclear if any of these sequences are important for centromere function. Here we study the evolution of centromere composition to gain insights into the role of DNA sequence in centromere biology. We characterized centromere organization in three sister species: *D. simulans, D. sechellia,* and *D. mauritiana*, which diverged only 240,000 years ago. To identify centromere candidates in each species, we performed CENP-A CUT&Tag and took advantage of our recent heterochromatin enriched assemblies. We discovered that *simulans* clade centromeres have similar organization as *D. melanogaster*'s: islands of complex repeat flanked by tandem repeats. However, none of the *D. melanogaster* centromere islands are conserved in the *simulans* clade. Instead, the *simulans* clade centromeres are mainly enriched in complex satellites. G2/Jockey-3 is enriched in *D. simulans* centromeres, but much less so in *D. sechellia* and *D. mauritiana.* Although the large-scale structure of centromeres is likely conserved between species, our results highlight the rapid turnover of centromeric sequences among the *simulans* clade species and *D. melanogaster*. Determining the detailed centromere organization within closely related species gives us the unique opportunity to study the short-term dynamics of centromeric DNA. Identifying the functional centromeric DNA will give insights into their roles in chromosome function and evolution.

85 Multiple defects in ribosome assembly or function affect translation and cell competition through Xrp1 and eIF2α

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Cell competition is the active elimination of cells that differ from their neighbors in a mosaic tissue. It is proposed to have both tumor-promoting and tumor suppressive role, as well as to regulate growth and developmental defects. In 1975, Morata and Ripoll described the first example of cell competition, the elimination of cells with heterozygous mutations in Ribosomal Protein genes ($Rp^{+/-}$) from growing Drosophila tissues. Cell competition was later shown to occur by apoptosis at the interface between $Rp^{+/-}$ and wild type cells. Recently, we showed that cell competition is not a direct response to reduced ribosome number, but a regulatory response controlled by the bZip domain putative transcription factor Xrp1. Xrp1 activation is even responsible for the reduced translation and growth of $Rp^{+/-}$ cells and contributes to the developmental delay of $Rp^{+/-}$ flies. The downstream effectors of Xrp1 have not previously been described. Here we show that Xrp1-dependent activation of PERK leads to phosphorylation of the α subunit of eukaryotic initiation factor 2 (eIF2α), a key regulator of CAP-dependent translation. We show that eIF2α phosphorylation is the cause of the reduced translation rates of Rp mutant cells. In addition, we found that depletion of factors participating in either ribosome biogenesis pathway (rRNA synthesis or ribosome maturation) or ribosome function (initiation or elongation of translation) also activate the Xrp1/p-eIF2α pathway and remove via cell competition deficient cells when normal cells are nearby. Furthermore, by depleting the eIF2α phosphatase (PPP1R15) in Drosophila imaginal disc cells, we showed that eIF2α phosphorylation is sufficient for cell competition. It does that by upregulating Xrp1 levels, which further increases the p-eIF2α levels and triggers the competitive elimination of the PPP1R15 depleted cells. Therefore, Xrp1 reacts to disruption of ribosome integrity at many levels and safeguards the ribosome quality at organismal level by removing those ribosome-compromised cells. In addition to identifying the next step in cell competition, these findings suggest potential targets for the treatment of human ribosomopathies caused by mutations in components of the ribosome biogenesis pathway.

86 Defining the role of Nuclear-pore complex (NPC) components in fly models of ALS

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Tar DNA binding Protein-43 (TDP-43) is a major DNA/RNA binding protein involved in multiple cellular processes including transcriptional regulation, mRNA splicing and stress granules formation. Mutations in TDP-43, such as TDP-43^{M337V}, cause Amyotrophic Lateral Sclerosis (ALS). Abnormal accumulation and phosphorylation of TDP-43 is also associated with Frontotemporal Dementia (FTD) and Alzheimer's disease (AD). Despite its contributions to several devastating diseases, the toxic properties of TDP-43 are less understood, and hence, lesser is known about modifiers of its toxic effects. Here, we report the first genetic screen of over six thousand next generation RNAi lines in a Drosophila model expressing human TDP-

43^{M337V}. We found ~200 genetic modifiers of TDP-43 toxicity using a degenerative fly eye phenotype as screening platform. We discovered nearly 30 NPC components and nuclear transport related genes comprising the second largest ontology group among all our modifiers. Most of the modifiers from this category were suppressors, but interestingly, an RNAi against importin Fs(2)Ket enhances the eye phenotype. We further validated our results by generating an overexpression line of Fs(2)Ket and established it as a strong suppressor. We also proved that overexpression of Fs(2)Ket suppresses phospho-TDP-43 staining and reduces TDP-43 toxicity in the fly CNS, which partially restores lifespan and locomotor deficits in TDP-43-expressing flies. We are currently working on other nuclear transport-related modifiers to understand the pathway contributions more comprehensively. We anticipate that modifying or altering the nuclear pore complex components will suppress the toxic effects caused by pathological TDP-43 mammalian models and may lead to the development of potential therapeutic approaches against TDP-43 proteinopathies. This work was supported by NIH grant R01059871.

87 Analysis of a transmembrane protein that stabilizes damaged photoreceptors

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Vitamin A (vitA) is essential for vision since photoreceptors require vitA-derived retinal to form light-sensitive Rhodopsins. VitA deficiency causes night blindness, damages the photoreceptors, and is the leading cause of preventable childhood blindness. Like humans, flies cannot synthesize vitA and must obtain it through their diet. However, unlike human photoreceptors, *Drosophila* photoreceptors do not fully degenerate in the absence of vitA despite severe damage and abnormal Rhodopsin expression.
We discovered that the novel transmembrane protein Mps is upregulated >140-fold in vitA-deprived eyes and localizes to the damaged light-sensing compartments (rhabdomeres) of the photoreceptors. To test the function of Mps, we generated mosaic eyes composed of eye-specific *Mps* null mutant clones and adjacent wild-type tissue by FRT recombination. Consistent with our hypothesis that Mps stabilizes damaged photoreceptors, the rhabdomeres of vitA-deprived *Mps* mutant photoreceptors were significantly shorter and fused into a ring-like shape. Ultrastructural analysis revealed that the photoreceptor membranes had collapsed and exhibited curtain-like folds. In contrast, vitA-replete *Mps* mutant tissue did not show these defects; we noted variable rhabdomere size with reduced F-actin levels, suggesting an additional role of Mps in actin organization.
Next, we stained *Mps* null mutant mosaic eyes with antibodies for photoreceptor-specific and phototransduction-related proteins. We found reduced levels of Trp, a light-sensitive Ca^{2+} cation channel, in both vitA-deprived and vitA-replete *Mps* mutant tissue. Furthermore, vitA deprived *Mps* null mutant tissue exhibited an upregulation of MyoV, an unconventional myosin involved in membrane transport to developing rhabdomeres. This is potentially due to the need for further membrane support due to the collapsed and curtain-like membrane folds in *Mps* mutants.
Taken together, we found cell autonomous, drastically exacerbated photoreceptor defects due to the loss of Mps in vitA-deprived photoreceptors. Mps thus preserves photoreceptors by stabilizing the damaged light-sensing compartments. We also identified additional roles of Mps in actin organization, MyoV regulation, and expression of Trp channels in the eye.

88 Investigating neuro-consequences of spaceflight and altered gravity using *Drosophila melanogaster*

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A comprehensive understanding of the effects of spaceflight and altered gravity on human physiology is necessary for continued space exploration and habitation. Spaceflight includes multiple factors such as microgravity, ionizing radiation, physiological stress, and disrupted circadian rhythms, contributing towards pathophysiological responses that target immunity, bone and muscle integrity, cardiovascular and nervous systems. 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) spaceflight conditions, MVP-Fly-01 mission which includes exposure to microgravity and in-flight space 1g controls, and 2) hypergravity, using the ground-based chronic acceleration model. In the MVP mission, we observed 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 microgravity adults show a decrease in neuronal ($p<0.05$) and dendritic field ($p<0.01$), dopaminergic neurons ($p<0.01$), and glia ($p<0.001$) in adult brains coupled

with increased oxidative damage ($p<0.01$) and apoptosis ($p<0.001$) compared to in-flight 1g controls, suggesting increased neuronal loss under spaceflight conditions. Further, transcriptomic analyses of spaceflight flies revealed differentially expressed genes that affected multiple biological pathways related to oxidative stress, neuronal regulation, and cell death. 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 ($p<0.001$). Also, qPCR analysis shows that parkin gene expression is significantly reduced in these fly brains($p<0.05$). Additionally, chronic hypergravity resulted in a depressed locomotor phenotype in these flies ($p<0.05$) in conjunction with decreased dopaminergic neuron counts ($p<0.0001$), increased apoptosis ($p<0.0001$), altered mitochondrial membrane potential ($p<0.05$) and decreased ATP levels ($p<0.05$) in these fly brains. Overexpression of SOD2, an antioxidant gene, in the neuronal population resulted in the rescue of hypergravity associated phenotypes. 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 elucidate the different approaches to protect the nervous system in astronauts during spaceflight, while also contributing to a better understanding of CNS disorders on Earth.

89 Using Natural Variation & Deep Learning to Construct Gene Regulatory Networks in *Drosophila*

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Maps of gene regulatory networks (GRNs) in most organisms remain incomplete. The discovered network components have mostly been identified using knockouts that perturb the network sufficiently to have observable phenotypic effects. While such techniques work well to identify the major components, a knockout might lead to several lost connections and that makes the discovery of other components hard.

We intend to use naturally occurring variation contained within the Drosophila Genetic Reference Panel (DGRP), which contains over 200 lines, to identify novel regulatory components. Deep Learning would be employed over three large datasets - genomic, transcriptomic, and spatial gene expression domains genes, within the DGRP. Specifically, we are investigating the well-studied anterior-posterior (AP) segmentation network, which determines body segments in the early embryo. Maternal coordinate proteins, Bicoid (Bcd) and Hunchback (Hb), activate Gap genes, and together they activate pair-rule genes such as *even-skipped (eve).* Spatial expression patterns of these network components are obtained by quantifying image data from fluorescent staining while the genome of all of the lines has already been sequenced.

Preliminary studies over only 13 fly lines, searching within a 20 kb region of the genome, has identified *pangolin* (*pan*) as a component of the AP patterning network. The statistical power from analyzing all 200 fly lines, over the entire genome, would identify several new components. We have developed techniques to reliably extract spatial quantitative data from a large number of stained embryos, without manual supervision. Using deep learning on this dataset we intend to discern subtle compensatory regulation in the AP segmentation network, by correlating DNA polymorphisms across fly lines with subtle, but detectable, changes in spatial expression patterns. This technique could then be applied to other biological networks where high-resolution data may be obtained.

90 Analysis of cell-type-specific chromatin modifications and gene expression in *Drosophila* neurons that direct reproductive behavior

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A major remaining challenge in the field of neuroscience is determining how neural circuits are specified to establish and maintain innate behaviors. Here, we investigate this by examining *Drosophila melanogaster fru P1*-expressing neurons that underlie reproductive behaviors in both sexes. We developed a method to purify cell-type-specific chromatin (Chromatag), using a tagged histone H2B variant that is expressed using the versatile Gal4/UAS gene expression system. Using Chromatag, we evaluate five chromatin modifications: H3K27ac, H3K4Me3, H3K36me3, H3K9me3, H3K27me3, at three life stages in both sexes. We find substantial changes in chromatin modification profiles across development and fewer differences between 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. We compare chromatin modifications to the gene expression data and find patterns of chromatin modifications associated with gene expression. An examination of the genic features where chromatin modifications resides shows certain chromatin modifications are maintained in the same genes across development, whereas others are more dynamic, which may point to modifications

important for cell fate determination in neurons. Using a computational analysis to identify super-enhancer-containing genes we discovered differences across development, and between the sexes that are cell-type-specific. A set of super-enhancer-containing genes that overlapped with those determined to be expressed with the TRAP approach were validated as expressed in *fru P1* neurons.

91 Dilp8 controls a time window for tissue size adjustment in *Drosophila*

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The control of organ size mainly relies on precise autonomous growth programs. However, organ development is subject to random variations, called developmental noise, best revealed by the fluctuating asymmetry observed between bilateral organs. The developmental mechanisms ensuring bilateral symmetry in organ size are mostly unknown. In Drosophila, null mutations for the relaxin-like hormone Dilp8 increase wing fluctuating asymmetry, suggesting that Dilp8 plays a role in buffering developmental noise. Here we show that size adjustment of the wing primordia involves a peak of Dilp8 expression that takes place sharply in the epidermis at the end of juvenile growth. We identify ecdysone signaling as both the trigger for epidermal dilp8 expression and its downstream effector in the wing primordia, thereby establishing reciprocal feedback between the two hormones as a systemic mechanism controlling organ size precision. This reciprocal feedback serves to limit the speed of development in early pupal wings, which is in turn required for the proper execution of a noise buffering mechanism. Our results reveal a hormone-based time window ensuring fine-tuning of organ size and bilateral symmetry.

92 Evading Death in the *D. Melanogaster* Nervous System

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The regulation of cell death and survival is critical for tissue development and function. Our lab has characterized a process named 'anastasis,' which is defined as cell survival following executioner caspase activation, previously thought to be the irreversible marker of apoptosis. Based on studies carried out in multiple laboratories, anastasis has been found to function during development, oncogenesis, and cell survival following injury in a variety of animal and cell culture models. Most anastasis research has focused on mammalian cancer cell lines and fly epithelial tissue, both highly proliferative cell types. However, the significance of anastasis has yet to be determined in the nervous system. During development, progenitor neurons compete with each other for survival, with only 50% surviving to adulthood. Additionally, post-mitotic neurons have been shown to initiate apoptosis and activate executioner caspases without executing apoptosis in a neurodegenerative disease model. We propose that anastasis might occur during neurodevelopment and that anastasis might enhance survival of neurons with tau tangle formation where it might function to slow neurodegeneration. We performed an RNAi screen in *D. melanogaster* neurons to test the functions of genes that affect anastasis in proliferating wing imaginal discs for effects on neurodegeneration. The results suggest that the InR/Pi3K pathway, which is pro-survival in the proliferative wing disc tissue, enhances tau toxicity in the tauopathy model. Members of Hippo and innate immunity signaling pathways appear to suppress tau toxicity. Additionally, our lab developed an *in vivo* caspase biosensor which revealed widespread anastasis in many tissues in response to stress and as a part of normal fly development, including development of the larval brain and eye disc. Further progress in understanding the relationship between cell death and survival in proliferative vs postmitotic cells will be presented.

93 iPLA2-VIA acts in distinct neuronal subtypes and in muscle to maintain locomotor ability with age, in a partially catalytic-independent manner

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Neurodegenerative disease (ND) is a growing health burden worldwide, but its causes and treatments remain elusive. Although most cases of ND are sporadic, rare familial cases have been attributed to single genes, which can be investigated in animal models. We have generated a new mutation in the *Drosophila* ortholog of the calcium-independent phospholipase A2 (iPLA2) VIA gene *CG6718*, the ortholog of human *PLA2G6/PARK14*, mutations in which cause a suite of NDs collectively called PLA2 associated neurodegeneration (PLAN). Our mutants display age-induced locomotor decline, similarly to others reported in *Drosophila* and mouse. We have confirmed reports that knockdown specifically in neurons phenocopies the age-induced locomotor decline of the mutant. As part of a Course-based Undergraduate Research Experience (CURE) in a genetics course

laboratory, twelve undergraduate students knocked down *iPLA2-VIA* in six distinct neuronal subtypes. Our results demonstrate that locomotor activity is particularly sensitive to loss of *iPLA2-VIA* in GABAergic neurons, whereas it is surprisingly resistant to loss of *iPLA2-VIA* in other neuronal subtypes, including dopaminergic neurons, which have been implicated previously in PLAN. We also find that ubiquitous knockdown results in a stronger locomotor defect than neuronal knockdown, suggesting a contribution from other tissues. Indeed, muscle specific knockdown can reproduce the age-induced locomotor decline, indicating that *iPLA2-VIA*'s function is not limited to neurons. Finally, although loss of iPLA2-VIA phospholipase activity has been proposed to underlie PLAN, we find that the locomotor decline in our mutants can be rescued by a transgene carrying a serine-to-alanine mutation in the catalytic residue, suggesting that iPLA2-VIA's cytoprotective function is at least partially independent of phospholipase activity. Altogether, our results show that iPLA2-VIA protects from age-related degeneration in both neuronal and non-neuronal tissues, and they suggest that specific neuronal subtypes, including GABAergic neurons, contribute more strongly to PLAN than others.

94 Yorkie drives tumorigenesis by non-autonomous induction of autophagy-mediated cell death.

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Mutations in the tumor-suppressor Hippo pathway lead to activation of the transcriptional coactivator Yorkie (Yki), which stimulates cell proliferation and survival, as well as non-autonomous induction of cell death in surrounding cells. The non-autonomous cell death by Yki-activated cells is called 'super-competition', while its role and mechanism have remained unclear. To address these, we performed a genetic screen in *Drosophila* eye discs for 'non-autonomous' modifiers of Yki-mediated tumorigenesis when mutated in surrounding wild-type cells. As a result, we found that mutations in autophagy-related genes in surrounding wild-type cells significantly suppressed Yki-mediated tumorigenesis caused by the Hippo pathway mutant *fat*. Autophagy activity was indeed elevated in wild-type cells nearby *fat* mutant cells and blocking autophagy significantly suppressed cell death in wild-type cells. Mechanistically, elevated autophagy in wild-type cells upregulated proapoptotic gene *hid* via NFκB, which was similar to losers of cell competition triggered by mutations in the ribosomal protein genes or *Hel25E* gene. We further found that the TOR-S6K pathway is activated in *fat* mutant cells, which is required for the induction of non-autonomous autophagy and cell death in surrounding wild-type cells. Our data indicate that Yki-activated cells elevate TOR-S6K signaling that induces autophagy-mediated cell death in surrounding wild-type cells, which is required for Yki-induced tumorigenesis.

95 A transposon expression burst accompanies the activation of fertility genes in Drosophila spermatogenesis

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Transposable elements (TEs) must be active in germline tissues to propagate new insertions to offspring. In *Drosophila melanogaster* ovaries there is narrow spatiotemporal window known as the piwi-less pocket during which TEs are most active in the female germline. However, the activity of transposable elements in the distinct cell types of the *Drosophila melanogaster* male germline is not well understood. To address this gap in knowledge, we use publicly available single-cell RNA-seq datasets to identify the cell types of the *Drosophila melanogaster* larval testes that express TE-derived transcripts. We identify a population of larval spermatocytes that express TEs at higher levels than other germline and somatic components of the larval testes. Additionally, we discover that larval TE expression is associated with a gene expression program that includes fertility factors and spermatocyte-specific transcriptional regulators. The TEs expressed by this population are enriched on the Y chromosome and depleted on the X chromosome relative to other active TEs. These TEs may represent a class of selfish elements that achieve high activity in males by taking advantage of male germline-specific transcriptional programs.

96 Cell wound repair: Dealing with life's daily traumas.

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Injuries to individual cells can occur as a consequence of daily wear-and-tear, accidents/trauma, violence, clinical interventions, and pathological conditions ranging from infections to diseases and cancers. These wounds can be particularly concerning when occurring in a non-renewing, irreplaceable cell type, or alongside fragile cell disease states. Decades of work on wound repair in a variety of models has revealed a generally conserved framework for wound closure. Our work has established the early syncytial *Drosophila* embryo as a robust cell wound repair model. Using the genetic amenability of this system, we have defined four major steps that are essential to return cells to their pre-wounded states: sensing wounds, resealing membranes, closing wounds, and remodeling plasma membrane/cortical cytoskeleton. These processes rely on dynamic changes of the

membrane/cortical cytoskeleton that are indispensable for carrying out the repairs within minutes. In particular, we have found that E-cadherin is required to anchor the actomyosin ring to the overlying plasma membrane, that RhoGEFs prepattern Rho family GTPases that are necessary for actomyosin ring formation/translocation, that Annexin proteins are required for actin stabilization within seconds of wounding, that translation rather than transcription is needed for repair initiation, and that autocrine signaling from the classical insulin signaling pathway is necessary to control actin dynamics, as well as highlighting how different mechanisms have been co-opted by different organisms to achieve the same end. We are currently using a genetic approach to identify new components/machineries of repair and to define then characterize the regulatory mechanism(s)/pathways underlying cell wound repair.

97 Investigating the role of SPECC1L Drosophila homolog, Split Discs, in the regulation of non-muscle myosin II contractility

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Point mutations to sperm antigen with calponin homology and coiled-coil domain 1 like (SPECC1L), have been associated with a spectrum of crainio-facial disorders including Tessier type IV clefts, Oblique facial clefts, Opitz G/BBB syndrome, and Teebi hypertelorism all of which commonly share pathologies such as cleft palate or lip, hypertelorism (wide- spaced eyes), and defects of larynx, and esophagus. These phenotypes are suggestive of defects in the migration and adhesion of neural cranial crest (NCC) cells. Interestingly, morpholino knock-out of the zebrafish homolog of SPECC1L led to defects in the formation of head and jaw structures, and depletion of the Drosophila homolog of SPECC1L, Split Discs (SPDS), led to defects in the formation of the proboscis and blistering in wings. Collectively, these phenotypes are indicative of defects in cellular adhesion and migration. In mammalian cells, SPECC1L co-localized with both actin and microtubules, and as such was hypothesized function as a cytolinker. When we expressed SPDS in Drosophila S2 cells we observed a localization pattern more consistent with nonmuscle myosin II (NMII). Given this potential localization we hypothesized that SPDS may affect cell migration through the regulation of NMII contractility. To test these hypotheses, we carried out a series of colocalization experiments between SPDS and NMII, including point mutations analogous to those associated with cranial-facial disorders in humans. Furthermore, we used a migratory Drosophila cell line (D25) and tracked focal adhesion dynamics. We found that the phosphorylation status of SQH had no effect on the association between SPDS and SQH, while point mutations to SPDS did. Our data indicate that SPDS does colocalize with NMII, with the point mutation Q266P showing the highest degree of colocalization while G915S showed the lowest. We also found that while depletion of SPDS by RNAi had no effect on focal adhesion assembly rates, however, the rates of focal adhesion disassembly were increased. Furthermore, the overall rate of cell migration was decreased following depletion of SPDS, suggesting that altered focal adhesion dynamics may be affecting cell migration. We conclude that SPDS localizes to NMII and that it plays a role in focal adhesion dynamics that may be mediated by this interaction.

98 More than skin deep: Using transparent animals to probe neuronal polarity

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Prototypical neurons have dendrites that receive information and axons that send it. This separation of function allows efficient directional signaling and requires distinct proteins and organelles to be targeted to the two compartments. The prototype of the polarized neuron was derived primarily from studies of mammalian neurons, which were most accessible in culture. It has subsequently become clear that many features that distinguish axons and dendrites in mammalian neurons are polarized in the same way in Drosophila. What underlies this evolutionarily conserved ability to generate two different types of compartments from materials made in a central cell body? We hypothesize that the layout of microtubules in axons and dendrites is critical to directing polarity. To test this hypothesis, we have used multiple approaches. First, we stepped further afield in animal evolution to examine polarity in the sea anemone Nematostella vectensis. Using live imaging in this transparent animal with a simple nervous system, we identified a neuron type that lacks polarity: it has two to three neurites that all have the same contents. Consistent with the idea that microtubules are critical for polarity, all the neurites have the same microtubule arrangement. In comparison, live imaging of microtubules in Drosophila sensory neurons in whole larvae shows that they have opposite organization in axons and dendrites. To more rigorously test the significance of this difference in microtubule organization, we used a Drosophila genetic background in which a subset of dendrites takes on the axonal microtubule organization. By comparing dendrites with normal and reversed polarity, we could show that most polarized features of axons and dendrites depend on the underlying arrangement of microtubules. Thus, microtubule organization is likely critical for establishing neuronal polarity across animal evolution.

99 How Flies get Fat: from Genes to Neurons

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Genetic background is a strong predisposing factor for obesity, but we currently understand only a few affected pathways. Our overall goal is understand how biological homeostatic mechanisms are established and maintained and what leads them to fail. We focus on energy homeostasis and obesity, and aim to identify new hereditary risk factors for obesity and determine how they work. We use the Drosophila larva as a model to investigate how an organism balances energy expenditure and storage. Preventing obesity requires a precise balance between deposition into and mobilization from fat stores, but regulatory mechanisms are incompletely understood. Parallel genetic screens pointed us to genes and groups of neurons with undefined roles in energy balance. As a result, we are investigating the roles of several RNA-binding proteins (RBPs) in controlling energy balance. One RBP, Spen (split ends), we found to act autonomously in the fat body to regulate fat mobilization. After carefully defining the metabolic changes that make Spen-depleted animals fat, we were able to design custom diets that partially alleviated these metabolic defects. We are now delving into the molecular details of how Spen and a related protein, Nito (Spenito), bind specific RNAs to regulate fat storage. Nito is part of the m6A RNA methyltransferase complex and is involved in sex determination. One of our areas of focus is understanding the molecular basis of the differences in fat storage between males and females and how Nito contributes to this dimorphism. Another RBP, Shep (Alan shepard), has known roles in neuronal remodeling via chromatin-level regulation of gene expression, but we find that Shep is involved differently in different tissues in regulating fat storage, and Shep levels and alternative splicing appear to respond to the nutritional status of the diet. Here also we seek to understand how Shep's RNA targets control energy storage at the organismal level. Finally, our unbiased neuronal screens identified groups of neurons in the brain whose activity prevents excess fat accumulation, and led us to identify a role for Arc1. In parallel, we identified the center of learning and memory in the *Drosophila* brain – the mushroom body – as a signaling nexus for both learning/memory and fat storage, with Arc1 as a potential mediator between the two. Given the newly appreciated roles for Arc1 in intercellular communication, including extracellular transport of RNAs, we are now investigating how diet-induced changes in Arc1 production in brain neurons might ultimately control fat storage in the fat body while coupling energetic needs to sustain brain biology required for memory and learning. Together, these efforts aim to provide a more holistic understanding of inter-organ communication in the context of organismal energy homeostasis.

100 Diet composition plastically resizes the Drosophila midgut by affecting cell gain and loss, stem cell-niche coupling and enterocyte size

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The gut is the primary interface between an animal and food, but how it adapts to qualitative dietary variation is poorly defined. We find that the *Drosophila* midgut plastically resizes following changes in dietary composition. A panel of nutrients collectively promote gut growth, which sugar antagonizes. Diet influences absolute and relative levels of enterocyte loss and stem cell proliferation, which together determine cell numbers. Diet also influences enterocyte size. Sugar inhibits translation and uncouples ISC proliferation from expression of niche-derived signals but, surprisingly, rescuing these effects genetically was not sufficient to modify diet's impact on midgut size. However, diet's impact on enterocyte size was enhanced when stem cell proliferation was deficient and reducing enterocyte-autonomous TOR signaling was sufficient to attenuate diet-dependent midgut resizing. These data reveal a new mode of plastic, diet-dependent organ resizing and clarify the complex relationships between nutrition, epithelial dynamics, and cell size.

101 Transcriptional regulation of muscle metabolism using a *Drosophila* model of tumor-induced organ wasting

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Cancer-induced cachexia is a systemic metabolic syndrome associated with higher energy expenditure, organ wasting and reduced life expectancy. Furthermore, muscle wasting can be induced by increased lipolysis in adipose tissue and elevation of fatty acid oxidation in muscle, suggesting a link between loss of fat and loss of muscle mass. Nevertheless, the mechanisms driving organ wasting in cachexia are not fully understood and no treatments are currently available. Using a previously characterized *Drosophila* model of organ wasting triggered by *Yorkie*-induced gut tumors, we identify the transcription factor REPTOR as a potential mediator of muscle wasting. We show that *REPTOR* is upregulated in muscle cells of the thorax of adult

flies with gut *yki*-tumors. Increasing REPTOR levels specifically in muscle is sufficient to induce myofiber degradation, alter mitochondrial morphology and affect glucose metabolism. The expression of *REPTOR* is modulated by lipolysis, starvation or by the gut tumor-derived ImpL2, an IGFBP-like protein that contributes to wasting by reducing systemic insulin signaling. Consistent with our findings in *Drosophila,* the expression of CREBRF, the mammalian ortholog of REPTOR, is also induced by starvation in C2C12-derived myotubes. We observe that sustained expression of CREBRF in myotubes reduces glycolysis while boosting mitochondrial respiration and oxidative metabolism, indicating that CREBRF may work in the muscle as a modulator of energy metabolism. Altogether, our findings reveal REPTOR/CREBRF as key regulators of muscle metabolism and provide novel mechanistic insight into muscle wasting and metabolic dysfunction in cancer-induced cachexia.

102 Mechanisms of Pericentrin degradation control its proximal centriolar localization and its reduction from basal bodies for sperm motility and male fertility.

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Meiotic centrosomes undergo extensive remodeling processes for basal body development prior to sperm formation. Early remodeling involves the elongation of centrioles followed by assembly and disassembly of centriolar proteins. The mechanism and biological significance of the meiotic centriole remodeling are mostly unknown. Here, we use the Drosophila model system to study centriole remodeling, as this process appears to be highly conserved. We used quantitative fluorescence microscopy to carefully characterize a range of centrosome proteins, in particular, a group of pericentriolar material (PCM) regulators known as the Bridge proteins (Pericentrin, Asterless, Spd2, and Sas4). We hypothesized that the bridge proteins spatially and temporally coordinate early centriole remodeling to facilitate sperm functions. Here, we focused our study on Pericentrin given its known functions in male fertility. To characterize the dynamics of Pericentrin, we performed a structure-function study of Pericentrin in developing basal bodies. We found that the N-terminal region of Pericentrin harbors several degradation signals that act in parallel during spermatogenesis. We observed that the degradation of Pericentrin is essential to limit its localization to the proximal end of centrioles, primarily to dictate the spatial recruitment of PCM during meiosis. We found that Pericentrin degradation also accounts for its reduction from basal bodies prior to sperm formation, which is essential for sperm motility and male fertility. To identify the precise degradation mechanisms, we performed a candidate RNAi screen composed of degradation related proteasome components. We found Rad6 and its related E3 ligases to be required for Pericentrin degradation in male germ cells. In this study, we have shown compelling evidence for Pericentrin degradation that facilitates basal body development in male meiosis, while our previous study by Galletta,2020 identified the importance of transcriptional control of Pericentrin for the spatial restriction of PCM at the centrosomes. Collectively, our studies reveal Pericentrin homeostasis as a regulatory mechanism that controls meiotic centrosome remodeling and the conserved basal body development.

103 High-throughput transcriptional profiling of multiple stages of neuronal development in a single experiment

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Recent single-cell sequencing efforts uncovered a large diversity of neurons in the adult Drosophila brain. However, neuronal transcriptional programs are highly dynamic during development and quite different from their adult counterparts. Thus, we need transcriptional atlases covering multiple stages of development. The generation of such atlases can be laborious and expensive. We devised an efficient strategy for single-cell RNA sequencing of neurons at multiple time points in a single experiment. This strategy exploits natural genetic variation in wild-type strains from the Drosophila Genetic Reference Panel to mark cells from different developmental stages. Brain samples from multiple time points and replicates were pooled together and processed as a single sample from tissue dissociation to sequencing. The resulting single-cell transcriptomes for each individual sample were separated based on SNPs captured in mRNA sequences and matched with the genotypes of parental strains.

We used this approach to generate a comprehensive transcriptional atlas of the developing Drosophila visual system covering more than 150 distinct neuronal populations and nine stages of pupal development. We matched 58 transcriptional clusters to morphological cell types with known connectivity patterns. We uncovered new regional subtypes for well-characterized T4/T5 and Tm9 neurons with distinct spatial distributions. The analysis of this dataset revealed a synchronous pan-neuronal program encoding the core components of synaptic machinery and membrane excitability. This program was overlaid by diverse cell-type-specific programs encoding rapidly changing cell recognition landscapes.

Similar strategies for pooled profiling of multiple samples can be easily adapted for a variety of experimental designs, including comparative studies in different mutant backgrounds or in altered environments. The rapid and economically

feasible generation of temporally resolved transcriptional datasets can be used to tackle a variety of longstanding questions in developmental biology.
Reference: Kurmangaliyev, Y. Z., Yoo, J., Valdes-Aleman, J., Sanfilippo, P., & Zipursky, S. L. (2020) Transcriptional Programs of Circuit Assembly in the Drosophila Visual System. Neuron, 108, 1–13.

104 The central role of the R7 photoreceptor in insect eye development and evolution

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One of the most heavily studied cell fate specification events in biology is recruitment of R7 photoreceptors in the developing *Drosophila* retina. New evidence suggests that R7 specification is also a target of evolutionary change and that modification of R7 has been used to enable a wide range of visual adaptations, such as to change the distribution of ommatidial types across the retina in several species of Diptera, for improved target detection in house flies, and for expanded color vision in butterflies. We first present evidence that the genes and signaling pathways involved in initial R7 specification are highly conserved across the insects. Here, we use antibody stains and CRISPR/Cas9 tests of gene function in butterflies and wasps. Despite deep conservation in how retina cell types are specified, considerable diversity exists in insect eyes across species that live in a wide range of environments and which have varying natural history requirements. We characterize Rhodopsin diversity across 25 families of Diptera and compare expression patterns in five species to what is known from *Drosophila*. In one example, even flies which distribute their receptors similarly to *Drosophila* sometimes make different color comparisons, such as Olive Flies, which compare one UV wavelengths (Rh3) to another UV (Rh4) instead of comparing UV to blue wavelengths in the "pale" ommatidial type. In another example, male *Musca domestica* house flies have instead sacrificed color vision entirely in favor of motion detection in a region of their eye dedicated to pursuing females, known as the "Love Spot". We used single cell sequencing to identify male-specific cell types and evaluate changes in gene expression in Love Spot R7s during development. We have identified genes that specify Love Spot fate and have used genetic tools in *Musca* and *Drosophila* to test regulatory relationships between candidate factors. We argue that Love Spot R7s are a novel neural type and uncover the genetic modifications used to produce this example of increased neural complexity. Despite deep conservation of a retina "ground plan" across the insects, specific changes in a highly conserved developmental program have allowed an impressive diversity of specialized features and functions to evolve.

105 Ran and associated karyopherins, Cadmus and Tnpo-SR, maintain ovarian cyst connectivity

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A hallmark of germ cell development in vertebrates and invertebrates is the formation of clusters of undifferentiated cells that remain interconnected during mitotic division. Germ cells proceed through mitotic expansion via incomplete cytokinesis, manifested by abscission failure, cleavage furrow arrest, and transformation of the contractile network into stable intercellular bridges that connect cells in a syncytium. Recent discoveries in cells that undergo complete cytokinesis suggest that the actomyosin contractile network is temporally and spatially regulated by Ras-related nuclear protein (Ran), a small G-protein implicated in protein transport during mitosis and interphase. Ran partners with karyopherins to transport protein cargo across the nuclear membrane and is essential for assembly of the mitotic spindle. The biological relevance of Ran to germline cyst formation and incomplete cytokinesis has not been established. Here, we identify Ran as a novel regulator of germline stem cell maintenance and germ cell cyst formation in the *Drosophila* ovary. We find that depletion of *Ran* or the karyopherins *Tnpo-SR* and *Cadmus* generates egg chambers of variable numbers, suggesting abnormal cyst development and cyst fragmentation. As a consequence of disrupting cyst division by depletion of *Ran* and *Tnpo-SR*, the oocyte fails to accumulate the oocyte-specific RNA binding protein Orb and oocyte DNA fails to condense into a karyosome. We propose that Ran, via Tnpo-SR and Cadmus, stalls cleavage furrow ingression by stabilizing Anillin localization at ring canals, thus allowing germ cell cysts to remain interconnected and oocytes to properly differentiate. Given the conservation of germline cyst formation across a diverse set of organisms, our data suggest that Ran-mediated intracellular transport may play a previously unappreciated role in the process of generating new gametes.

106 The CHD8/CHD7/Kismet family links blood-brain barrier glia and serotonin to ASD-associated sleep defects

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Sleep disturbances in autism and related neurodevelopmental disorders are common and adversely affect quality of life in patients and families, yet the underlying mechanisms are understudied. We found that individuals with mutations in *CHD8*, among the highest-confidence autism risk genes, or *CHD7* suffer from disturbed sleep maintenance. These defects are recapitulated in *Drosophila* mutants affecting *kismet*, the sole *CHD8/CHD7* orthologue. We show that Kismet is required in glia for early developmental and adult sleep architecture. This role localizes to subperineurial glia constituting the blood-brain barrier. Moreover, we show that pan-glial Kismet loss leads to hyperserotonemia, a well-established but genetically unsolved autism endophenotype. Using a number of pharmacologic, thermogenetic and genetic approaches, we demonstrate that increased serotonin levels during development underlie Kismet's sleep problems. Finally, we show that Kismet-related sleep defects, despite their developmental origin, can be reversed in adulthood by a behavioral regime resembling human sleep restriction therapy. Our findings provide fundamental insights into glial regulation of sleep and propose a causal mechanistic link between the CHD8/CHD7/Kismet family, the blood-brain barrier, developmental hyperserotonemia, and autism-associated sleep disturbances.

107 Embryos organize their glycogen and triglyceride reserves during development

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In both cells and organisms, metabolic pathways are spatially segregated, into distinct organelles or tissues. In contrast, many animal eggs appear to display little spatial specialization of metabolism, providing an opportunity to observe how such spatial organization arises during development. Drosophila embryos contain large, maternally provided stores of three major nutrients: proteins (in endosome-derived yolk vesicles (YVs)) , triglycerides (in lipid droplets (LDs)), and carbohydrates. We confirmed previous observations that YVs and LDs are initially largely homogenously distributed, but segregate to distinct regions at cellularization: YVs are almost entirely restricted to the central yolk cell, while LDs are predominantly present in the nascent blastoderm epithelium. We followed glycogen distribution by fluorescence microscopy, using chemical carbohydrate staining (PAS) or a YFP fusion of the glycogen protein glycogenin. By both approaches, we detect discrete granular structures of 1-5µm diameter, similar to the glycogen granules (GGs) previously described by electron microscopy. GGs first appear in late stage oocytes and are uniformly distributed in newly laid embryos. During syncytial stages, they deplete from the embryo periphery and are allocated almost entirely to the yolk cell. During this time, they also undergo a morphological transition, from discrete spherical granules to a diffuse broad distribution. Serendipitously, we discovered that this segregation of LDs and GGs is prevented in mutants lacking the LD protein Jabba. Here, most LDs are embedded in the surface of GGs, as confirmed by PAS staining, in vivo observation with YFP glycogenin, and electron microscopy. No such association is observed in mutants for other LD proteins. These LD-GG complexes appear stable, surviving centrifugation and traveling as units during cytoplasmic streaming. Intriguingly, while these interactions have very little effect on the motion or developmental distribution of GGs, LDs are severely affected: their streaming velocities are greatly reduced, and at cellularization, they predominantly allocate to the yolk cell. *Jabba* mutant embryos consume their mislocalized LDs more slowly than wild type and hatch with residual LDs. Altogether, we find that *Drosophila* embryos spatially segregate two of their major caloric reserves (triglycerides and glycogen) into spatially distinct compartments and that perturbations to this nutrient layout affect embryonic metabolism.

108 Defining the role of Flamingo during tumor progression and cell competition

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Solid tumors harm the host not only by local invasion of healthy tissue and metastasis to distant sites but also through cell competition. Cell competition is a process in which cells with greater fitness (winners), eliminate less fit neighboring cells (losers) during development to preserve fitness and optimize tissue function. Tumors and super-competitor cells use cell competition to take over the surrounding tissue. We have identified an essential role for the atypical cadherin Flamingo (Fmi), a central component of the Planar Cell Polarity (PCP) signaling pathway, in *Drosophila* tumor and cell competition models. PCP signaling polarizes cells within the plane of the epithelium to orient cellular structures, cell divisions, and cell movement during development and homeostasis. Intercellular Fmi homodimers scaffold the assembly of an asymmetric set of core PCP proteins.

Fmi homodimers transmit different signals in opposite directions to communicate the presence of both protein complexes, linking the proximal and distal complexes in adjacent cells and mediating asymmetric signaling between them. In addition to establishing planar polarity, numerous reports have linked PCP signaling to cancer. In spite of the growing body of literature, the role of PCP in cancer remains unclear.

We have found that Ras^{V12}-dependent tumors and super-competitor cells completely lose their ability to eliminate less fit cells if they lack Fmi. We confirmed this phenomenon in several cell competition scenarios. Additionally, *fmi*-null tumors and super-competitors undergo apoptosis. Interestingly, loser cells are not affected by the lack of Fmi. This could drive new therapeutics to directly target tumor cells, with no detrimental effect on the host tissue.

We are currently studying the mechanisms by which Fmi is required by winner cells to outcompete less fit cells by performing genetic assays in *Drosophila* tumors and super-competition models. We are evaluating two non-exclusive mechanisms: (1) Fmi enhances cell adhesion between winner cells or winner and losers, and/or (2) Fmi is required to establish a communication hub that lets cells communicate their fitness to their neighbors in a PCP-like mechanism.

To translate our findings to vertebrates and evaluate possible therapeutics, we are extending our studies to human and mouse tumors in an organoid tumor model. This rapid and efficient system will allow us to define the potential role of Fmi in cell competition and tumor progression. Characterization of such a role will define a critical and therefore therapeutically targetable pathway facilitating solid tumor progression.

109 *Drosophila* adipokinetic hormone signaling pathway regulates the sex differences in triglyceride metabolism

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In many species, including *Drosophila*, females have increased triglyceride storage and reduced triglyceride breakdown compared with males. Here, we use *Drosophila* as a model to identify metabolic pathways that contribute to these sex differences in triglyceride metabolism. Our analysis of triglyceride metabolism in both sexes identified a key role for adipokinetic hormone (AKH) in regulating the sex differences in both triglyceride storage and breakdown. Normally, mRNA levels of both AKH and the AKH receptor are higher in males than in females. In addition, we found that activity of the ~14 AKH-producing neurons was higher in males than in females. To determine whether this sex-specific regulation was significant for triglyceride metabolism, as AKH is a known regulator of fat storage and breakdown, we ablated and silenced the AKH neurons. Both genetic manipulations caused a male-specific increase in triglyceride storage and a male-specific delay in triglyceride breakdown. In contrast, overactivation of AKH neurons decreased triglyceride storage in both sexes and caused a female-biased increase in triglyceride breakdown. Notably, loss of AKH, but not another AKH neuron-derived peptide limostatin, phenocopied the male-specific effects of silencing the AKH neurons. This suggests that the sex-specific regulation of AKH is an important factor in establishing the sex differences in triglyceride metabolism. Importantly, we identified reproductive effects of AKH in both sexes that suggest one reason for its sex-specific regulation: high AKH levels in males are required for their normal mating and fertility, whereas low levels in females optimizes their fertility. This identifies a previously unrecognized, sex-specific role for the AKH signaling pathway in regulating the male-female differences in triglyceride metabolism. Furthermore, we also identified that the sex-specific regulation of AKH optimizes fertility in both sexes, demonstrating distinct approaches to reproductive success in males and females.

110 Multiple stages of germ cell differentiation in *Drosophila* testes require gap junction-mediated soma-germline communication

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Gametogenesis is a developmentally conserved process in animals that requires coordinated signaling between germ cells and somatic cells. It was shown in *Drosophila melanogaster* that communication between soma and germline regulates cell proliferation and differentiation, failure of which can lead to infertility or cancer.
Gap junctions (GJs) mediate cell-cell communication, as they allow the passage of small molecules between cells. GJs in *Drosophila* are made of innexin proteins that are functionally homologous to vertebrate connexins. We previously showed that Innexin4/Zero population growth (Zpg) mediates bi-directional signaling between soma and germline. Flies lacking *zpg* expression are sterile due to the arrest of germ cell differentiation at an early stage and impaired germ cell maintenance.
Our hypothesis is that specific signals pass through GJs, consisting of Zpg on the germline side and Inx2 in the soma, to control

the developmental program of gametogenesis. We used insight into innexin protein structure to design mutations aimed at selectively perturbing the passage of different cargos to different extents. Our goal is to identify mutations that block some cargos but not others. Using a structure-function analysis approach, we replaced the endogenous Zpg protein in the testes with mutated protein versions and analyzed germline and soma development.
To date we have analyzed nearly two-dozen different *zpg* mutants. While many led to arrest of early germ cell development, we identified a subset that disrupt specific stages of germline development. E.g. flies with mutations in residues in the channel pore which, according to 3D protein homology modeling data, are predicted to directly interact with cargo or to regulate pore permeability, exhibited normal early germline development. However, in these mutants, entry to meiosis was strongly inhibited, resulting in reduced fertility or sterility. Our work shows that specific signals that pass through GJs regulate the transition between different stages of gametogenesis.
To further analyze the function of Zpg, we are using fluorescent biosensors to find out which cargos pass through the GJ. Our data shows that the intracellular pH in somatic cells of *zpg* mutants is increased. Treating wild type testes with the gap junction blocker CBX also led to increased pH in somatic cells. Further exploring the link between pH and GJ-mediated soma-germline communication will give us insight into the mechanism of germ cell differentiation in the testis.

111 Muscle innervation: From stem cell to connectivity

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Drosophila locomotion requires the coordinated excitation of muscles by motor neurons (MNs). Each of the six legs contains 14 muscles innervated by ~ 50 MNs. Surprisingly each MN has a unique morphology defined by their dendritic arborizations in the ventral nerve cord and their axonal targetings into the leg, which is stereotyped between animals. All these MNs are produced from 10 stem cells, called neuroblasts (NBs). One of these NBs generates a lineage called LinA/Lin15 containing more than 2/3 of the adult leg MNs. We use this lineage to understand how a single stem cell generates at each division a MN with a unique and stereotyped morphology.
To understand how the stereotypic wiring of muscle innervation by Lin A MNs is transcriptionally coded, we first performed an expression screen to identify transcription factors (TFs) expressed in immature LinA MNs just before they morphologically differentiate. Combining the expression profile of diverse TFs and the spatiotemporal relationship between MNs birth order and their relative spatial distance from the NB, we developed a method to assign a specific TF code to each immature MN. We identified 18 different TF codes. The functional analysis of these TFs confirmed that their expression profile in immature MNs is instructive for their role in specifying axonal targeting. We then analyzed further the expression dynamic of these TFs in immature MNs from birth until they begin their morphological differentiation. Our results indicate that the TF code is progressively established in immature MNs as they age. The establishment of this code requires a post-transcriptional regulation in postmitotic neurons of these TFs by two mRNA binding proteins (Imp and Syp), which are known to be involved in specifying temporal identity among mushroom body (MB) neurons by regulating post-transcriptionally the expression of Chinmo. Our results revealed that the post-transcriptional regulation of TFs by RNA binding proteins contributes to generate a unique code of TFs controlling MN morphologies.

112 Tolloid-related proteases process Slit to generate novel axon guidance activities

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The secreted midline chemorepellent protein Slit is proteolytically cleaved to produce positive signals that stimulate axon growth and branching, and other diverse biological functions. Slit is cleaved into two fragments, Slit-N and Slit-C. In *Drosophila*, Slit-N can form a complex with the Dscam1 and Robo1 receptors that is required for longitudinal axon growth. We report the tolloid-related protease, *tolkin* (*tok*; also known as *tolloid-related* or *tlr*) is the sole gene responsible for Slit proteolysis *in vivo* and *in vitro*. Mutants for *tok* display disrupted longitudinal axon tracts, which can be rescued by midline expression of Slit-N, and Slit-N cannot substitute for Slit-FL midline repulsion *in vivo*. Our results identify Tok as the Slit protease and demonstrate differing biological functions of full-length Slit and Slit-N *in vivo*, with preliminary murine data to support the conserved function of Tolloid-like 1 on Slit2 processing in vertebrates. These findings explain how a single ligand can have distinct effects during neural development and will influence fields as diverse as angiogenesis and cancer.

113 Sex Peptide-containing microcarrier secretion in the *Drosophila* accessory gland is regulated by the ceramide

galactosyltransferase homologue UGT50B3

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The seminal fluid component Sex Peptide (SP), which is secreted by the main cells of the male accessory gland, plays a critical role in inducing long-term post-mating responses in females, including increased ovulation, elevated feeding and reduced receptivity to remating. We have recently shown that SP has an additional signalling role in males. It regulates the assembly of large neutral lipid-containing secreted structures called microcarriers that carry SP and other proteins on their surface. Microcarriers are stable storage vehicles for specific seminal peptides in the accessory gland, but rapidly dissipate in the female uterus after mating, releasing their contents, so that SP, for example, can bind to sperm and be stored in the sperm storage organs. Here we show that Ugt50B3, the *Drosophila* homologue of human UGT8, a ceramide galactosyltransferase, which is the most highly conserved lipid glycosyltransferase in the animal kingdom, is expressed at high levels in main cells and controls microcarrier release. When *Ugt50B3* is knocked down in main cells, microcarriers remain attached to the main cell surface via long projections that differ in composition to the rest of the plasma membrane. During mating, these microcarriers are not transferred to females, even though sperm are, and in the most extreme cases, this leads to complete sterility. Although Ugt50B3 is expressed at high levels exclusively in the adult accessory gland, UGT8 is much more broadly expressed in human tissues, where it appears to play roles in breast milk lipid droplet and bile formation, and in myelination. Furthermore, it is frequently highly upregulated in breast and other specific cancers. Our work raises the possibility that UGT8 plays a secretory role in these different tissues and that microcarrier structures may have signalling roles outside the *Drosophila* male reproductive system.

114 Expansion and interpretation of novel *ATAD3A* alleles using Drosophila models

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The ATPase family AAA-domain containing protein 3A (ATAD3A) is a nuclear encoded mitochondrial membrane anchored protein. ATAD3A is involved in diverse processes including mitochondrial dynamics, mitochondrial DNA organization, and cholesterol metabolism. We and others previously discovered that biallelic deletions (null), recessive missense variants (hypomorph), and heterozygous missense variants or heterozygous duplications (antimorph) in *ATAD3A*lead to neurological syndromes in humans. Here we report an additional 13 individuals from 8 unrelated families with biallelic *ATAD3A* variants. Four of the identified missense variants, p.(Leu77Val), p.(Phe50Leu), p.(Arg170Trp), p.(Gly236Val), were inherited in *trans* to loss-of-function alleles (copy number variant or single nucleotide variant) in *ATAD3A*. A fifth missense variant, p.(Arg327Pro), was homozygous. Affected individuals exhibited findings that were previously associated with *ATAD3A* pathogenic variation, including developmental delay, hypotonia, congenital cataracts, hypertrophic cardiomyopathy, and cerebellar atrophy. One family exhibited hearing loss, which may represent an expansion of the phenotypic spectrum. To determine whether the missense variants were indeed pathogenic, we created a *Drosophila Atad3a Gal4* trap null allele using CRISPR-Cas-9 gene editing technology. Phe50Leu, Gly236Val, and Arg327Pro are severe loss of function alleles leading to early developmental lethality accompanied with neurogenesis defects, whereas Leu77Val and Arg170Trp are partial loss of function alleles that

cause progressive locomotion defects. Moreover, Arg170Trp and Leu77Val expression leads to an increase in autophagy and mitophagy in adult muscles. Our findings expand the allelic spectrum of *ATAD3A* variants for human mitochondrial diseases, and exemplify the use of a functional assay in *Drosophila* to aid variant interpretation.

115 Coordinated shifts in redox metabolites during quiescence are heritable factors that drive the reprogramming of progeny metabolism

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Maternal metabolic stress has a profound impact on progeny health and disease susceptibility. While studies have shown that maternal metabolic dysfunction induces a heritable change in progeny physiology, these studies fail to examine how the quiescent nature of oocytes impact this process. This knowledge gap largely stems from the inability to isolate pure populations of quiescent oocytes for in-depth mechanistic studies.
To address this issue, we used *Drosophila* oogenesis as a system to isolate large quantities of staged oocytes for biochemical and systems-based studies of oocyte metabolism. Based on our previous research, we hypothesized that changes in metabolites in mature oocytes function as heritable factors that drive the reprogramming of progeny metabolism. In testing this model, we found that oocytes acquire a unique redox state during mitochondrial respiratory quiescence (MRQ) in late oogenesis. Interestingly, inducing metabolic stress in females caused MRQ to occur several stages earlier in oocyte development and disrupts this unique redox state. In particular, the precocious onset of MRQ induces reductive stress and impairs NAD^+ biosynthesis. We also showed that altering NAD^+ levels are both necessary and sufficient to induce the reprogramming of progeny metabolism. We have found that these reprogrammed progeny survive much better on a low nutrient diet. At the same time, these progeny display a shortened adult lifespan suggesting an adaptive trade-off that helps progeny adapt to the nutrient environment. These effects on progeny are primarily mediated by a loss of H3K27 methylation and de-repression of genes involved in several aspects of intestinal metabolism during embryogenesis. Using mammalian cells, we found that inducing cellular quiescence has a conserved role in creating a metabolic environment that induces the reprogramming of progeny metabolism in development and models of cancer recurrence. Overall, our data show that cellular quiescence has a conserved role in inducing the reprogramming of progeny metabolism and that redox metabolites, such as NAD^+, can function as heritable factors that link maternal metabolic stress to changes in progeny health and disease susceptibility.

116 The role of intestinal TOR signaling in metabolic responses to bacterial infection.

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Animals in their natural ecology are often exposed to environmental stressors (e.g. starvation, extreme temperature, hypoxia, pathogens) that can affect their physiology, development and lifespan. An important question in biology is how animals sense these stresses and, in response, adapt their metabolism to maintain homeostasis and survival. In some cases, specific tissues function as stress sensors to control whole body adaptive responses. One well-studied example is the Drosophila intestine. In response to oral pathogenic bacterial infection, the fly gut controls both local and systemic anti-bacterial immune responses. Recent work shows that the gut also controls whole-body metabolic changes to promote infection tolerance. Here, we show that one way that the fly gut mediates these adaptive metabolic responses is via induction of target-of-rapamycin (TOR) kinase signaling. TOR is a well-established regulator of metabolism that has classically been shown to be activated by growth cues and suppressed by stress conditions. Interestingly however, we found a rapid increase in TOR activity in the fly gut in response to bacterial infection stress, Furthermore, we showed that blocking this TOR induction reduced survival upon infection. Our data suggest that these protective effects of gut TOR signaling on organismal survival may be mediated through altered whole-body lipid metabolism. We found that bacterial infection increased expression of lipases, and induced both intestinal and whole-body depletion of lipid stores. In contrast we found that genetic activation of TOR in the intestine induced expression of transcription factors and enzymes that promote lipid biogenesis, and led to increased whole body lipid stores. Moreover, we found that TOR was required for upregulation of several gut-derived signaling peptides that have been shown to communicate with the brain and fat to control lipid metabolism. Our data supports a model in which induction of TOR signaling in the intestine represents a host adaptive response to counteract infection mediated loss of whole-body lipid stores in order to promote survival.

117 Fine mapping of crossover and noncrossover distributions around heterozygous inversion breakpoints

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Accurate chromosome segregation during meiosis is ensured by using recombination to create crossovers (COs) between homologous chromosomes. Recombination is initiated by a DNA double-stranded break (DSB) that can be repaired either as a CO or a noncrossover (NCO), but how any given DSB is slated for CO or NCO repair has remained enigmatic despite a detailed understanding of the genetic networks involved. It is well established that heterozygous inversions have multiple effects on the recombination landscape. First, they suppress COs locally, both inside the inversion and immediately outside of the inversion. Furthermore, heterozygous inversions induce the interchromosomal effect, whereby CO frequency is dramatically increased on structurally normal chromosomes through a *pch2* mediated checkpoint delay of prophase. We recently showed that heterozygous inversions suppress COs locally outside the inversion breakpoint by altering repair outcome in favor of NCOs and suggested that the change in the recombination landscape due to heterozygous inversions is mediated by CO patterning mechanisms. In order to understand how heterozygous inversions shuttle DSB repair away from a CO repair outcome, we have generated a high-resolution map of both COs and NCOs outside of the *chrX dl-49* inversion by recovering 100 COs that occurred between *yellow* and the distal end of *dl-49* or between the proximal end of *dl-49* and *forked.* We have also recovered 100 non-recombinant offspring, of which a fraction will have NCOs. We have sequenced these individuals to generate a high-resolution map of the CO and NCO distribution outside of inversion breakpoints. Preliminary data show that CO suppression is highly patterned, with no COs within 500 kb of the breakpoint and a gradual increase in frequency away from the breakpoint. Simultaneously, these flies will have also experienced the interchromosomal effect due to the *dl-49* inversion. To ask if a CO on an inversion chromosome alleviates the interchromosomal effect, we are analyzing the CO frequency on *chr2* in the 100 individuals with a CO and the 100 individuals without a CO. Together, these data will provide insight into the mechanisms used to change the recombination landscape in response to heterozygous inversions.

118 Role of prostaglandins in germline stem cells of the *Drosophila* ovary

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Prostaglandin (PG) signaling regulates a variety of physiological processes. Specific prostaglandins are produced downstream of cyclooxygenase (COX) enzymes. Long-term inhibition of COX activity produces beneficial effects such as a reduced inflammation at the cost of side effects including gastric ulcers and decreased female fertility. It is crucial that we determine the molecular details of PG signaling to understand tissue-specific effects associated with PG signaling. Capitalizing on the genetic tools of *Drosophila*, we are using the ovary as a model tissue to identify downstream targets of PGs. *Drosophila* contains a single COX-like enzyme, Peroxinectin-like (Pxt), which is necessary for PG signaling during oogenesis. Pxt is expressed throughout oogenesis and weakly accumulates in the germarium. Homozygous females for *pxt* mutant alleles 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 follicles. Recently, we found that PG signaling plays a role in germline development. Two to three germline stem cells (GSCs) reside in the anterior tip of the germarium to give rise to female gametes. Loss of Pxt causes elongated germaria and disrupts the organization of dividing cysts. In germaria lacking Pxt, there is an increased number of GSCs trapped in late stages of division. Stem cysts of undifferentiated cells form in *pxt* mutants similar to those formed from the disruption of abscission machinery or nucleolar structure-function. The nucleolus is a target of PG signaling in later stage follicles, suggesting this as a potential mechanism for regulating germline differentiation. Another potential target of PGs are nuclear actin pools. Nuclear actin is implicated in a variety of nuclear activities such as chromatin remodeling and interactions with RNA polymerases. A distinct pool of actin recognized by the C4 antibody is present within the germarium, accumulating in both the nucleoplasm and nucleolus of GSCs. These C4 positive pools diminish as cysts begin to differentiate suggesting that nuclear actin may play a role in stemness. In *pxt* GSCs, the C4 actin pool in the nucleoplasm is decreased. These data indicate an important role for prostaglandin signaling in germline development and provide new insight into mechanisms regulating oogenesis.

119 Innexins are required for glia function and ensheathment of the peripheral nerve

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Glia are key to protecting and enabling nervous system function. Each peripheral nerve in the Drosophila larva is ensheathed by three glial layers, which structurally support, insulate and create the blood-nerve barrier. How peripheral glia communicate with each other and between layers is not well established and we investigated the role of the gap junction proteins, Innexins, in mediating glial function in the Drosophila periphery. Of the eight Innexins we tested, we found two (Inx1 and Inx2) play a number of roles in Drosophila peripheral glia. Both Inx1 and Inx2 are distributed throughout the glial layers with Inx1+Inx2 plaques in the subperineurial glia and wrapping glia. We observed that Inx2 is key to the Ca+2 pulses we observed in the peripheral subperineurial glia but did not disrupt subperineurial glial morphology. Conversely while Ca+2 pulses were not

observed in the wrapping glia, loss of Inx2 disrupted wrapping glial ensheathment of axons. However loss of Inx2 in the subperineurial glia also disrupts the morphology of the neighboring wrapping glia, resulting in fragmented wrapping glial processes. This suggests that Inx2 mediates communication between these two glial layers. We will present evidence that supports a role for Inx2 in mediating subperineurial-wrapping glia adhesion that is either independent or dependent on gap junction channel function. Overall we have identified Inx1 and Inx2 as key components in the proper function and ensheathment of mutiple glial layers in the peripheral nerve.

120 Characterization of *Drosophila* 3rd instar larval ventral cord motor interneurons using single-cell RNA-seq profiling

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To gain insight into the cellular diversity of the third instar larval ventral cord of *Drosophila*, we have sequenced ~30,000 single cells and assigned them to 15 clusters using Seurat, a toolkit for single cell genomics. We have used the expression of *VGlut, Gad1, VAChT and Ddc* to define glutamatergic, gabaergic, cholinergic, and serotonergic/dopaminergic neurons. We have identified motoneuron, interneuron and glial clusters, as well as a developmental trajectory initiated from neuroblast precursors and leading to differentiated ventral cord neurons. One large cluster is made up of putative interneurons and is marked by expression of all four types of neurotransmitters, with each cell capable of expression of a single neurotransmitter. The interneuron cluster has been sub-clustered into more than 30 clusters, each consisting of gabaergic, cholinergic, glutaminergic or dopaminergic neurons. The large number of gabaergic and cholinergic clusters suggests an extensive diversity of these cell types. Two of the dopaminergic clusters are composed of neurosecretory cells and most other clusters express one or two neuropeptides as well. For each of the interneuron clusters, we identify signaling inputs by identifying ionotrophic and GPCR neurotransmitter receptors. Enriched transcription factor profiles for each cluster suggests that each cluster has a unique identity. Our analysis provides information on larval interneurons that will lead to enhanced understanding of the function of these cells in the larval ventral cord.

121 Constraints in protein biosynthesis of multi-functional fat body tissue lead to a trade-off between reproduction and immunity

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Using the same tissue for multiple purposes can result in sub-optimal performance of each process. Although such tradeoffs are common, it is largely unknown how tissues balance competing roles. Immune performance and reproductive output are often negatively correlated in diverse biological systems, generating a classic life history tradeoff. The insect fat body tissue is a highly polyfunctional tissue whose roles include the production of egg yolk proteins and mounting of systemic immune responses, as well as metabolic control, nutrient storage, detoxification of xenobiotics, and other functions. We hypothesized that the fat body achieves its multiple functions through the division of labor, with distinct cellular subpopulations performing specific functions. We further hypothesized that physiological perturbations affect specific subsets of the tissue, including the robust systemic immune response triggered by a bacterial infection. To test this, we sequenced individual nuclei from the dissected fat body tissues of *Drosophila melanogaster* females were either mated in order to induce active reproductive investment (M_) or held as virgin to limit reproductive investment (V_), and were either given a systemic bacterial infection with *Providencia rettgeri* to stimulate an immune response (_I) or were held uninfected (_U). Each factorial treatment (VU, VI, MU, MI) was generated in two independent biological replicates consisting of 40 flies per replicate. Confirming our initial hypothesis, we identified transcriptionally heterogeneous cell populations indicative of cellular subfunctionalization within the tissue. The most abundant fat body subpopulations across all treatments were marked by the expression of genes encoding yolk proteins. Mating induced increased expression of metabolic genes across multiple subpopulations. Bacterial infection induced increased tissue-wide expression of immune-related genes including anti-microbial peptides, although the expression of specific sets of genes was heterogeneous across cellular subpopulations. Strikingly, the fat bodies of MI females, which are under joint reproductive stress and immune challenge, show transcriptional signatures of lowered ribosome biogenesis and reduced translational capacity. We confirmed using Western blots and cycloheximide treatment that MI females indeed are translationally stressed, and suggest that a failure of translational capacity leads to reduced immunocompetence in mated females and a higher risk of death from infection. Such physiological limitations could explain the wide prevalence of tradeoffs, including the commonly observed reproduction-immunity tradeoff.

122 A novel role for MICOS complex *CHCHD6* in cardiac function and structure

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Hypoplastic left heart syndrome (HLHS) is a severe birth defect that accounts for up to 4% of congenital heart diseases (CHDs). HLHS is a complex, multifactorial genetic disease, however, our ability to understand the genetic complexities and pathogenic mechanisms leading to this disease is limited. Therefore, there is a great need to uncover additional genes that contribute to the molecular, cellular, and morphological processes underlying HLHS. A candidate list of 11 genes was generated based on whole genome sequencing of a patient with sporadic HLHS and their unaffected family. We use *Drosophila* as a cardiac model system to test candidates upon spatially and temporally targeted gene knockdown (KD). Of the candidate HLHS genes tested, cardiac-specific KD of coiled-coil-helix-coiled-coil-helix-domain-containing protein 6 (*CHCHD6*) in *Drosophila* results in drastically compromised heart contractility and altered filamentous (F) actin-based sarcomeric myofibrillar structure. CHCHD6, previously not known to be associated with heart disease, is part of the MICOS complex and functions to maintain mitochondrial cristae morphology and respiratory complex assembly. Mitochondrial function and localization were impaired in *Drosophila* when *CHCHD3/6* was knocked down in all muscle cells. KD of other MICOS complex components, *Mitofilin* and *Sam50*, also exhibit significantly reduced contractility, however, sarcomeric F-actin patterning/ structure appears normal. Extended bioinformatic analysis on a cohort of 183 HLHS patients revealed additional variants in *CHCHD6* and other components of the MICOS complex. These findings suggest that CHCHD3/6 plays an important role in maintaining heart function and structure, likely due to the energy-demanding nature of the heart.

123 Defective peroxisomal import accelerates abnormal lipid accumulation in *Drosophila* oenocytes

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Recent advances suggest that peroxisome plays an important role in the progression of many human disorders, including cancer, obesity-related diabetes, and degenerative neurologic disease. In cooperation with mitochondria and lipid droplets, peroxisomes contribute to the oxidation of fatty acids and biosynthesis of lipids. Previous studies have shown that peroxisomal dysfunctions induce abnormal retention of lipids, indicating an imbalance of synthesis and elimination of triglycerides. However, knowledge about mechanisms underlying the association between peroxisome and abnormal lipid accumulation (steatosis) remains limited. In this study, we find that defective peroxisomal import (*Pex5* knockdown) accelerates steatosis in *Drosophila* oenocytes (hepatocyte-like cells). In addition, we identify two transcriptions factors that regulate *Pex5* knockdown-induced steatosis: Daughters against dpp (Dad) and Mondo/ChREBP. Both transcription factors are up-regulated under oenocyte-specific *Pex5* knockdown. Dad is an inhibitor of SMAD complex activity in the TGF-ß signaling pathway. Oenocyte-specific knockdown of *Dad* further promotes *Pex5* knockdown-induced steatosis. Mondo/ChREBP is a transcription factor that regulates genes involved in carbohydrate metabolism and lipid metabolism. Oenocyte-specific knockdown of *Mondo/ChREBP* is sufficient to block *Pex5* knockdown-induced steatosis. Taken together, our data suggest that TGF-ß signaling pathway, carbohydrate metabolism and/or lipid metabolism participate in steatosis caused by peroxisomal import dysfunction in *Drosophila* oenocytes.

124 The Regulation of Lipid Breakdown and Transport by Heterogeneous Nuclear Ribonucleoproteins (hnRNPs) in *Drosophila*

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The storage of excess nutrients as triglycerides is essential for all organisms to survive when food is scarce; however, the mechanisms by which triglycerides are stored are not completely understood. Genome-wide RNAi screens in cultured *Drosophila* cells have identified genes that are important in the regulation of triglyceride storage, including 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 seven hnRNPs specifically in the *Drosophila* fat body. Decreasing the levels of *hnRNP-K, glo,* and *rump* resulted in a lean phenotype, whereas decreasing the levels of *sm*, *Hrb27C*, and *Hrb98DE* resulted in a triglyceride accumulation 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, the adipose triglyceride lipase brummer (bmm), and fatty acid synthase (dFAS), was altered. Hrb27C-RNAi fat bodies had a decrease in bmm levels, suggesting that the excess triglyceride observed in these flies is due to reduced lipid breakdown. In addition, we also observed that despite having a lean phenotype, glo-RNAi flies stored excess fat in each fat body cell but less fat in non-adipose tissues, suggesting

an alteration in lipid transport from the fat body to other tissues in the fly. In insects, lipids are transported through the hemolymph on apoB-containing lipoproteins called lipophorins and the packaging of lipids on lipophorins is regulated by lipid transfer particle (LTP) and microsomal transfer particle (MTP) genes. Interestingly, glo-RNAi flies had reduced expression of the lipophorin, LTP and MTP genes, but not the adipose triglyceride lipase brummer, consistent with a lipid transport and not a lipid breakdown defect in these flies. Together, these results suggest that the hnRNP family of splicing factors have varying metabolic functions and may act on specific lipid metabolic genes to control their expression and processing.

125 An *in vivo* repurposing screen identifies novel therapeutic candidates for NGLY1 deficiency

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Rare diseases collectively impact more than 30 million Americans, however treatment options are limited or non-existent in approximately 90% of cases. NGLY1 deficiency, a rare disease with no effective treatment, is caused by autosomal recessive, loss-of-function mutations in the gene *N-glycanase 1* (*NGLY1*) and is characterized by global developmental delay, hypotonia, alacrima, and seizures. NGLY1 is a deglycosylase that removes GlcNAc sugar chains from glycoproteins that have been retrotranslocated from the endoplasmic reticulum (ER) lumen to the cytoplasm. Recent work demonstrated that the transcription factor NRF1 is translated into the ER membrane, glycosylated, retrotranslocated to the cytoplasm, and immediately degraded. Under proteasome stress, NRF1 is deglycosylated by NGLY1, allowing it to be cleaved and subsequently act as a transcription factor to upregulate proteasome genes. Unfortunately, therapeutic approaches targeting the known interaction between NGLY1 and NRF1 have yielded limited results. In this work, we took an unbiased approach and conducted an *in vivo* small molecule screen to identify therapeutic compounds for NGLY1 deficiency. We utilized a *Drosophila melanogaster* model of NGLY1 deficiency that harbors a null allele of *dNGLY1* (*Drosophila NGLY1* ortholog) and displays lethality at the late pupal stage. In efforts to limit the time and cost associated with bringing a new drug to the NGLY1 deficiency population, we employed the Prestwick Chemical Library, consisting of 1280 off patent small molecules, 99% of which are approved by FDA/EMA regulatory agencies, to screen for compounds that rescue lethality in *dNGLY1^{KO}* flies. We identified multiple compounds that modulate the serotonergic and dopaminergic signaling pathways, as well as iodine containing molecules, that rescued lethality in *dNGLY1^{KO}* flies. Although serotonin and dopamine signaling impacts numerous cellular pathways, we hypothesize that serotonin and dopamine modulators rescue lethality in *dNGLY1^{KO}* flies by inhibiting glycogen synthase kinase 3 (GSK3). GSK3 negatively regulates NRF1 activity, and disinhibiting NRF1 through GSK3 inhibition may rescue defects in *dNGLY1^{KO}* flies. Experiments are currently underway to elucidate the mechanism of action of hit compounds. This study demonstrates the power of *Drosophila* in therapeutic development for rare diseases, and similar small molecule screening strategies can be applied to other disorders.

126 Regulation of *Drosophila* Synaptonemal Complex disassembly during prophase I

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Meiosis is essential for gamete production, maintenance of diploidy and generation of genetic diversity through recombination of the homologous chromosomes. The Synaptonemal Complex (SC) has a pivotal role during meiotic recombination. It is assembled between the homologous chromosomes during early meiosis, functioning primarily as a scaffold that allows interacting chromatids to perform crossover activities. SC proteins are poorly conserved across the eukaryotic tree of life, yet they typically contain coiled-coil domains and have the ability to form dimers. SC structure and oligomerization properties are nevertheless highly conserved, suggesting SC biophysical properties are likely to be highly relevant for its function. Consistently, very recently it was shown in *Caenorhabditis elegans* that the SC has liquid crystalline properties (PMID: 28045371).

In *Drosophila melanogaster*, pairing of the homologous chromosomes is associated to centromeres clustering and requires oligomerization of the SC lateral elements, with subsequent zipper-like pairing of the homologous chromosomes. Very recently, it was shown that E3-ubiquitin ligase Sina is required during early meiosis to avoid the abnormal assembly of SC subunits into large structures named polycomplexes (PMID: 31107865). Formation of such complexes is likely to be deleterious for meiosis and gametogenesis.

Our working hypothesis is that SC proteins have an intrinsic tendency to self-organize into polycomplexes, and that meiotic cells have multiple mechanisms to help avoid their formation. Consistent with this hypothesis, we have identified a highly conserved chromatin remodeling protein whose function within the female germ-line is important for the correct disassembly of the SC and avoidance of polycomplex formation. Depletion of this protein is associated with a delay in SC disassembly, formation of large polycomplexes during mid-oogenesis and reduced female fertility. Super-resolution analysis and live-cell imaging showed that although these complexes are highly dynamic they have nevertheless a well-defined crystal organization. Finally, and since polycomplex formation has been described in multiple organisms beyond *Drosophila*, we will discuss the possibility that their formation is the result of an intrinsic conserved feature of the SC and that the protective mechanisms against polycomplex formation are likely to be highly conserved in meiotic cells.

127 R7 photoreceptor axon targeting requires matching levels of the novel protein Lost and found in R7 and its synaptic partners

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The foundation of proper brain function is the correct formation of neural circuits. Growing neurites must use molecular cues, such as the presence or absence of cell surface proteins, to establish connections with their synaptic partners in the complex environment of the developing brain. Here, we examine this process in the context of the *Drosophila* visual system. In an RNAi screen for proteins required in the ultraviolet-sensitive R7 photoreceptors for their normal axon projection pattern, we identified *lost and found (loaf),* which encodes a transmembrane protein with CUB and LDLa domains. We found that when *loaf* is removed only from photoreceptors, R7 axons terminate prematurely in the M3 layer of the medulla instead of reaching their normal layer, M6. Analysis of pupal brains shows that this phenotype arises in later stages of R7 targeting and likely reflects an inability of R7 terminals to form or maintain stable connections. However, when the whole animal is mutant for *loaf,* R7s target correctly. This suggests that Loaf is not essential for axon targeting, but a mismatch in Loaf levels between R7 and another cell type disrupts the process. To identify this cell type, we restored Loaf to specific neuronal populations in a *loaf* mutant background and looked for defects in R7 targeting. The results suggest that R7s achieve proper targeting by matching their Loaf levels with their post-synaptic partners Dm9 and Tm5a/b. Surprisingly, both in cultured cells and in vivo, Loaf does not localize to the plasma membrane but accumulates in endosomes, suggesting it may act by controlling the trafficking or activity of a cell surface protein. We found that the effects of the synaptic organizer Lrp4 on R7 axon guidance are *loaf*-dependent, suggesting Lrp4 could be one of the downstream effectors of Loaf. These results define a novel mechanism in which matching levels of an intracellular protein in pre- and postsynaptic cells are necessary for the formation of stable connections between them.

128 Genetic dissection of egg-laying decisions

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The ability to “choose the greater of two goods” – i.e., value-based decision-making – allows animals to optimize the process by which they acquire and allocate precious resources. While neural signals that correlate with the values of options have been described in the mammalian brains, the specific functional properties of a neural circuit that can implement value-based decisions – as well as the genetic program that specifies such properties – remain incompletely understood. Our lab has been using egg-laying site selection by *Drosophila* females as a simple model to study the neural and genetic basis of value-based decision-making. *Drosophila* females lay one egg at a time and will sample available options prior to depositing each egg. Importantly, their decision of whether to accept or reject an option when sampling it depends critically on the quality of the other options they recently experienced. For example, when selecting for egg-laying site in our two-choice arena, they readily accept a sucrose-containing substrate when the other option also contains sucrose, but robustly reject a sucrose-containing substrate when the other option is sucrose-free. Successful rejection of the “inferior” sucrose-containing option in the *sugar vs. plain* task relies on females’ ability to sense sweetness but curiously not on functional mushroom bodies, a brain center known to support taste learning, or dopaminergic (DA) neurons, a neural substrate known to encode valence. In this talk, I will discuss our effort in combining behavioral, anatomical, and genetic approaches to understand how females are able to reject an acceptable but less-preferred sugary option according to recent experience as well as our recent attempt in exploring how they are able to remember the spatial location of the preferred plain option.

129 Drosophila and its parasitic wasps: Understanding the host-parasite interface

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Parasitic wasps constitute a large and diverse group of insects that attack and devour their hosts. As keystone species, they influence trophic relationships in natural communities. More than 50 species of parasitic wasps attack *Drosophila* spp., most infecting more than one host species. These wasps present fascinating opportunities for discovery at all scales of biological organization—from the molecular to ecological. We work on solitary wasps of the genus *Leptopilina* that attack larval stages of *Drosophila*. To understand why closely related *L. heterotoma* (*Lh*) enjoys success on many more *Drosophila* spp. than *L. boulardi* (*Lb*) does, we are studying wasp proteins in relation to the fly's immune response to wasp attack. While the venom of both *Lh* and *Lb* contains the so-called "virus like particles" (VLPs), only those in *Lh* are linked to strong immune suppression whereby most blood cells of *D. melanogaster* larvae are destroyed after an *Lh* attack. VLPs may not be "virus-like" but instead appear to be more similar to extracellular vesicles (EVs), composed of a plethora of proteins, some with cellular functions and others with proposed immune-suppressive functions. Using an antibody marker for *Lh* EVs, we find that these parasite-derived cellular structures spread within the hosts' hemolymphatic system with high concentration around the tightly clustered cells of the bifunctional posterior signaling center (PSC) of the larval lymph gland. The PSC cells disperse post infection. A normal PSC serves as a niche in uninfected animals, but in wasp-infected animals, it directs hematopoietic differentiation to promote wasp egg encapsulation. Lymph gland progenitors and macrophages (but not lamellocytes) endocytose the EVs where they damage the cells' phagolysosomal compartments. Comparative proteomics of *Lh* and *Lb* EVs revealed key differences in potential virulence proteins. We will present results of functional analyses of candidate *Lh* EV proteins that help explain how *Lh*'s simultaneous and multipronged effects block cellular immunity. Our recent isolation of *Lh* germline mutants and RNA interference approaches add to the functional genomics toolkit available for the analysis of *Drosophila* parasitoids. As biocontrol agents, parasitoids contribute to pest management practices providing alternatives to synthetic agrochemicals. The *Drosophila*-parasitic wasp model can thus contribute to improving human and planetary health.

130 Metabolic regulation of growth and development in *Drosophila* larvae

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During *Drosophila* larval development, body growth and size are determined in large part by dietary nutrients: in rich nutrient conditions, larvae grow rapidly and develop to pupae in 4-5 days, but in poor nutrients, growth is reduced and development is delayed. While the signaling pathways that couple nutrients to growth are well studied, the metabolic processes that drive tissue and body growth are less clear. In this talk, I will describe our work exploring how mitochondrial metabolism controls larval body growth.

Mitochondria are central regulators of cellular metabolism. They are primarily known as bioenergetic organelles that generate ATP to provide energy to cells. But they also play a biosynthetic role by generating metabolite precursors for synthesizing amino acids, lipids and nucleotides.

We have discovered a central role for mitochondrial metabolism in the fat body in the control of larval growth and development. We find that dietary nutrients can reprogram fat body mitochondrial morphology and activity. We also see that lowering mitochondrial bioenergetic activity just in the fat body is sufficient to remodel adipose glucose metabolism and to accelerate whole body growth and development. These growth effects occur in part due to alteration of fat body-derived cytokine signaling, which leads to enhanced systemic insulin signaling, the main hormonal regulator of fly body growth. Our findings indicate that reprograming of fat body mitochondrial metabolism can couple nutrient signaling to the control of body growth.

131 Developmental genetics of regulated exocytosis

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The stimulus-dependent release of specialized proteins via regulated exocytosis is a fundamental but poorly understood cellular process that is essential for animal development, physiology, and human disease. We have taken a forward genetic approach to uncover novel genes and biological processes that regulate secretion. Our work has identified a number of new mechanisms that promote "readiness" for exocytosis, including an unexpected role for *hobbit*, a highly conserved but poorly studied protein, in trafficking of secretory granule membrane proteins during secretion.

132 Genome-wide screen uncovers genes involved in the loss of dopaminergic neurons

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The hallmark of Parkinson's Disease (PD) is the loss of dopaminergic (DA) neurons in the brain. However, it remains unknown as to why DA neurons are selectively vulnerable to PD. To identify genes that are associated with DA neuron loss, we screened through 201 wild-caught populations of *Drosophila melanogaster* as part of the Drosophila Genetic Reference Panel (DGRP). Here we identify the top associated genes containing SNPs that render DA neurons vulnerable. We tested these genes further by using mutant analysis and tissue-specific knockdown for functional validation. We found that this loss of DA neurons caused progressive locomotor impairment in mutants and in gene knockdown analysis. We also investigate *sestrin,* one of the most significant candidate genes from our screen. *Sestrin* is a known regulator of TOR signaling and maintains levels of reactive oxygen species, therefore we choose to examine the mechanism for how the functional loss of *Sestrin* causes DA neuron death. Further analysis of these genes should help to identify factors that render DA neurons selectively vulnerable in conditions such as PD.

133 Distinct neuromodulatory input pathways to mushroom body regulate sleep need and arousal in *Drosophila*

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A key feature of sleep-wake regulation isthe ability to rapidly transition from one state to the other and persist inthat state. One currentmodel in mammals relies on mutually inhibitory interactions between sleep and wake-promoting neuronal populations that implement a bistable flip-flop circuit. Each half of this circuit (sleep-and wake-promoting neurons) strongly inhibitsthe other via interneurons thereby creating a self-reinforcing behavioral switch preventing intermediate states. Once the wake microcircuit is activated it remains unclearhow these neurons persist in an active state to support extended periods of arousal. Evidence from multiple animal models shows that the arousal phase is associated with concomitant and persistent activity of specific neuromodulators primarily dopamine andnoerpinephrine but it is not clear if their function is permissive to promote wakefulness, sleep homeostasis or state transitions. We recently identified two distinct synaptic microcircuits within mushroom body in Drosophilawhere sleep-promoting KCs increase sleep by preferentially activating a class of cholinergic ONs and wake-promoting KCs decrease sleep by preferentially activating a class of glutamatergic ONs. In addition to the core sleep and wake synaptic microcircuitswithin the MB we have also identified 5 classes of dopaminergic neurons (DANs) and octopaminergic neurons (VPMs) that project to the MB and regulate sleep. Using genetic, physiological and behavioral approaches we find that the molecular signaling and circuit connectivity within these sleep networks regulates both persistence of arousal and sleep need. We will present these results and discuss potential mechanisms of sleep regulation by aminergic inputs to the mushroom body.

134 Defining distinct phospholipid-dependent signaling that regulates plasmatocytes activation, migration and cytokine release during bacterial infection.

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Lipids have emerged as important factors in immunity. While certain lipid metabolic programs initiated during infection are needed to satisfy the energetic demands of immune defense, accumulating evidence shows that immune cells also generate specialized lipid species with direct functional roles in immunity during infection. Leukocyte recruitment, phagocytosis, receptor-mediated signal transduction, and the inflammatory response all require distinct changes in lipid metabolism.
We uncovered a peroxisome-dependent metabolic requirement for the plasmatocyte immune response during infection. Plasmatocytes detect and destroy pathogens, recruit other immune cells, and regulate inflammatory responses. Our findings revealed that plasmatocytes rely on peroxisome activity for phagocytosis and for the production of inflammatory and antimicrobial molecules. Although peroxisomes are evidently important for these immune processes, it is unclear to what extent peroxisomes control macrophage function and how peroxisomes mediate these processes.
We identified the molecular determinants underlying the peroxisome-dependent immune metabolic networks. We surveyed the global cellular transcript and lipid profiles of peroxisome-deficient *Drosophila* macrophages under steady-state conditions and during bacterial infection. A lack of functional peroxisomes in macrophages perturbed key phospholipid regulators of signal transduction. Cell biology and Biochemica approaches determined that peroxisomes are needed to form distinct membrane phospholipid nanodomains to activate RhoI-dependent signals that in turn drive plasmatocyte activation. Genetic depletion of peroxisomes eliminates these nanodomains and affects cell migration and cytokine release during microbial challenge. Moreover we also identified the conserved p190RhoGAP, an inhibitor of RhoI, as novel and unexplored immune responsive regulator of plasmatocyte activation. Analysis of macrophages from peroxisome-mutant mice established that

this peroxisome-phospholipid-RhoI signaling axis is conserved in macrophage activation in mammals. Our study shows that peroxisomes control distinct lipid metabolic pathways necessary for macrophage activation.

135 Splicing mediated by the U2-associated Scaf6/CHERP is necessary for myogenesis in Drosophila and vertebrates

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Muscle structure and functional properties are defined through the combinatorial regulation of transcription and alternative splicing. Mis-regulation of splicing results in severe diseases such as myotonic dystrophy or dilated cardiomyopathy, illustrating the importance of proper splicing. Despite intense study of a select few RNA binding proteins associated with disease, many RNA binding proteins have never been studied in muscle, creating a gap in our understanding of splicing regulation. Here we identify Scaf6/DmCHERP, previously shown to promote cancer cell proliferation, to play an essential role in muscle development. Whole animal Scaf6 mutant flies are flightless and display severe muscle atrophy. We show that Scaf6 is required for myoblast proliferation as well as later during fiber differentiation for proper myofibril maturation. Aberrant spontaneous contractions during myofibril assembly lead to muscle fiber detachment by 48h APF. mRNA-seq data demonstrate that Scaf6 regulates alternative splicing and suppresses cryptic splicing, and we show that these molecular defects lead to the loss of specific sarcomere proteins. Interestingly, Scaf6 has distinct phenotypes in muscle and neurons, and neuronal-specific RNAi results in climbing, grooming, eclosion and axonal morphology defects. Additionally, siRNA knockdown of CHERP in mouse C2C12 cells results in proliferation defects and early differentiation, indicating that CHERP function in muscle development is likely conserved. From pull-down mass-spectrometry experiments in mouse and human muscle cells, we demonstrate that CHERP is part of the U2 complex of the spliceosome and tightly associated with the Sf3b1 complex guiding 3' splice site recognition. Our results demonstrate a function for Scaf6 in the development of multiple Drosophila tissues, highlight the importance of alternative splicing during myogenesis and suggest pleotropic functions for vertebrate CHERP beyond the described role in cell proliferation.

136 A single cell atlas of the proximal wing disc uncovers early transcriptional events driving fibre-type divergence in myoblasts

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The adult muscle precursor cells associated with the wing disc give rise to the fibrillar indirect flight muscles (IFM) and the tubular direct flight muscles (DFM). We performed single- cell RNA-sequencing experiments to understand early transcriptional events underlying this muscle diversification. We built a transcriptional cell atlas of the proximal wing disc at third instar larval stage. In addition to epithelial and tracheal cell clusters, we identified distinct transcriptional signatures for IFM and DFM myoblasts. Various states of differentiation of myoblasts were uncovered, thus illustrating previously unappreciated spatial and temporal heterogeneity among myoblasts. Novel markers for both IFM and DFM myoblasts were identified and validated at various states of differentiation by immunofluorescence and genetic cell-tracing experiments. Finally, a panel of markers from the reference cell atlas was systematically used to screen for relevant genes. We found Amalgam and Arginine kinase as novel genes to be functionally important in muscle development. In sum, our work illustrates the power of combining single-cell genomics with genetic approaches and cell lineage tracing experiments to address important questions in developmental biology.

137 Dual roles of nitric oxide in *Drosophila* blood progenitors

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Nitric oxide (NO) is a gaseous free radical produced by nitric oxide synthase (NOS) and is involved in multiple biological processes, including neuronal activity,
vasodilation, and immune cell activation. Despite previous investigations, the function of NO in stem cell development and maintenance has not been comprehensively characterized, particularly in the *in vivo* context. In *Drosophila*, the lymph gland houses blood progenitor cells and functions as a site for the larval hematopoiesis. With the use of the lymph gland model system, we identified that nitric oxide synthase (NOS) is expressed in the lymph gland progenitors, and loss of which leads to precocious differentiation of progenitors. Interestingly, the free radical form of NO is only detected in the intermediate progenitors - differentiating progenitors that lose their quiescence- but not in the core progenitors even with the broad expression of NOS. We found that NO is utilized for post-translational modification of protein targets to promote progenitor cell maintenance. In the intermediate progenitors, free radical NO is sensed by a soluble guanylate cyclase and functions

in blood cell maturation through the cGMP signal. Overall, our findings reveal essential roles of NO in the progenitor maintenance and differentiation *in vivo*, which establishes the signaling roles for NO in stem/progenitor cells.

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138 A gut-secreted peptide controls arousability through modulation of dopaminergic neurons in the brain

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Since sensory information is always present in the environment, animals need to internally regulate their responsiveness to fit the context. During sleep, the threshold for sensory arousal is increased so that only stimuli of sufficient magnitude can cross it. The mechanisms that make arousability flexible are largely mysterious, but they must integrate sensory information with information about physiology. We discovered a gut-to-brain signaling pathway that uses information about ingested nutrients to control arousability from sleep, without affecting sleep duration. Protein ingestion causes endocrine cells in the *Drosophila* gut to increase production of CCHa1, a peptide that decreases sensory responsiveness. CCHa1 is received by a small group of brain dopaminergic neurons whose activity gates behavioral responsiveness to mechanical stimulation. These dopaminergic neurons innervate the mushroom body, a brain structure involved in determining sleep duration. This work describes how the gut tunes arousability according to nutrient availability, allowing deeper sleep when dietary proteins are abundant. It also suggests that behavioral flexibility is increased through independent tuning of sleep depth and duration.

139 Neuronal lipid droplets promote a pathological conversion in alpha-synuclein via a feed-forward mechanism

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Neurodegenerative diseases are associated with lipid metabolism dysregulation and the accumulation of lipids in cytoplasmic organelles called lipid droplets (LDs) is emerging as an important hallmark of neurodegeneration. Parkinson's disease is a neurodegenerative disorder characterized by accumulation of alpha-synuclein (αSyn) aggregates and by abnormalities in lipid storage. To investigate the potential pathophysiological consequences of interactions between αSyn and proteins that regulate the homeostasis of LDs, we employed a transgenic *Drosophila* model of PD in which human αSyn is specifically expressed in photoreceptor neurons. We found that overexpression of the LD-coating proteins perilipin 1 and 2 (dPlin1/2) markedly increased LD accumulation in the neurons. Perilipins also co-localized with αSyn at the LD surface in both Drosophila photoreceptor neurons (dPlin2) and human neuroblastoma cells (PLIN3). Co-expression of human αSyn and dPlin2 in photoreceptor neurons synergistically amplified LD content through a mechanism involving LD stabilization, independently of Brummer-mediated lipolysis or *de novo* synthesis of triacylglycerols. Accumulation of LDs also increased the resistance of αSyn to proteolytic digestion, a phenomenon associated with αSyn aggregation in human neurons. Our results suggest that binding of αSyn to PLIN-coated LDs stabilizes the LD structure and may contribute to the pathogenic misfolding and aggregation of αSyn in neurons.

140 Partial double-strand break repair enables broken chromosome segregation during mitosis

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Cells must promptly respond to DNA damage to maintain genome integrity. Actively cycling cells frequently activate interphase cell cycle arrest checkpoints in response to DNA breaks. However, some DNA breaks do not trigger these checkpoints, leading to mitosis with broken chromosomes. More recently, cellular responses to DNA breaks that persist into mitosis have been appreciated, but their molecular regulation remains to be fully determined. Uncovering regulation of these responses is crucial, as failure of such responses can lead to micronuclei, which are aberrant, disease-associated nuclear structures formed from persistent broken DNA.

We established *Drosophila melanogaster* rectal papillar cells (hereafter papillar cells) as a highly accessible model to study how broken chromosomes segregate and avoid micronucleus formation during mitosis. We previously identified that papillar cells lack interphase checkpoints and therefore frequently enter mitosis with broken chromosomes. Strikingly, these aberrant chromosomes segregate properly despite lacking centromeres/kinetochores, using a mechanism that involves conserved Fanconi Anemia (FA) proteins. Papillar cells also have a long (several day) delay between DNA breakage and mitosis, which allows us to easily investigate interphase and mitotic DNA breakage responses separately.

Here, we report that despite lacking a cell cycle arrest response, papillar cells still recruit a subset of early-acting double-strand break repair proteins to damaged chromosomes. These proteins (mre11 and RPA3) persist on damaged chromosomes significantly longer than in cells with intact checkpoints. Further, these repair proteins persist into mitosis but with distinct kinetics - while mre11 is resolved prior to nuclear envelope breakdown, RPA3 persists into anaphase on broken DNA. Both mre11 and the FA protein Fancd2 are required for: proper RPA3 localization at DNA breaks, segregation of broken papillar chromosomes, micronuclei prevention, and subsequent cell survival. From a candidate screen of distinct DNA damage repair pathways, we find that the alternative end-joining repair protein DNA polymerase theta is also required for RPA3 regulation, micronucleus prevention, and cell survival following mitosis. Our data reveal a mechanism for surviving mitosis with broken chromosomes, one which is likely relevant to any cell with inactive cell cycle checkpoints or with DNA damage present in mitosis.

141 Clock proteins regulate spatiotemporal organization of clock genes to control circadian rhythms

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Our goal is to visualize circadian clocks within living organisms *continuously* and with *sub-cellular resolution* to understand the cellular mechanisms underlying circadian rhythms. Circadian clocks are internal timekeepers within our cells, which orchestrate daily rhythmic expression of a large number of genes and affect much of our behavior, physiology, and metabolism. While many of the advances in understanding the molecular mechanisms of circadian rhythms have come from either genetic screens or biochemical studies, we still lack a basic understanding of how spatiotemporal organization of clock components affect circadian rhythms. Recent work from our lab have uncovered some surprising new discoveries about how clock proteins regulate circadian rhythms by controlling the spatiotemporal organization of clock genes.

Using high-resolution live imaging techniques we demonstrate that *Drosophila* clock proteins are concentrated in a few discrete foci and are organized at the nuclear envelope; these results are unexpected as previous studies have suggested that clock proteins are diffusely distributed in the nucleus. We also show that clock protein foci are highly dynamic and change in number, size, and localization over the circadian cycle. Further, we demonstrate that clock genes are positioned at the nuclear periphery by the clock proteins precisely during the circadian repression phase, suggesting that subnuclear localization of clock genes plays an important role in the control of rhythmic gene expression. Finally, we show that Lamin B receptor, a nuclear envelope protein, is required for peripheral localization of clock protein foci and clock genes and for normal circadian rhythms. These results reveal that clock proteins form dynamic nuclear foci and play an important role in the subnuclear reorganization of clock genes to control circadian rhythms, identifying a novel mechanism of circadian regulation. Our results further suggest a new role for clock protein foci in the clustering of clock-regulated genes during the repression phase to control gene co-regulation and circadian rhythms.

The manuscript is currently in revision in PNAS. Uploaded the manuscript on bioRxiv - https://www.biorxiv.org/content/10.1101/2020.10.09.333732v1

142 The Drosophila Baramicin polypeptide gene protects against fungal infection

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The fruit fly Drososphila melanogaster combats microbial infection by producing a battery of effector peptides that are secreted into the haemolymph. The existence of many effectors that redundantly contribute to host defense has hampered their functional characterization. As a consequence, the logic underlying the role of immune effectors is only poorly defined, and exactly how each gene contributes to survival is not well characterized. Here we describe a novel Drosophila antifungal peptide gene that we name Baramicin A (BaraA). We show that BaraA encodes a precursor protein cleaved into multiple peptides via furin cleavage sites. BaraA is strongly immune-induced in the fat body downstream of the Toll pathway, but also exhibits some expression in the nervous system. Importantly, we show that flies lacking BaraA are viable but susceptible to a subset of filamentous fungi, including entomopathogenic fungi. Consistent with BaraA being directly antimicrobial, overexpression of BaraA promotes resistance to fungi and the IM10-like peptides produced by BaraA synergistically inhibit growth of fungi in vitro when combined with the membrane-disrupting antifungal pimaricin. Surprisingly, BaraA males but not females display an erect wing phenotype upon infection, pointing to a role for BaraA in the wing muscle or the nervous system. Collectively, we identify a new antifungal immune effector downstream of Toll signalling, improving our knowledge of the Drosophila antimicrobial response.

143 Myofibril and mitochondria morphogenesis are coordinated by a mechanical feedback mechanism in muscle

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Complex animals build specialised muscles to match specific biomechanical and energetic needs. Hence, composition and architecture of sarcomeres are muscle type specific. *Drosophila* build two major muscle type organizing cross striated or fibrillar myofibrils. The muscle selector Spalt instruct muscle specification via transcription and alternative splicing regulation of sarcomeric genes. Interestingly we find mitochondria organisation dependent of the muscle type and regulated by *spalt*. Mechanisms coordinating mitochondria with sarcomere morphogenesis are elusive. Here we use *Drosophila* muscles to demonstrate that myofibril and mitochondria morphogenesis are intimately linked. High resolutive microscopy and serial block face electronic microscopy (SBF EM) reveal with detail mitochondria organisation specificity. In flight muscles, *spalt* instructs mitochondria to intercalate between myofibrils, which in turn mechanically constrain mitochondria into elongated shapes. Conversely in cross-striated muscles, such as found in the leg, mitochondria networks surround myofibril bundles, contacting myofibrils only with thin extensions. To investigate the mechanism causing these differences, we manipulated genetically the GTPase drp1 and marf to modify mitochondrial dynamics. We found that increased mitochondrial fusion during myofibril assembly prevents mitochondrial intercalation in flight muscles. Strikingly, this coincides with ectopic expression of cross-striated muscle specific sarcomeric proteins and loss of fibrillar muscle specific protein expression. Consequently, flight muscle myofibrils convert towards a partially cross-striated architecture similar to tubular leg muscle. Together, these data suggest a biomechanical feedback mechanism downstream of *spalt* synchronizing mitochondria with myofibril morphogenesis.

144 Septins regulate heart contractility through modulation of cardiomyocyte store-operated Ca^{2+} entry

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An important mechanistic underpinning of heart failure is dysregulation of the Ca^{2+} transport mechanisms that drive cardiomyocyte contractility. Better understanding of cardiomyocyte Ca^{2+} physiology is therefore essential for development of new heart failure therapies. Recent data from our lab and others demonstrate that store-operated Ca^{2+} entry (SOCE) is an essential Ca^{2+} transport mechanism in cardiomyocytes. SOCE refers to Ca^{2+} influx that is activated specifically by depletion of sarco/endoplasmic reticulum (S/ER) Ca^{2+} stores. SOCE is mediated by STIM proteins, which function as S/ER Ca^{2+} sensors, and Orai Ca^{2+} influx channels. Importantly, both upregulation and downregulation of SOCE in cardiomyocytes results in impaired heart contractility and heart failure, demonstrating that precise SOCE regulation is critical. However, mechanisms that regulate SOCE in cardiomyocytes are poorly understood. Septins have recently emerged as key modulators of SOCE, but functional roles of septins in cardiomyocytes have not been analyzed. Using genetic tools and *in vivo* analysis of heart contractility and architecture in *Drosophila*, we show that RNAi-mediated suppression of *Drosophila* septins 1, 2, or 4, previously shown to be positive SOCE regulators, results in heart dilation and reduced fractional shortening. These results demonstrate for the first time that septins are required for proper heart contractility. Further, these findings are nearly identical to the effects of SOCE suppression by STIM or Orai RNAi, suggesting that septin RNAi-mediated phenotypes may be due to SOCE suppression. In support of this, SOCE restoration by constitutively active Orai expression partially suppressed the septin 2 RNAi phenotype. Interestingly, septin 7 suppression has been shown to result in SOCE upregulation as opposed to inhibition. Consistent with this, we found that septin 7 RNAi resulted in heart wall thickening and constriction of the heart lumen based on micro-computerized tomography (microCT) imaging, similar to cardiac hypertrophy that we see when SOCE is upregulated by expression of constitutively active STIM or Orai mutants. The hypertrophy phenotype caused by septin 7 RNAi was suppressed by co-expression of Orai RNAi, further implicating SOCE dysregulation as the cause of the Septin 7 phenotype. These results collectively suggest that proper heart contractility depends on septin regulation of SOCE in cardiomyocytes.

145 The *Serratia marcescens* outer membrane vesicles paralyze and kill the flies through complex interactions with the host

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One of the important characteristics of Gram-negative bacteria is the secretion of outer membrane vesicles (OMVs). These vesicles contain biologically active proteins and execute diverse biological processes. The injection of few *Serratia marcescens* (*S.m*) bacteria kills the flies in less then a day. Our laboratory is interested in studying the pathogenicity of *S.m* OMVs in the fruit fly. To our surprise we found that the injection of OMV preparations triggers paralysis then death of the host within a few hours. Interestingly, we identified several host factors that protect the fly from the toxicity of OMVs and

others that contribute to the pathogenicity of the injected OMVs. Indeed, our findings indicate that the IMD pathway and melanization are both required for the host to alleviate the detrimental effect of OMVs. In contrast, we found that ROS, the cellular immune response and JNK pathway activation enhance the deleterious effect of the bacterial vesicles. Furthermore, we showed that the virulence of these vesicles is mediated by the bacterial prtA protease.

146 Local 5-HT dynamics in a high brain learning center that critically modulate time dependent synaptic integration revealed by a GRAB sensor

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Serotonin (5-HT), an important biogenic monoamine neuromodulator across animal phyla, has been implicated to participate in diverse physiological processes, including learning and memory. In *Drosophila*, a single serotonergic DPM neuron innervates the olfactory learning center mushroom body (MB) of each hemisphere. Previous work established that perturbation of 5-HT metabolism or signaling by genetically or pharmacological means could lead to impaired olfactory learning, however, the dynamics of 5-HT *in vivo* and how it is regulated remains largely unknown, nor is clear how 5-HT may affect learning circuit in the MB. Here, capitalizing on transgenic flies that expressed our newly developed GRAB-5-HT sensor, we found physiological relevant stimuli, such as odor or aversive electrical body shock, readily triggered compartmental 5-HT dynamics *in vivo*. This compartmental 5-HT release relies on Ca2+ influx through DPM presynaptic nicotinic receptors. The DPM nicotinic receptors are in turn activated by local ACh release from upstream MB Kenyon cells (KCs). Next, we found locally released 5-HT from DPM could reciprocally act on KC terminals to turn down cAMP level as well as ACh release. Finally, by perturbation of 5-HT release from DPM neuron, we found 5-HT signaling is critical to control the length of time window where the plasticity of KC to MB output neuron may occur during odor-shock paring in γ1 compartment of MB. Thus, our work reveals *in vivo* 5-HT dynamics that may be important for optimal time window of synaptic plasticity during olfactory learning in *Drosophila*.

147 Methylation Pathways and Amino Acid Metabolism Intersect to Alter Behavioral Responses to Alcohol

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Alcohol use disorders (AUDs) exact an immense toll on individuals, families, and society. Genetic factors determine up to 60% of an individual's risk of developing problematic alcohol habits. These risk factors can manifest as reduced naïve sensitivity to the intoxicating effects of alcohol and increased functional tolerance (i.e., brain-mediated decreases in sensitivity after repeat exposure). The neurobiology underlying how AUD-associated genes alter these phenotypes remains poorly understood.

Genes implicated in AUDs include epigenetic modifiers, which profoundly influence gene expression, neuronal plasticity, and addiction formation. We found that global knockout (KO) of Lysine-specific Histone Demethylase 3 (*KDM3*) in *Drosophila* increases naïve ethanol sensitivity and rapid tolerance, suggesting that *KDM3* modulates AUD risk. RNA-seq analysis on *KDM3*^{KO} fly heads revealed disproportionate upregulation of genes associated with one-carbon metabolism, such as the folate and methionine cycles. These cycles are critical for production of S-adenosyl methionine (SAM), the universal methyl donor required for epigenetic methylation. Many of the dysregulated enzymes act on amino acid substrates to affect the levels of cycle metabolites, including SAM. Influencing the cycles by altering amino acid intake modulated flies' alcohol sensitivity and tolerance in a *KDM3*-dependent manner. Global and neuron-specific manipulation of the aforementioned enzymes similarly changed alcohol sensitivity and tolerance. Together, these results indicate that altered neuronal activity and expression of genes associated with the folate and methionine cycles may mediate our observed amino acid feeding and *KDM3*^{KO} phenotypes, revealing an uninvestigated role of neuronal one-carbon metabolism in alcohol phenotypes.

Finally, our results indicate that the aforementioned sensitivity and tolerance phenotypes may coincide with altered SAM levels and methylation potential. We are currently testing whether direct manipulation of SAM levels in neurons is sufficient to modulate these phenotypes, which would suggest a role for SAM-mediated methylation in AUD-associated behaviors.

These studies elucidate a novel neuronal mechanism by which epigenetic modifiers may contribute to AUDs. Furthermore, by assessing the unique role of the folate and methionine cycles in mediating alcohol responses, these results could support interventions as simple as altering amino acid consumption to mitigate AUD behaviors and risk.

148 The Upd3 cytokine couples inflammation to maturation defects

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Developmental transitions such as puberty or metamorphosis are tightly controlled by steroid hormones and can be delayed by the appearance of growth abnormalities, developmental tumors or inflammatory disorders, such as inflamed bowel disease or cystic fibrosis (Andersen et al., 2013; Ballinger et al., 2003; Brain and Savage, 1994; Tennessen and Thummel, 2011).
Here we used a highly inflammatory epithelial model of malignant transformation in Drosophila (Dekanty et al., 2012; Muzzopappa et al., 2017) to unravel the role of Upd3 - a cytokine with homology to interleukin 6 - and the JAK/STAT signaling pathway in coupling inflammation to a delay in metamorphosis. We present evidence that Upd3 produced by malignant cell populations signals to the prothoracic gland - an endocrine tissue primarily dedicated to the production of the steroid hormone ecdysone - to activate JAK/STAT and bantam miRNA and to delay metamorphosis. Upd cytokines produced by the tumor site contribute to increasing the systemic levels of Upd3 by amplifying its expression levels in a cell autonomous manner and by inducing Upd3 expression in neighboring tissues in a non-autonomous manner, culminating in a major systemic response to prevent larvae from initiating pupa transition.
Our results identify a new regulatory network impacting on ecdysone biosynthesis and provide new insights into the potential role of inflammatory cytokines and the JAK/STAT signaling pathway in coupling inflammation to delays in puberty.

149 Female Drosophila respond to ejaculate with copulation song

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In most animal species, males and females communicate during sexual behavior to negotiate reproductive investments. Pre-copulatory courtship may settle if copulation takes place, but often information exchange and decision-making continue beyond that point.
We have shown that female Drosophila sing by wing vibration in copula. This copulation song is distinct from male courtship song and requires neurons expressing the female sex determination factor DoublesexF. Copulation song depends on transfer of seminal fluid components of the male accessory gland. Playback of female copulation song to a mating couple increases the time the female takes to remate with subsequent males and thereby increases the reproductive success of the first male. This suggests that auditory cues from the female modulate male seminal fluid transfer (strategic ejaculate allocation).
We hypothesize that female copulation song serves as a signal in mate choice, giving the female the opportunity to influence the composition and postmating effect of ejaculate.
Our findings reveal an unexpected fine-tuning of reproductive decisions during a multimodal copulatory dialog. The discovery of a female-specific acoustic behavior sheds new light on Drosophila mating, sexual dimorphisms of neuronal circuits and the impact of seminal fluid molecules on nervous system and behavior.
Current efforts are directed at identifying which seminal fluid components affect female song (mass-spectrometry), as well as uncovering the receptors and sensory neurons in the reproductive tract necessary for rapid ejaculate evaluation by the female.
References: Kerwin et al. 2020, Nat Comm (https://doi.org/10.1038/s41467-020-15260-6), Kerwin and von Philipsborn 2020, BioEssays (https://doi.org/10.1002/bies.202000109)

150 The Sodium-dependent mutlivitamin transporter (SMVT) regulates tissue homeostasis by maintaining intestinal stem cell lineage and microbiota homeostasis

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Emerging evidence suggests that alterations in healthy microbiota composition, known as commensal dysbiosis, makes the intestine susceptible to acute and chronic infection that can elicit intestinal inflammation. We characterized the stem cell-specific transporter SMVT as a regulator of intestinal homeostasis and infection-induced regeneration in *Drosophila*. SMVT-deficient flies exhibit impaired regeneration and abnormal intestinal stem cell (ISC)/differentiated cell balance both in physiological conditions, as well as upon pathogenic infection. ISC-specific SMVT knockdown leads to reduced mitosis compensated by downregulation of *CycE* expression and increased enterocyte (EC) endoreplication. Gene expression analysis of known ISC regulators, revealed that the JAK/STAT ligand *upd1* and downstream effector *socs36E* are reduced upon SMVT knockdown. Importantly, ISC-specific *upd1* overexpression rescues the SMVT knockdown phenotype, highlighting that JAK/

STAT functions downstream of SMVT. Interestingly, ISC-specific SMVT deficient flies exhibit microbial dysbiosis whereby the growth of opportunistic pathogens, such as *Providencia sneebia*, is favored. In addition, SMVT-deficient flies showed increased expression of the NADPH enzyme Nox, which in turn induces reactive oxygen species, leading to apoptosis of ECs. Since SMVT is the only known transporter of biotin, we assumed that absence of SMVT inhibits absorbance of biotin by ISCs, which may lead to deregulation of ISC proliferation and differentiation. Commensal microbes, such as *E. coli*, are known to produce biotin. We found that when dietary biotin is scarce, commensals are capable of producing biotin and, in turn, autonomously maintain ISC proliferation. Overall, our work highlights the importance in the interplay between nutrients, intestinal microbiota and tissue homeostasis.

151 Parsing the functions of lipid droplets in a high-fat diet model of renal disease

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Lipid droplets are induced at ectopic sites in many different metabolic diseases but their roles in this context are far from clear. One important question is whether lipid droplets function primarily to exacerbate disease or to protect against it. Here, we study the role of lipid droplets in a high-fat diet model in *Drosophila* that recapitulates several aspects of mammalian chronic kidney disease (CKD). Cell-type specific genetic manipulations show that lipid can overflow from adipose tissue and is taken up by renal cells called nephrocytes. A high-fat diet drives pericardial nephrocyte lipid uptake via the multiligand receptor Cubilin, leading to the ectopic accumulation of lipid droplets. These nephrocyte lipid droplets correlate with ER and mitochondrial deficits, as well as with impaired macromolecular endocytosis, a key conserved function of renal cells. Nephrocyte knockdown of diglyceride acyltransferase 1 (DGAT1, Mdy), overexpression of adipose triglyceride lipase (ATGL, Bmm) and epistasis tests together reveal that fatty acid flux through the lipid droplet triglyceride compartment protects the ER, mitochondria and endocytosis of renal cells. Strikingly, boosting nephrocyte expression of the lipid droplet resident enzyme ATGL is sufficient to rescue high-fat diet induced defects in renal endocytosis. Moreover, endocytic rescue requires a conserved mitochondrial regulator, peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC1alpha, Srl). This study demonstrates that lipid droplet lipolysis counteracts the harmful effects of a high-fat diet via a mitochondrial pathway that protects renal endocytosis. It also provides a general strategy for determining whether lipid droplets in different biological contexts function primarily to release beneficial or to sequester toxic lipids. Together, our findings also highlight that caution is needed when assigning protective or harmful roles to lipid droplets and argue for a more nuanced interpretation that parses their various subfunctions.

152 The septate junction protein Snakeskin is critical for epithelial barrier function and tissue homeostasis in the Malpighian tubules of adult *Drosophila*

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In multicellular animals, ageing is a progressive decline in tissue homeostasis and organ function, leading to increasing probability of disease and death. Maintenance of a healthy intestine has been established as a critical determinant of lifespan across taxa, with a causal link between age-related tissue degeneration and cellular junctional remodelling indicating that dysregulation of junctional proteins in self-renewing tissues may be a principal driver of ageing. However, whether non-proliferative tissues, like the kidneys, also undergo age-related cellular and tissue degeneration that impacts viability remains unresolved.

Transporting epithelia provide a protective physical barrier while directing appropriate transport of ions, solutes and water. In invertebrates this is dependent on formation, and maintenance, of 'tight' septate junctions (SJs). Using *Drosophila* Malpighian (renal) tubules we demonstrate for the first time that tubules undergo an age-dependent decline in secretory transport capacity, which correlates with mislocalisation of SJ proteins and coincident progressive degeneration in cellular morphology and tissue homeostasis. By restrictively impairing Snakeskin (Ssk), a cell adhesion protein essential for smooth SJ formation, in principal (PC) or stellate (SC) cell-populations of adult tubules, we can phenocopy age-related effects such as mislocalisation of junctional components. Critically, failure of tubule junctional integrity leads to an accelerated reduction in secretory capacity and concomitant loss of systemic fluid homeostasis, ultimately resulting in significant reduction in viability. Furthermore, cell-specific depletion of *Ssk* leads to a block in SC maturation, loss of apicobasal polarity and an increase in SC clustering; indicating a key role for *Ssk* in maintaining MT function and stability. Finally, knocking down *Ssk* also led to a proliferation of tiny (renal stem) cells and increase in tubule tracheation, suggesting that MTs can autonomously respond to tissue damage with Ssk acting as a novel regulator of tubule tissue homeostasis.

Our investigations demonstrate a crucial link between cell-cell junction integrity, epithelial transport competence and tubule

homeostasis in a classically non-proliferative tissue; highlighting the tubule's essential role in maintenance of organismal health, while providing measurable markers of compromised epithelial barrier and tissue function that manifest in advanced morbidity and death.

153 The Role of Bacterial Genotype in Persistence of the Microbiota of *Drosophila melanogaster*

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The microbiome of *Drosophila melanogaster* can have significant effects on the host, and many of these have been studied. However, the reason why the bacterial species associate with and persist in *D. melanogaster* has not been studied in depth. Here we define persistence as how long a microbe associates with a host. The early assumption has been that the *D. melanogaster* gut microbiome is established solely through diet, but recent work suggests that other factors may be at play in the microbiome establishment. This experiment aims to study the correlation between bacterial genotype and persistence in the *D. melanogaster* microbiome. In this study, a metagenome wide association (MGWAS) was done using 40 different strains of bacteria to find distinct bacterial genes that are significantly correlated with persistence. To do this, each strain was mono-associated with twenty-four individual flies. The flies were reared for fourteen days, transferred onto new food three times a day for two days, homogenized, and plated. Using the significant genes found through the MGWAS, the same experiment protocol was used to test mutants of these genes for their effect on persistence. These data showed that the four flagellar genes, one urea carboxylase gene, one phosphatidyl inositol gene, one bacterial secretion gene, and one AMP resistance gene were all significantly correlated with persistence in *Drosophila melanogaster.*

154 A co-transmitting neuron regulates aggression through pre- and postsynaptic mechanisms

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Neuromodulators such as monoamines are often co-expressed in neurons with at least one small fast-acting neurotransmitter. The release of more than one transmitter (a phenomenon known as co-transmission) allows neurons to alter their signaling capacities to downstream circuitry. The increase in complexity from co-transmission suggests that release of transmitters must be tightly regulated through presynaptic cellular mechanisms and/or through selective postsynaptic receptor expression. Here we use neural circuits that control aggressive behavior to examine how co-transmission complexity is regulated at the individual neuron level. First, we demonstrate that the release of octopamine (OA) and the excitatory transmitter glutamate from a single neuron, VPM4, is regulated presynaptically via autoreception mechanisms. Second, we determined the downstream neuron, MBON-11, is required for aggression and expresses both glutamate and OA receptors.

Males lacking either OA or glutamate in brain OA-glutamate neurons (OGNs) exhibit decreased aggression. Our current studies address the mechanisms used by co-transmitting neurons to regulate and distribute aggression-promoting signals. To identify presynaptic mechanisms, we first reduced expression of the metabotropic glutamate receptor (mGluR) specifically in a single OGN, OA-VPM4. As mGluRs function to presynaptically inhibit neurotransmittion, we predicted that reducing mGluR from this single OGN would increase glutamate release and thus increase aggression. Our results demonstrate this is indeed the case as males with reduced mGluR in OA-VPM4 exhibit increased lunge number as well as an increase in *high-intensity aggressive behaviors such as* holding and boxing. Similar results were obtained by reducing expression of the OA autoreceptor OA alpha-2R within VPM4, with flies showing an increase in lunge number and holding. Together, these results indicate that presynaptic feedback mechanisms within a single neuron can be used to regulate the activity of entire aggression circuit(s).

To examine information transfer to downstream neurons by a single co-transmitting neuron, we used the NeuPrint connectome to identify the mushroom body output neuron MBON-11 as a target of OA-VPM4. Silencing the activity of MBON-11 resulted in a decrease in aggressive behavior, while activating MBON-11 increased aggression. A screen for glutamate receptors within MBON-11 identified expression of NMDAR2. Studies are underway to identify which OA receptor is expressed in MBON-11, the distribution of each receptor, and their requirements for aggression.

By using the OGNS, known for their role in aggressive behavior, our work will uncover fundamental principles by which neurons that use the same co-transmitters and contact the same target neurons can elicit distinct and shared responses from a behavioral circuit.

155 Cooperation between Oncogenic Ras and p53 Stimulates JAK/STAT Non-Cell Autonomously to Promote Ras Tumor Radioresistance

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Oncogenic RAS mutations are associated with tumor resistance to radiation therapy. The underlying mechanisms remain unclear. Emergent cell-cell interactions in the tumor microenvironment (TME) profoundly influence therapy outcomes. The nature of these interactions and their role in Ras tumor radioresistance remain unclear. We used *Drosophila*oncogenic Ras tissues and human Ras cancer cell radiation models to address these questions. We discovered that cellular response to genotoxic stress cooperates with oncogenic Ras to activate JAK/STAT non-cell autonomously in the TME. JAK/STAT accelerates the growth of the less-damaged Ras tumor cells, leading to rapid tumor recurrence. Specifically, p53 is heterogeneously activated in Ras tumor tissues in response to irradiation. This mosaicism allows high p53-expressing Ras clones to stimulate JAK/STAT cytokines, which activate JAK/STAT in the nearby low p53-expressing surviving Ras clones, leading to robust tumor re-establishment. Blocking any part of this cell-cell communication loop re-sensitizes Ras tumor cells to irradiation. This finding suggests that coupling STAT inhibitors to radiotherapy might improve clinical outcomes for Ras cancer patients.

156 Maintenance of Terminal Differentiation by Retinoblastoma and Hippo Tumor Suppressors

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Both the Retinoblastoma (pRB) and Hippo tumor suppressor pathways have been implicated in regulation of differentiation and stemness. However, the role of pRB and Hippo pathways in maintaining terminal cell specification is currently unknown. Mature *Drosophila melanogaster* photoreceptor neurons with dual mutation in *Rbf* and *wts* abruptly lose neuronal specification and dedifferentiate into an uncommitted cell type. This does not occur in *Rbf* or *wts* single mutant photoreceptors. We employed single-cell RNA-sequencing on wildtype, Rbf^{120a}, wts^{x1}, and $Rbf^{120a}wts^{x1}$ eye tissue to identify dedifferentiated photoreceptor neurons and understand the transcriptional changes accompanying loss of neuronal identity. We found three novel cell populations specific to the double mutant eye disc, including a cluster of proliferating early photoreceptors, a putative dedifferentiated photoreceptor cluster, and a population expressing both undifferentiated and differentiated cell markers. We suggest that these populations reflect the process of dedifferentiation. We also identified that dedifferentiated photoreceptors aberrantly express genes specific to anterior, undifferentiated cells such as *wingless*, *homothorax*, and *homothorax* target genes. Finally, we employed a constitutively active yorkie (yki) to generate a new, robust model for studying photoreceptor dedifferentiation. Taken together, our work suggests that wts maintains terminal cell specification through cytosolic retention of yki, while Rbf prevents expression of hth and its target genes.

157 Synaptic development depends on activity coordinated by a discrete neuronal population

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The stereotyped synaptic connections that define neural circuit function are established during development. In vertebrates, stimulus-independent activity is found throughout the developing brain and has been implicated in circuit formation. In *Drosophila*, patterned, stimulus-independent neural activity accompanies synaptogenesis, suggesting that developmental activity is a fundamental feature of neural circuit formation in complex brains. However, the importance of developmental activity to synapse and circuit formation remains an open question, particularly at the level of defined cell types. Here we show that neurons expressing the cation channel Trpγ are essential to developmental activity in the fly, and that this activity modulates synaptogenesis in a cell-type-specific manner. Developmental activity is globally attenuated in *trpγ* mutants. In the visual system, stereotyped activity patterns and synaptic structure are altered in a cell-type-specific fashion, consistent with an instructive role for developmental activity not previously appreciated. *Trpγ* is expressed in a small population of neurons which arborize extensively throughout the fly brain in an apparently space-filling fashion. Silencing these *Trpγ+* neurons leads to near-complete loss of developmental activity, whereas their activation induces oscillatory activity throughout the brain. Together, these results indicate that this small population of neurons coordinates brain-wide developmental activity. We propose that stereotyped patterns of developmental activity are driven by a discrete, genetically specified network to instruct neural circuit assembly at the level of individual cells and synapses. This work establishes the fly brain as an experimentally tractable system

for studying how activity contributes to synapse and circuit formation.

158 Manipulating animal sex lives: unraveling variation in the strength of *Wolbachia*-induced cytoplasmic incompatibility

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Maternally transmitted *Wolbachia* bacteria infect ~40-65% of insect species and commonly hijack host reproduction to favor their spread. Many *Wolbachia* cause cytoplasmic incompatibility (CI) that reduces the survival of uninfected embryos fertilized by *Wolbachia*-infected males. *Wolbachia* infections in host females rescue CI, increasing the relative fitness of infected females and encouraging *Wolbachia* spread. Researchers are now using CI to drive *Wolbachia* from *Drosophila,* and their pathogen blocking phenotypes, to high frequencies in mosquito populations to curb the spread of arthropod borne disease. Despite the success of this application and the importance of CI for *Wolbachia* spread through natural systems, the genetic and cellular basis of pervasive CI-strength variation remains elusive. Here, we leverage *Drosophila* transgenics and natural *Wolbachia* diversity across the *Drosophila* genus to unravel why CI strength varies from very small reductions in embryo survival to complete death. These works inform CI's mechanism and *Wolbachia*'s status as the World's greatest pandemic.

159 A Role for the *Drosophila* Blood-Brain Barrier in the Regulation of Sleep

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Sleep is vital for most animals, yet its mechanism and function remain unclear. We show that the permeability of the BBB – the organ required for maintenance of homeostatic levels of nutrients, ions, and other molecules in the brain – displays circadian clock-dependent rhythmicity reflecting sleep/wake cycles. The blood-brain barrier GPCR *moody* is upregulated in sleep-deficient *insomniac (inc)* null *Drosophila*, suggesting a link between the blood-brain barrier (BBB) and sleep. We observed defects in BBB integrity in each of four sleep mutants *inc*, *wakeD1*, *wakeD2* and *sleepless.* Acute and chronic sleep deprivation increase BBB permeability, while rebound sleep and administration of the sleeping aid gaboxadol reduce it. Mild sleep deprivation increased drug penetration into the brain, suggesting a novel method of drug delivery for brain pathologies. Glia-specific modulation of *moody*, *lachesin,* or *Gαo*, each a well-studied regulator of BBB function, increased BBB permeability and produced robust sleep phenotypes. Together these studies indicate a novel role for BBB activity in the modulation of sleep.

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FlySection: A Database of Gene Expression Patterns in Embryonic *Drosophila* *Lossie Rooney*[1], Prasad Bandodkar[2], Gregory Reeves[2] 1) North Carolina State University, Raleigh, NC; 2) Texas A&M University, College Station, TX.

Fluorescence microscopy images are frequently used for quantitative genetics and modeling of gene regulatory networks (GRNs) in the *Drosophila* blastoderm; however, few consolidated sources of these data exist that allow easy curation of datasets. We present a database, called "FlySection," that will be publicly available for access through the Reeves' lab website to contain quantitative data on gene expression patterns extracted from images generated by our lab. These data and the original images will be searchable and available for download. FlySection consists of a JavaScript webapp where users can search for relevant images using a few categorizing labels or quickly create datasets using criteria for parameters extracted from the images during analysis. The app accesses a Firebase Real-time Database where the results of our image analysis are stored. We expect that this database will assist members of the *Drosophila* research community who are studying gene regulatory networks in development to explore existing hypotheses and uncover new hypotheses for further study by collecting a large volume of image data in one place that is easily accessible and sorted by fly genotype, gene, fixed vs. live imaging, protein vs. mRNA imaging, or specific parameter values from which custom datasets can easily be constructed.

We present some examples, including pooled datasets drawn from FlySection that allow for greater statistical power than images acquired and analyzed by a single researcher, datasets that contain a wider scope of images than might be expected to apply to a given question, and novel questions generated by examining datasets created with FlySection.

161 Shine-Gal4: A light-controlled Gal4/UAS system for fast spatiotemporal control of gene expression *in vivo*

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The ability to control gene expression in space and time is essential to understand the molecular basis of tissue dynamics, organ development, patterning and adult homeostasis. This explains why the binary Gal4/UAS system has become such a popular research tool in Drosophila and other model organisms. The relevance and versatility of this system have been boosted by the availability of large collections of "responder lines" to express cDNA, reporters, or dsRNA. Here we describe a Gal4/UAS system that can be controlled by light, allowing efficient and exquisite spatiotemporal control of Gal4 activity in whole animals. We have developed Shine-Gal4, which can be used for induction of UAS transgenes in a fast and reproducible manner, in all tested *Drosophila* tissues and developmental stages *in vivo*. Shine-Gal4 can be activated ubiquitously or locally by modulating the field of illumination. In addition, the activity of Gal4 produced by a specific promoter can be gated by light. Finally, this new optogenetic tool enables efficient activation of loss and gain of function UAS transgenes at specific times, allowing to generate diverse phenotypes within scales ranging from individual cells to the whole animal. In conclusion, Shine-GAL4 enriches the *Drosophila* genetic toolkit by opening the possibility to control gene expression with an unprecedented temporal and spatial resolution.

162 Iterative assay for transposase-accessible chromatin by sequencing allows to home in on specific neurons

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Small neuronal subpopulations, and even individual neurons, can have profound effects on many different behaviors. Thus, numerous studies focus on identifying which specific subpopulations underlie various behavioral outputs and on understanding how these neurons function. Answering these questions requires tools that specifically target individual or a few neurons in a high-throughput manner. Previous approaches have been based on large collections of Gal4, or split-Gal4 lines, which are generated in a mostly random shotgun manner, and then need to be stained and/or screened by the hundreds or thousands. These approaches have included dividing certain genes into small potential enhancer DNA elements that drive expression in subsets of brain neurons (often unpredictable). However, potential enhancer DNA elements may or may not drive expression. We hypothesized that determining the open chromatin state in specific cells will lead to the identification of enhancer elements that drive expression in subsets of the starting population. To test this hypothesis, we analyzed the open chromatin state in *Drosophila melanogaster* neurons using Assay for Transposase-Accessible Chromatin followed by deep sequencing (ATAC-seq). Sorting nuclei from two neuronal Gal4 drivers (*elav*- and *nSyb*-) and from two whole-body drivers (*actin*- and *Tubulin-Gal4*) followed by ATAC-seq revealed 13,221 DNA sequence peaks from more open chromatin regions in neurons. These sequences drove *peak-Gal4* expression specifically in neurons, from 1000s down to 10s of different neurons. Conversely, peaks that were more open in whole-body samples drove expression in different non-neuronal tissues. To further subdivide a given neuron population expressing open *peak-Gal4*, we performed iterative ATAC-seq from this specific *peak-Gal4* population to identify second-round DNA sequence peaks that were more open in this given population of neurons compared to all neurons. We will present data suggesting that this iterative approach can be used to systematically refine a given population of neurons into more and more specific subpopulations in a non-random, data-driven fashion. This iterative ATAC-seq approach is applicable to other, non-neuronal cell-types and tissues, and it allows for identification and targeting of small, specific types of cell populations throughout the animal.

163 Precise genome engineering in *Drosophila* using prime editing

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Precise genome editing is a valuable tool to study gene function in model organisms. Prime editing, a precise editing system developed in mammalian cells, does not require double strand breaks or donor DNA and has low off-target effects. Here, we applied prime editing for the model organism *Drosophila melanogaster* and developed conditions for optimal editing. By expressing prime editing components in cultured cells or somatic cells of transgenic flies, we precisely introduce premature stop codons in three classical visible marker genes, *ebony*, *white*, and *forked*. Furthermore, by restricting editing to germ cells, we demonstrate efficient germ line transmission of a precise edit in *ebony* to 36% of progeny. Our results suggest that prime editing is a useful system in *Drosophila* to study gene function, such as engineering precise point mutations, deletions, or epitope tags.

164 High-Throughput Absorbance-based Quantification of Consumption in *Drosophila* using a Microplate Feeder Assay

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Food intake is affected by a wide range of physiological and environmental conditions and can be effectively quantified

in *Drosophila melanogaster*. However, few methods are currently available to measure consumption in *Drosophila* that are compatible with high-throughput experimental designs. We developed a Microplate Feeder Assay (MFA) that can be adapted to measure consumption of liquid food with high throughput. In this assay, flies are allowed to consume liquid food medium, which contains a dilute tracer dye, from select wells of a 1536-well microplate. By loading a known volume of liquid food medium into each well, absorbance measurements acquired before and after consumption can be used to calculate the resulting change in volume (*i.e.* volume consumed). To enable high throughput analysis with this method, we designed a 3D-printed coupler that allows flies to be cultured individually in 96-well microplates and that precisely orients 96- and 1536-well microplates giving each fly access to up to 4 wells for consumption, thus enabling food preference quantification in addition to regular consumption. To test the MFA, we performed a pilot study with Canton-S flies (n = 93 males and n = 111 females), which were sorted for individual culture into wells of a 96-well microplate containing 60 µL of 1.5% agarose. Using the 3D-printed coupler, flies were allowed to recover 24 h with access to solid fly food. Subsequently, solid food was replaced with a 1536-well microplate containing 4% sucrose + 1% yeast extract + 0.004% FD&C Blue #1 (10 µL/well; 1 well/fly). Empty wells (n = 63) were included as evaporation controls. After 18 h of exposure, consumption was quantified and adjusted for evaporation. Evaporative changes were -0.030 µL ± 0.022 µL (mean ± SEM), while males and females respectively consumed 0.423 µL ± 0.044 µL (mean ± SEM) and 0.869 µL ± 0.070 µL (mean ± SEM). These data indicate the suitability of the MFA for quantifying consumption in *Drosophila*. In principle, this method can be extended to other *Drosophila* species as well as other small insects, such as mosquitoes. Additionally, the coupler could be re-designed to accommodate a variety of microplate formats and culture vessels. Supported by NIH grants DA041613 and GM128974.

165 The Fly Cell Atlas: single-cell transcriptomes of the entire adult Drosophila

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Single-cell technologies have ushered in a new era for Drosophila, allowing researchers to obtain transcriptomes for all stable cell types, as well as dynamic cell states. Here we present the first release of the Fly Cell Atlas (FCA), which includes >570,000 nuclei sampled across all tissues of the entire adult fly. Using a unified platform of single-nucleus transcriptomics (snRNA-seq), we obtained 350k single-cell transcriptomes extracted from 15 individually dissected sexed tissues and 220k of the entire head and body, covering the whole fly. We designed an automated analysis pipeline to preprocess, filter, and harmonize all samples, yielding on average 20 main clusters per tissue, with the highest cell diversity in the antennae. Further sub-clustering allowed for the identification of 100s of clusters, resulting in more than 250 distinct annotated cell types across all tissues as driven by a series of crowd annotation jamborees with field experts. Few cell types were uniquely detected in the entire head and body samples, suggesting high cell type saturation of the atlas. Comparing every cell type against the whole fly leads to the characterization of body-wide marker genes, returning an average of 10 stringent markers per cell type, with 266 having at least one unique marker. Differences between male and female tissues beyond the reproductive tissues were mainly found as differential gene expression within shared cell types, rather than as sex-specific cell types or composition biases. We also analyzed main cell type lineages that are shared between tissues, including blood cells, muscle cells, neurons, glia, epithelial cells, and trachea, allowing the retrieval of rare cell types and tissue-specific subtypes. Furthermore, gene regulatory network analysis provided new insights into transcription factor pleiotropy, and cross-species comparisons highlighted conserved cell types between flies and mammals in the digestive and urinary systems. This atlas provides a valuable resource for the entire Drosophila community and will be made available for user-friendly querying, analysis, and download via multiple data portals, including SCope and ASAP. The annotations are linked to FlyBase identifiers for integration with existing resources. Finally, we provide a set of cell type classifiers to map newly generated data onto the Fly Cell Atlas. In conclusion, we present the first whole-animal single-cell atlas that contains to our knowledge the majority of Drosophila cell types outside the CNS, and that will be instrumental as a reference to study genetic perturbations and disease models at single-cell resolution.

166A Drosophila glue prevents from predation and evolves rapidly

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Drosophila larval salivary glands produce a proteinaceous glue that attaches the pupa to a substrate during metamorphosis. Drosophila glue is made of 7 proteins called Sgs (salivary gland secreted) proteins and Eig71Ee protein. So far nothing is known about the role of Drosophila pupal attachment and the forces underlying the evolution of glue genes.
We examined the effect of pupa attachment on predation. In a forest near Paris, we found that attached pupae disappear twice less than detached ones and that ants are potential predators of pupa. Using ant colonies in the laboratory, we found that detached pupae are brought by ants to the nest while attached pupae are eaten on site. Eating pupae outside the nest requires longer time and 4 times more ants, thus representing a higher risk for ants in nature. Overall, our results indicate that Drosophila glue can prevent pupal predation.
Analysis of Sgs sequences revealed that glue genes seem to evolve under different selection pressures: *Sgs1*, *Sgs3*, *Sgs7* and *Sgs8* present a low nucleotide diversity while *Sgs5* and *Sgs5bis* experience balancing selection, suggesting that some glue genes may confer general properties and some other genes would confer environment-specific properties. Using wild strains from diverse geographic locations, we found that adhesion varies by three-fold between *D. melanogaster* populations. Additionally, we found that silencing *Sgs1* caused a decrease in adhesion. For the first time, we demonstrate that a *Sgs* gene plays a role in adhesion.
Drosophila fly glue represents a good and simple system to study evolution as a few genes seem to be involved in adhesion and as the adhesion trait is directly connected to the environment.

167B Genetic variation among *w*Mel strains of *Wolbachia pipientis* differentially rescues a *bag of marbles* partial loss of function mutant in *Drosophila melanogaster*

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Wolbachia is an intracellular, maternally inherited endosymbiotic bacteria that infects over 65% of insects and manipulates their reproduction for its own transmission. In *Drosophila melanogaster, Wolbachia* genetically interacts with the adaptively evolving germline stem cell gene *bag of marbles* (*bam*). Since *Wolbachia* must enter the host female germline to propagate, one hypothesis is that *Wolbachia* and *bam* are in a genetic conflict for control of oogenesis. In order to understand if *Wolbachia* could be driving the adaptive evolution of *bam*, we must understand the nature of the genetic interaction between *bam* and *Wolbachia*. Previously, we documented that infection with the *w*Mel strain of *Wolbachia* rescued the fertility and cytological ovarian defect of a *bam* hypomorphic mutant. However, this mutant was generated over 20 years ago in an uncontrolled and variable genetic background, and thus we have been unable to perform controlled experiments to further assess the interaction. Here, we used CRISPR/Cas9 to engineer the same single amino acid *bam* mutation into the w^{1118} isogenic background as well as generated a *bam* null allele in the same background. We assessed the female fertility of wildtype *bam*, a *bam* transheterozygous hypomorph/null mutant, and a homozygous *bam* hypomorphic mutant, each infected individually with 10 diverse *Wolbachia* variants. Overall, we found that the *Wolbachia* variants tested do not generally increase bam^{-} female fertility, but they do rescue *bam* hypomorphic defects with variation in the effect size of some variants on female fertility. Therefore, the interaction between *bam* and *Wolbachia* appears to be modulated by both *bam* and *Wolbachia* genotypes. We are currently working to identify the mechanisms of this interaction by utilizing these genetic tools to look for changes in expression in *bam* hypomorphic ovaries associated with *Wolbachia* infection. Additionally, to help us understand if *bam* repeatedly participates in cellular interactions with *Wolbachia* or if the interaction is unique to particular *Drosophila* lineages, we are generating *bam* hypomorphic mutants in other *Drosophila* species and asking if *bam* and *Wolbachia* genetically interact.

168C Experimental Evolution of an Adaptive Inversion Polymorphism

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Chromosomal inversions suppress recombination in heterozygous state and thus potentially allow adaptive alleles to be co-inherited. Yet, at the same time, inversions also prevent the purging of deleterious alleles which might accumulate on both inverted and non-inverted chromosomes. In *Drosophila melanogaster*, the *In(3R)Payne* inversion polymorphism exhibits strong latitudinal clines on multiple continents and has been found to be maintained adaptively. However, the precise evolutionary mechanisms that maintain this polymorphism in natural populations remain poorly understood. To begin to address this matter, we have established two panmictic populations, one for each arrangement, in which chromosomes isolated from a natural population in Florida can recombine freely and adapt to laboratory conditions in large population cages. This thus creates recombinant standard and inverted homokaryotypes (but preventing gene flux between karyotypes) which can be used for large-scale phenotyping and population genomics. To characterize evolutionary changes, we surveyed egg-to-adult survival (viability) across generations, both for panmictic standard and inverted homokaryons as well as for heterokaryons derived from mass crosses between the standard and inverted populations. We observed that viability of all genotypes increased over

20 generations of recombination, probably reflecting the purging of deleterious alleles within arrangement types. Interestingly, this increase in viability was more pronounced under high larval density conditions, with heterokaryons displaying the strongest change. These preliminary observations suggest that *In(3R)Payne* might be maintained by overdominant viability selection and/or associative overdominance in a density-dependent manner. We are currently further exploring this hypothesis, using a combination of phenotyping and resequencing of the evolved populations; these genomic analyses might shed some light on the fate of potential deleterious alleles.

169A Drift in Individual Preference as a Population-level Strategy for Environmental Adaptation

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Phenotypic variation at the level of individuals (individuality) buffers species from fluctuations in the environment by ensuring a portion of the population is suited to survive sudden changes in selective pressures. Individuality can arise from a number of sources, including genetics, plasticity, and stochastic variation in development. These phenomena operate on different timescales relative to the lifespan of an organism and may underpin adaptive responses to different timescales of environmental fluctuation. Here, we examine an additional source of individuality, random drift in phenotype over the lifetime of an organism. Using a modeling framework based on shifts in behavioral preferences observed in Drosophila melanogaster, we show that random drift in the preferences of individual flies can be advantageous to populations. We compare the relative efficacy of drift compared to two other population-level strategies to deal with changes in the environment: bet-hedging (variation in phenotype among genetically identical individuals at birth) and adaptive tracking (genetically driven changes in population phenotype). We demonstrate a potential evolutionary benefit for drift and suggest that the degree of drift may be subject to evolutionary control depending on the structure of environmental pressures. These findings suggest that observed variation in individual behavioral preference over time may reflect an evolutionary drift strategy. With its deep genetic toolkit, Drosophila is a promising system for studying the mechanistic basis of phenotypic drift in an evolutionary context.

170B Differing lifestyles and metabolisms of *Drosophila lutzii*, a Floridosa group of species, and sympatric *D. simulans*, a generalist.

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The *Drosophila* genus of the family Drosophilidae comprises around 1600 described species. These species differ in their geographic distribution and ecologies, and consequently, in ecological niches and lifestyles. As they evolve in different environments, species may differentially regulate their metabolism and behavior as they adapt to these local conditions. Here, we characterize *Drosophila lutzii*, a Neotropical Phloridosa group of species of *Drosophila*. As its group of species implies, they are not saprophytic, but rather feed on flowers. We made a comparative study between *D. lutzii,* a specialist, and sympatric *D. simulans*, a generalist. We analyzed metabolic and behavior parameters. *Drosophila simulans* is a saprophytic generalist, with feeding based on rotting plants and fruits, while *Drosophila lutzii* is a phytophagous specialist. We have found *D. lutzii* eggs, larvae, pupae and adults inside *Ipomoea sp.* flowers. This suggested a restricted diet, and thus, an interesting avenue for research in metabolism, in comparison to generalist species of flies. We found that freshly caught *D. lutzii* from the wild have higher carbohydrates levels, but similar lipid content, as compared to sympatric freshly caught *D. simulans.* Consistent with a restricted diet and specialist lifestyle, *D. lutzii* flies are less capable of surviving in culture in diets that differ in the amounts of carbohydrates, and when fed diets with high sugar concentrations, contrary to *D. simulans*, they significantly accumulate them. Triglycerides levels also were differentially affected in both species when fed with diets that varied in sugar content. *D. lutzii* flies are significantly and dramatically less motile, but possess a circadian activity rhythm akin to *D. melanogaster or D. simulans*. Taken together, our results show that, in contrast to generalists, this specialist species, with more restricted habitat and feeding, is less capable of metabolic adjustments.

171C Mapping the loci contributing to wing form adaptation to high altitude

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Studies identifying the loci contributing to adaptive change in natural populations have often identified genetic variants of large phenotypic effect contributing to divergence. However, there are fewer examples of the size and number of loci contributing to adaptive divergence for complex traits. Wing shape and size in *Drosophila melanogaster,* are examples of complex traits with a well-studied genetic basis in both lab and natural populations. Both wing size and shape have large mutational target sizes and variation within natural populations is polygenic with many alleles of small effect contributing. Alleles of moderate effect have been implicated in size variation between populations. Populations from Sub-Saharan African have diverged for both shape and size along an altitudinal cline. Specifically, highland populations from Fiche, Ethiopia

(~3000m) have diverged from lowland ancestral populations. Ethiopian flies are larger for body size in general and have disproportionately larger wings than lowland Zambian flies. Additionally, there is also a consistent shape change between low and highland populations. This creates an excellent system with which to ask questions about the number and effect size of variants contributing to adaptive divergence for a complex trait.
Using strains derived from Zambian and Ethiopian populations, we took a bulk segregant mapping approach to identify loci contributing to adaptive divergence in wing form. Each selected Ethiopian line was crossed to each selected Zambian line, and allowed to recombine for 20 non-overlapping generation. F20 individuals were phenotyped and pools of individuals were created using the extremes of the shape and size distributions followed by sequencing of the pools. Using an ancestry-based mapping approach, we are able to identify the loci contributing to adaptive divergence and estimate effect sizes for shape and size between highland and lowland populations. This allows us to ask questions about the number and effect size of the loci contributing to divergence in this system. I will present the loci mapped in a number of our F20 crosses and compare these findings with others from the literature to better understand the genetic basis of adaptive divergence for a complex trait.

172A The genetic basis of cardiac glycoside resistance in wild-caught Drosophila melanogaster

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A significant number of unrelated herbivorous insects have repeatedly and independently evolved the ability to feed on plants that produce toxic secondary compounds called cardiac glycosides (CGs). CGs inhibit the function of Na+,K+-ATPase (NKA), a medically important enzyme in animals that is necessary for many processes including neural function and muscle contraction. Previous work identified a small number of adaptive amino acid substitutions in the alpha-subunit (ATPalpha) of NKA that confer the enzyme with resistance to CG inhibition. Further, engineering the native ATPalpha of D. melanogaster to carry one or more of these adaptive substitutions results in flies that are substantially resistant to CG toxicity. Here we show that wild-collected D. melanogaster strains harbor substantial genetic variation in sensitivity to CG-toxicity. Using whole genome sequences and expression data from 180 Drosophila melanogaster Genome Reference Panel lines, we show that this CG-insensitivity does not map to ATPalpha, but maps to a number of genes implicated in maintaining cell-cell barriers to solute diffusion and feeding behavior. This information can be used to predict additional targets of recurrent adaptation in diverse CG-tolerant taxa. In addition, these results yield insight into the development of drugs to treat a number of Na+,K+-ATPase-associated neurological and physiological disorders in humans.

173B Genomics of recombination variation in temperature-evolved *Drosophila melanogaster* populations

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Meiotic recombination is a critical process that ensures proper segregation of chromosome homologues through DNA double strand break repair mechanisms. Rates of recombination are highly variable among various taxa, within species, and within genomes with far-reaching evolutionary and genomic consequences. The genetic basis of recombination rate variation is therefore crucial in the study of evolutionary biology but remains poorly understood. To this end we therefore took advantage of a set of experimental temperature-evolved populations of *Drosophila melanogaster* with heritable differences in recombination rates depending on the temperature regime in which they evolved. We performed whole genome sequencing and identified several chromosomal regions that appear to be divergent depending on temperature regime. In addition, we identify a set of single nucleotide polymorphisms and associated genes with significant differences in allele frequency when the different temperature-evolved populations are compared. Further refinement of these gene candidates emphasizing those expressed in the ovary and associated with DNA binding reveals numerous potential candidate genes such as *Hr38, EcR,* and *mamo* responsible for observed differences in recombination rates in these experimental evolution lines thus providing insight into the genetic basis of recombination rate variation.

174C Identification of Mef2 and GGA as potential regulators of differential aging among closely related *Drosophila* species

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We employed a novel strategy to identify genes potentially involved in aging by analyzing divergent gene networks among *Drosophila melanogaster* and its sibling species *Drosophila simulans*. Previous studies have shown that these species have different stress tolerance levels, which has been linked to aging, as well as different lifespans. Here we show that hybrid males between *D. melanogaster* and *D. simulans* that carry an X-chromosome from *D. simulans* have significantly longer lifespans compared to their parental species. In contrast, hybrid *D. mel/D. sim* females carrying one X-chromosome

copy from each parental species have significantly reduced lifespans. These findings indicate that genes located on the X-chromosome must be involved in this dysregulated aging and therefore must be good candidates for studying their roles in the aging process. To identify such genes, we carried out a bioinformatics search for X-chromosome genes and their autosomal interacting partners that were most divergent between *D. melanogaster* versus *D. simulans* and its close sibling species *Drosophila sechellia*. Because systemic aging has been shown to be regulated by brain aging, we knocked down a series of candidate genes in brain neurons using *elav*-Gal4 expressing UAS-RNAi against our genes of interest. The knockdown of *Myocyte enhancer factor 2* (*Mef2*) and *Golgi-localized, γ-adaptin ear containing, ARF binding protein* (*Gga*) genes in *D. melanogaster* caused an increase in lifespan of males, but not females, similarly to our observations in male *D. mel/D. sim* hybrids. *Mef2* encodes a transcription factor with roles in muscle development and neuronal regulation. *Gga* encodes the only *Drosophila* clathrin-coated vesicle adaptor protein homolog to the human GGAs, which are expressed in neurons and required for sorting the sub-cellular distribution of beta-secretase that regulates the processing of APP. We are currently testing the longevity effects of *Gga* and *Mef2* through negative geotaxis behavioral assays in aging flies. Our preliminary results suggest that knockdown of these genes significantly improve climbing ability in aged flies. We are also determining the central brain regions that express Mef2 and Gga protein to conduct future experiments using G-TRACE, a method to track the expression changes within individual neurons combined with RNAi knockdown to investigate gene function. Collectively, our research has the potential to uncover genes not previously linked to brain aging.

175A Taxi regulates life span of the *Drosophila melanogaster* through *Adar*

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Ageing is a multifactorial physiological process, where maintenance of neuronal health, muscle, fat bodies and gut bacteria play a crucial role in progression of ageing. Disruption in any of these intrinsic factors and other extrinsic factors like diet can lead to loss of homeostasis in physiological processes. Both genetic and / or epigenetic factors have been shown to regulate these. Although the major pathways involved in ageing have been fairly studied, there are upcoming molecular players that would help us understand the cross-talk between such factors. We have used *Drosophila* as a model system to address this question due to the ease of genetic manipulations and evolutionarily conserved ageing mechanisms. We have identified that over-expression and knockdown of Taxi, a transcription factor, in neurons lead to a stark reduction in the life span. Taxi negatively regulates *Adar* (Adenosine deaminase acting on mRNA), an ortholog of the human ADAR2, implicated in neurodegeneration and other metabolic pathways. Over-expression of the *Adar* significantly rescued the reduction in life span caused by *taxi* over-expression in neurons. *Adar* is implied to have a function in the regulation of cellular stress response, such as autophagy. Our work suggests that finding out the targets of *Adar* from our RNA-seq data of *taxi* null would help us elucidate pathways involved in regulation of life span.

176B Differential Regulation of non-coding RNA (ncRNA) in aged *Drosophila melanogaster* in response to Infection by RNA Virus

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The model organism *Drosophila melanogaster* can elicit powerful innate immune responses against various microorganisms including RNA viruses and is useful for the investigation of host-pathogen interactions. Moreover, with its short lifespan, the fruit fly allows investigations of the mechanisms underlying the aging process. In humans, advanced organismal age is correlated with greater susceptibility and mortality to several viral infections, as seen in the recent pandemic caused by the RNA virus SARS-CoV-2. Although considerable progress has been made in understanding how aging impacts the innate immune system, which represents the first line of defense against pathogens, the exact factors and molecular events contributing to the more rapid death of the aged organism following virus infection are not fully understood.

Our research group has observed that aged *Drosophila* succumb more rapidly to Flock House virus (FHV) infection compared to young, infected flies without increase in viral load. To investigate the underlying molecular basis, we performed RNA sequencing (RNAseq) on young and aged flies that have been infected with FHV or control injected. We observed that aged flies mount a larger transcriptional response post FHV infection than younger flies, a signature that is different from the transcriptional changes taking place during the aging process itself. Gene Ontology analysis revealed that more than 50% of differentially regulated genes for each experimental condition were uncategorized and did not belong to specific biological processes. Among these genes we found non-coding RNAs including long non-coding RNAs (lncRNA) and anti-sense RNAs (asRNA). We compared the expression of several lncRNAs via RT-qPCR and confirmed the differential gene expression

specifically observed in aged, FHV-infected flies. This indicates that FHV infection affects the expression of genes encoding different categories of ncRNAs, and that specific ncRNAs are regulated in the aged organism after FHV infection.

Our work shows that *Drosophila* can be used as a model to investigate host-virus interactions during aging and opens the possibility for analysis of gene function, including lncRNA's function within the mechanisms that govern survival and control of virus infections at older age. Such analysis in our *Drosophila*-FHV model would give us valuable insight into the basis of the correlation of susceptibility and mortality to viral infection with advanced age.

177C Diet-Dependent Fat Body Transcriptome Analysis Reveals the Proteasome as a Molecular Link Between Circadian Rhythms, Longevity, and Dietary Restriction

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Dietary restriction (DR), reduction of diet intake without causing malnutrition, is one of the most robust anti-aging interventions in animal models. Recent studies suggest circadian clock is linked to aging and DR. The circadian clock controls a wide range of rhythmic physiological, metabolic, and behavioral parameters through clock control of output genes. Yet it is not known through which clock controlled genes the clock might influence DR responses. To identify clock controlled mediators for DR effects we performed RNA-sequencing from dissected abdominal fat bodies across the 24 h day after just 5 days under control or DR diets. Notably, we did not detect any significant changes in the rhythmic expression of core clock genes, suggesting that such changes evident after chronic DR are not mediating core clock effects on more rapid DR longevity responses. Yet we discovered that DR induced *de novo* rhythmicity in ~ 300 genes as well as increased expression of some rhythmic output genes in general. Pathway enrichment analysis and Weighted Gene Co-Expression Network Analysis (WGCNA) revealed that DR increased network connectivity in modules involved in protein life cycles including a module primarily comprised of the genes encoding multiple proteasome subunits. An RNAi screen of clock controlled genes demonstrated that proteasome subunits contribute to lifespan under standard and/or DR diets, including the response to DR. Thus our data suggest that clock control of output, in particular those involving the proteasome, link DR-mediated changes in rhythmic transcription to lifespan extension.

178A Searching for genetic factors that aggravate aging-related muscle loss.

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Aging in humans and animals is associated with a reduction in the size and mass of skeletal muscles. This condition (known as sarcopenia) impacts elders' wellbeing, but its genetic underlying is not clear. Our lab has developed an explicit *Drosophila* model to study sarcopenia. We demonstrate that adult flies sporadically lose individual fibers in the composite muscles of the thorax. Quantitative analysis has confirmed that such spontaneous muscle fiber loss neatly correlates with the life span of individual populations and inbred genetic lines. A limited genome-wide association study performed with fully sequenced genetic lines (DGRP lines) has outlined a pool of candidate genes that may influence the rate of aging-related muscle loss. Interestingly, most of these candidates are relevant to the central nervous system (CNS). In a follow-up study, we analyzed how an alteration of the normal functioning of the CNS can affect the rate of muscle loss in aging flies. We applied tissue-specific genetic knockdown (KD) of the gene *jus* (FBgn0039647), associated with the bang-sensitive phenotype (FBcv:0000391). We found that CNS-specific but not muscle-specific *jus* KD profoundly promotes the loss of muscle fibers over four weeks. Notably, deliberate chronic induction of seizures and paralysis in the *jus* KD flies was not a contributing factor. We conclude that even subtle abnormalities in the CNS›s functioning can stimulate aging-related muscle loss and promote sarcopenia.

179B The role of commensal microbes on the longevity effect of dietary restriction in *Drosophila melanogaster*

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Dietary restriction (DR), undernutrition without malnutrition, is the most well-known intervention to retard aging and to

extend the lifespan in diverse organisms. Commensal bacteria resided in the digestive tracts have been reported to affect the host fitness and lifespan and is changed by environmental factors such as diet. However, the relationship of commensal microbes and the longevity effect of DR are not illustrated yet. To test whether the longevity effect of DR is related with commensal microbes, we generated axenic flies and measured the lifespan under the various concentration of yeast, the protein source of flies' diet. The lifespan-extending effect of DR was diminished by the removing of commensal microbes and this effect of microbes did not accompany with reduced fecundity and feeding amount of flies. In addition, we observed that there is positive correlation between yeast concentration and the abundance of *Acetobacter* and *Lactobacillus*, main phylotype of microbes in *Drosophila*, and that lifespan altered by axenic culture in low yeast diet were partially rescued by the association of *Acetobacter*, but not of *Lactobacillus*. Furthermore, the level of phosphorylated Akt and intracellular localization of dFOXO showed that insulin/IGF-1 signaling is downregulated in axenic flies as similar with DR-experienced flies, but its reduction is recovered by association of *Acetobacter*. Taken together, our results showed that *Acetobacter* plays important role in DR-related lifespan extension through IIS modulation. Our results showing the relationship of commensal microbes and longevity effect of DR will provide fundamental knowledge to understand the underlying mechanisms of lifespan extension by DR.

180C Partial inhibition of RNA Polymerase I promotes animal health and longevity

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Ageing can be defined as the decline of physiological function, health and vitality over time. In humans, age is the biggest risk factor for several diseases, including cancer, cardiovascular or neurodegeneration. Biogerontology research has shown in the past decades that ageing can be modulated by specific interventions on key cellular and molecular processes. Additionally, these processes can be adjusted by change to adult-specific transcriptional regulation. We found that RNA polymerase (Pol) I is a key player in longevity. Pol I is the essential, evolutionarily conserved enzyme in charge of generating the pre-ribosomal RNA (pre-rRNA). We showed that reducing the levels of Pol I activity is enough to extend lifespan in *Drosophila*. Moreover, this effect could be recapitulated by partial, adult-restricted inhibition in the midgut, independently in enterocytes and stem cells. Importantly, reduction in Pol I activity delayed broad, age-related impairment and pathology, improving gut health and neuromuscular function. Therefore, our study demonstrates that Pol I activity in the adult drives systemic, age-related decline in animal health and anticipates mortality.

181A Non-cell-autonomous Intestinal Occluding Junction Modulation in Aging and Disease

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Aging is a process marked by a continuous decline in multiple physiological functions. Intestinal barrier function, for example, is tightly linked to longevity in *Drosophila melanogaster* and other organisms. We have previously shown that altered expression of occluding junctions in the guts of fruit flies can lead to various hallmarks of aging, including modulation of intestinal homeostasis, variations in microbial dynamics, changes in immune activity, and alterations in lifespan. Loss of a specific occluding junction, Snakeskin (Ssk), leads to rapid and reversible intestinal barrier dysfunction, altered gut morphology, dysbiosis, and a dramatically reduced lifespan. Remarkably, restoration of Ssk expression in flies showing intestinal barrier dysfunction rescues each of these phenotypes previously linked to aging. Intestinal up-regulation of Ssk protects against microbial translocation following oral infection with pathogenic bacteria. Furthermore, intestinal up-regulation of Ssk improves intestinal barrier function during aging, limits dysbiosis, and extends lifespan. Additionally, perturbing barrier function in the gut has non-cell-autonomous impacts, including alterations in the brain and muscle. Moreover, these analyses add more information about the impact of the gut on tissue outside the gut and begin to address communication between the gut and the brain and muscles in disease models. These findings indicate that intestinal occluding junctions may represent prolongevity targets in mammals, in addition to their possible roles in intestinal dysfunction, aging, and disease.

182B Retrotransposon Insertion and Expression in Aging in *Drosophila melanogaster*

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Across all metazoans, aging correlates with functional decline. One potential contributor to this is the expression and mobilization of retrotransposons (RTs). RT expression and copy number have been shown to increase with age within multiple organisms. However, it remains unknown which RTs are most active in different populations of aging somatic cells, where

in the genome new insertions occur and the biological consequence of their activation. While increased RT expression has been shown to elicit the inflammatory response, it is unlikely that this is the only contributor to functional decline. We utilize *Drosophila melanogaster* to dissect evolutionarily conserved mechanisms mediating aging. Our goal is to determine RT insertion number within different somatic tissues that show age related functional decline. To-date, we have focused on the effects of altered RT expression and insertion on health and lifespan. Using a single cell whole genome sequencing approach, we have shown that RT insertions increase with age within indirect flight muscles of female *Drosophila*, with the most active element being the long terminal repeat (LTR) element, 412. To determine the biological importance of 412 insertions, we ubiquitously knocked down the expression of this RT using inducible shRNAs. Using a ubiquitously expressed Gal4 driver, we confirmed the efficiency of the 412-knockdown using two non-overlapping hairpins compared to a scrambled shRNA control. This significantly extended female lifespan without a significant improvement in healthspan at old age. This would indicate that the phenotype we are studying is directly related to aging biology irrespective of health. Interestingly, the increased lifespan upon knocking down 412 expression was observed only in female flies, suggesting a sex dependent response. Additionally, the RNA expression of 412 does not increase with age, suggesting that the phenotype is based on 412 insertion rather than expression alone. Based on our data linking reduced 412 expression with lifespan extension, we have generated a complementary overexpression system to examine the acute and chronic effects of 412 activation. We are also investigating global changes elicited by the 412-knockdown on transcription. Importantly, our data highlight that the expression and/or mobilization of RTs is likely to be a key driver of aging irrespective of health decline. This is also the first study to show a positive impact on lifespan and health by knocking down a single RT, 412.

183C Genome-wide analysis reveals novel regulators of synaptic maintenance

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Maintaining synaptic structure and function is vital for overall nervous system function and survival. Recent evidence indicates that synaptic deficits are associated with both aging and neurodegenerative diseases. However, we do not yet have a strong understanding of the mechanisms responsible for how these structures are maintained. In order to identify genes responsible for sustaining synapses, we performed an unbiased forward genetic screen utilizing the *Drosophila* Genetic Reference Panel (DGRP) consisting of 200 lines that have been fully sequenced. We assessed neuromuscular junctions (NMJs) that innervate the indirect flight muscles utilizing a high throughput flight assay that provides a direct readout of synaptic integrity. We tested for the progressive loss of flight ability from early and late time points of all DGRP lines to identify Single Nucleotide Polymorphisms (SNPs) responsible for synaptic maintenance. Our screen generated a list of several candidate genes associated with the flight phenotype including *Futsch*, a microtubule binding protein required in motor neurons and *MSP300*, required in muscle tissue. We functionally validated the role of these genes through assaying the flight behavior of genetic mutants and tissue-specific RNAi. Our results implicate several novel genes not previously associated with synaptic maintenance. Thus, we seek to further characterize the nature of how these genes sustain synaptic integrity and the mechanisms responsible.

184A Identification of p38 MAPK Binding Partners During Aging and Oxidative Stress

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How and why an organism ages is not fully understood, though increased oxidative stress has been hypothesized to promote aging and age-related diseases. However, the exact mechanistic relationship between aging and oxidative stress is not fully understood. In order to understand how aging and oxidative stress are mechanistically linked, we performed quantitative mass-spec to identify binding partners of the Stress Activated Protein Kinase, p38Kb, which has been found to regulate both oxidative stress and aging. Loss of p38Kb leads to increased oxidative stress, premature locomotor dysfunction, and a shortened lifespan, while over-expression of p38Kb in the muscle leads to oxidative stress resistance and a 37% increase in lifespan. As p38Kb plays a role in both processes, we wanted to determine if p38Kb uses the same mechanisms to regulate oxidative stress and aging. Therefore, we compared p38Kb binding partners at different ages (1, 3, and 5 weeks) and during exposure to different oxidizing agents: paraquat and hydrogen peroxide. We find that there are unique p38Kb binding partners at each age and condition. We then performed a comparative analysis of p38Kb binding partners between ages and oxidative conditions. We find that there is very little overlap between p38Kb binding partners in paraquat versus hydrogen peroxide exposed flies. We also find limited overlap between p38Kb binding partners from aged flies as compared to oxidative stress conditions. These data suggest that p38Kb is regulating the response to aging, paraquat, and hydrogen peroxide through different genetic mechanisms. Interestingly, we find that though the binding partners are different, they are involved in similar cellular processes, which suggests that p38Kb may be regulating these processes through different mechanisms in order to

respond to aging and different sources of oxidative stress.

185B Deciphering the role Class II PI3K variants in Autophagy

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Autophagy is a process that serves as an adaptive response to various forms of cellular stresses. The autophagic pathway involves membrane-bound sequestration of cytoplasmic contents for degradation in the lysosome, called autolysosomes. In this way, autophagy trafficking converges with the late steps in endosomal trafficking for one common outcome of degradation, respectively. Autophagy is regulated between basal and induced levels to meet changing cellular demands, raising the question of how the shifting balance between endosomal and autophagic degradation is achieved. Membrane identities and trafficking dynamics depend in part on phosphoinositides, the seven phosphorylated forms of phosphatidylinositol (PI), many of which are implicated in autophagy. We found that the phosphoinositide enzymes, class II PI3-kinase (PI3KC2) and Mtm PI3-phosphatase, co-regulate basal autophagy levels by restricting autolysosome maturation. We identified two splice variants of PI3KC2: one with the catalytic kinase function (PI3KC2), and the other truncated without the kinase domain (PI3KC2-short). Variant-specific and null deletions revealed that PI3KC2-short is needed to promote autophagy, acting upstream of PI3KC2 and Mtm to relieve autolysosome maturation. PI3KC2-short forms a protein complex with and downregulates both PI3KC2 and Mtm catalytic activities, acting as a pathway to regulate basal versus induced autophagy levels. PI3KC2 and Mtm are also implicated in promoting endosomal transit toward cortical recycling. We are currently testing a model that PI3KC2-short co-regulates PI3KC2 and Mtm to tune the balance between endosomal trafficking and autolyososmal maturation. In addition, PI3KC2-short also inhibits PI3KC2 kinase activity – distinct from Mtm function – to promote levels of autophagy initiation. Together, we identified a novel pathway that helps coordinate levels of converging membrane trafficking steps that are needed for autophagy.

186C Pleiotropic role of Drosophila *phosphoribosyl pyrophosphate synthetase* in autophagy and lysosome homeostasis

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Phosphoribosyl pyrophosphate synthetase (PRPS) is a rate-limiting enzyme that plays a crucial function in nucleotide metabolism. The functional importance of PRPS is illustrated by the fact that human *PRPS1* is mutated in neurological disorders such as Arts syndrome, Charcot-Marie-Tooth disease and nonsyndromic sensorineural deafness. However, it is currently unclear how PRPS deregulation contributes to neuropathogenesis and, as a consequence, no treatment option is available for affected patients. We generated two fly models of Arts syndrome-derived mutations established via CRISPR/Cas9, $dPRPS^{Q165P}$ and $dPRPS^{R228W}$. Both mutant alleles demonstrate shortened lifespan and locomotive defects, common phenotypes associated with neurodegeneration. Careful analysis revealed that they have profound defects in lipolysis, cellular response to ROS, macroautophagy, and lysosome homeostasis. Additionally, the nervous system of *dPRPS* mutant flies is affected by these defects and accumulate aberrant lipid droplets and protein aggregates in the brain. Significantly, dietary supplementation of S-adenosylmethionine SAM can partially improve lysosome dysfunction and starvation defects of *dPRPS* mutant flies. SAM treatment was previously shown to delay onset of neurodegeneration in ARTS syndrome patients. Overall, we uncovered an unexpected link between nucleotide metabolism and autophagy/lysosome function and established a *Drosophila* model of PRPS-associated neurological disorder. Ongoing analysis aims to gain mechanistic insight behind *dPRPS*-associated defects.

187A How Myc influences glutamine metabolism to induce autophagy in tumor growth

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Drosophila melanogaster is emerging as a valuable model for studying different human diseases, including cancer and its metabolic adaptation to support cell growth. One of the major players in this process is Myc, which can promote tumorigenesis by triggering a metabolic reprogramming that allows cells to produce macromolecules, by modulating glycolytic flux, glutaminolysis, lipogenesis, and autophagy. So far, no connection between Myc, glutamine and autophagy was suggested, neither was addressed the intriguing question of whether Myc-glutamine relation is responsible for the initial confrontation between precancerous and wild-type cells to enhance cancer cell fitness.
To investigate the possible contribution of glutamine metabolism to Myc-induced autophagy, we downregulated glutaminase (GLS) gene in Myc-overexpressing clones in wing imaginal discs. The results showed that GLS is necessary for Myc-dependent autophagy, with a mechanism that is independent from mTOR pathway, one of the major regulators of autophagy.

We then investigated the role of Myc-induced autophagy in a tumor model expressing Ras^{v12} and disc large-RNAi. Here we found that Myc mediates the growth and hyperproliferation of the tumor cells, while its reduction leads to a decrease in autophagy. In order to better investigate the relevance of autophagy during the initial confrontation between precancerous cells and wild type cells, we analysed, in clones in the wing imaginal discs, how manipulation of GLS affects the growth of Ras^{v12} and disc large-RNAi expressing cells and their relation with the neighbouring cells. Our data can be important to establish how GLS contributes to Myc induced-autophagy and how this can enhance cancer cellular fitness.

188B SVIP is a Molecular Determinant of Lysosomal Dynamic Stability, Neurodegeneration and Lifespan

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Missense mutations in Valosin-Containing Protein (*VCP)* are linked to diverse degenerative diseases including IBMPFD, amyotrophic lateral sclerosis (ALS), muscular dystrophy and Parkinson's disease. Here, we characterize a VCP-binding co-factor (SVIP) that specifically recruits VCP to lysosomes. SVIP is essential to maintain the dynamic stability of a tubular lysosomal network and autophagosomal-lysosomal fusion. SVIP mutations cause muscle wasting and neuromuscular degeneration while muscle-specific SVIP over-expression increases lysosomal abundance and is sufficient to extend lifespan in a context, stress-dependent manner. We also establish multiple links between SVIP and VCP-dependent disease in our *Drosophila* model system. A biochemical screen identified a disease-causing VCP mutation that prevents SVIP binding. Conversely, over-expression of an SVIP mutation that prevents VCP binding is deleterious. Finally, we identify an SVIP mutation in a human patient and confirm the pathogenicity of this mutation in our *Drosophila* model. We propose a model for VCP disease based on the differential, co-factor-dependent recruitment of VCP to intracellular organelles.

189C Reduction of Glutamate Dehydrogenase Increases Autophagy in Neurons and Ameliorate Motility and Survival in a Drosophila Model for Huntington's Disease

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Autophagy is a fundamental cellular pathway involved in the clearance of dysfunctional organelles and protein aggregates, and it is particularly important in neurons. A lot of neurodegenerative diseases, such as Huntington's disease (HD), Spinocerebellar Ataxias (SCAs), and Amyotrophic Lateral Sclerosis (ALS) are characterized by an impaired autophagic flux that together with the formation of toxic protein aggregates, contribute to neuronal death. Glutamate Dehydrogenase (GDH) is an evolutionary conserved enzyme that catalyses the conversion of glutamate and ammonia to α-ketoglutarate and is also member of the Glutamate-Glutamine Cycle, a physiological process between glia and neurons that controls glutamate homeostasis. In a *Drosophila* model for HD we observed that the reduction of GDH in neurons ameliorates the motility defects and decreases the size of mutated Huntingtin's aggregates (mHTT) in brains. This recovery is associated with the ability of GDH reduction to induce autophagy. Indeed, GDH was found to be overexpressed in human HD post-mortem brains. Its reduction changes the amino acids levels, including a decrease of leucine. Leucine is proposed to act by binding to a 'sensor', such as Sestrin, to activated mTOR pathway, a central regulator of metabolism that promotes cellular growth and inhibits autophagy. Furthermore, reduced GDH expression results in a reduced localization of mTOR on lysosomes, a fundamental step for its activation. Thus, this study suggest a novel function for GDH to control autophagy in post-mitotic cells like neurons resulting, when inhibited, in the clearance of the toxic mHTT aggregates.

190A Addressing the physiological role of endosomal Microautophagy

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Autophagy is a catabolic process which degrades damaged organelles and aggregate prone proteins and thus is essential for development and cellular homeostasis. It is induced in response to different stressors including starvation, oxidative stress and accumulation of misfolded proteins. As such, it counteracts various human diseases, and its reduction leads to aging like phenotypes. Of the three major forms of autophagy, Macroautophagy can degrade organelles or aggregated proteins, and Chaperone-mediated autophagy is specific for proteins containing KFERQ-related targeting motifs. Endosomal Microautophagy (eMI), a form of autophagy during which substrates are taken up into multivesicular bodies for degradation in a KFERQ-specific manner or in bulk. Among the three form of autophagy the physiological role of eMI is poorly understood.
Using a KFERQ-tagged fluorescent biosensor, we are characterizing the physiological role of eMI with a focus on what types of cellular stress activate eMI. Our data suggest that oxidative stress and DNA damage, but not ER stress can elicit an eMI response in an ESCRT machinery dependent manner, implying a stress-selectivity of the process. Further, we are trying

to understand the mechanism of stress induced eMI by identifying novel regulators of the eMI pathway. We identified a candidate regulator, that, when upregulated, results in premature induction of starvation induced eMI. Since targeting autophagic pathways has been proposed as treatment strategy particularly in non-dividing neurons,we are also testing eMI candidate regulators as a possible modifiers of fly models of human neurodegenerative diseases. We anticipate that the physiological role of eMI in stress regulation and neurodegeneration are conserved in humans.

191B Mechano-chemical enforcement of tendon apical ECM into nano-filaments during *Drosophila* flight muscle development

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Contractile tension is critical for musculoskeletal system development and maintenance. In insects, the muscular force is transmitted to the exoskeleton through the tendon cells and tendon apical extracellular matrix (ECM). In *Drosophila*, tendon cells secrete Dumpy (Dpy), a zona pellucida domain (ZPD) protein, to form the force-resistant filaments in the exuvial space, anchoring the tendon cells to the pupal cuticle. By live imaging, we showed that Dpy undergoes filamentous conversion in response to the increase in tension during indirect flight muscle development. Additionally, we found another ZPD protein, Quasimodo (Qsm), which plays a long-range, non-cell-autonomous role in the enforcement of Dpy filaments *in vivo*. Qsm also promotes secretion and polymerization of Dpy-ZPD *in vitro*. The dual role of Qsm underlies the positive feedback mechanism of force-dependent organization of Dpy filaments, providing new insights into apical ECM remodeling through the unconventional interaction of ZPD proteins.

192C Establishing a mechanism for *Drosophila* midgut basement membrane repair

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Basement membranes are the oldest, most conserved forms of extracellular matrix and serve to separate tissue layers, provide mechanical support, direct signals to neighboring cells, and insulate tissues from signals. Although basement membranes are sometimes thought of as static, we have found that they maintain homeostasis through dynamic processes. Further, basement membranes are subject to mechanical damage, and require dynamic repair mechanisms to repair the damage. Work from our lab indicates that many aspects of basement membrane repair are shared during homeostasis. Yet, the exact differences and mechanisms remain unclear. This work aims to clarify the major differences between basement membrane homeostasis and repair by identifying the sources of major basement membrane components under homeostatic and damaged conditions, and elucidating mechanisms used by cells to respond to basement membrane damage. My work utilizes the *Drosophila* midgut basement membrane, which is located basally to the epithelial cells and surrounds the muscles responsible for gut peristalsis. We are identifying the sources of basement membrane components for this tissue under both maintenance and repair conditions. We hypothesize that the main difference between homeostasis and repair is the rate of assembly, and to test it, we have devised a system for measuring the assembly and disassembly rates of basement membranes under maintenance and repair conditions. We will continue to uncover fundamental principles of basement membrane repair through this work and develop a model for how repair occurs following basement membrane damage.

193A The ECM protein Fibulin plays important roles in trunk visceral mesoderm and somatic muscle development during embryogenesis

Bronwyn Tollefson[1], Courtney Peckosh[1], Annasha Doane-Ramkhalawon[1], Anmol Suri[1], Afshan Ismat[1] 1) The University of St.Thomas.

Uncovering the genes that promote, block, and influence muscle development is a necessary component of embryogenesis. The visceral mesoderm of Drosophila melanogaster forms the layers of muscle fibers surrounding the midgut. An important component of this structure is the trunk visceral mesoderm (TVM) which gives rise to the circular muscle fibers that line the midgut. This structure develops through the rearrangement and elongation of cell clusters in the dorsal mesoderm along the anterior-posterior axis of the embryo. The somatic musculature forms through the fusion of muscle founder cells (FCs) and fusion competent myoblasts (FCMs) to form multinucleate muscle fibers. In this study, we are examining the role of fibulin (fbl) (CG31999) on the development of the TVM and somatic musculature. Fibulin is a family of proteins in the extracellular matrix associated with elastic tissues and basement membranes. In the visceral mesoderm, knock-down of fbl resulted in gaps in the TVM. Over-expression of fbl in the TVM or caudal visceral mesoderm (CVM) resulted in scattered and mis-migrating CVM cells. In the somatic muscles, knock-down of fbl resulted in morphological changes in somatic muscle patterning. Over-expression of fbl in somatic muscles also displays muscle patterning defects, and, surprisingly, swollen and misshapen midguts. We are

currently in the process of examining CVM migration in the absence of fbl, as well as pinpointing the exact muscle fibers disrupted in the loss of fbl. The continuation of this research is necessary to provide more insight into the complex relationship between fibulin and somatic muscle development.

194B Wg secreted by conventional Golgi transport diffuses and forms Wg gradient, but Wg tethered to extracellular vesicles do not diffuse

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Wingless (Wg)/Wnt family proteins are essential for animal development and adult homeostasis. *Drosophila* Wg secreted from the dorsal-ventral (DV) midline in wing discs forms a concentration gradient that is shaped by diffusion rate and stability of Wg. To understand how the gradient of extracellular Wg is generated, we compared the secretion route of NRT-Wg, an artificial membrane-tethered form of Wg that is supposedly not secreted but still supports fly development, to that of wild-type Wg. We found that wild-type Wg is secreted by both conventional Golgi transport and via extracellular vesicles (EVs), and NRT-Wg can be also secreted via EVs. Furthermore, wild-type Wg secreted by Golgi transport diffused and formed Wg gradient but Wg-containing EVs did not diffuse at all. In case of Wg stability, Sol narae (Sona), a metalloprotease that cleaves Wg, contributes to generate a steep Wg gradient. Interestingly, Wg was also produced in the presumptive wing blade region, which indicates that NRT-Wg on EVs expressed in the blade allows the blade cells to proliferate and differentiate without Wg diffused from the DV midline. We propose that EV-associated Wg induces Wg signaling in autocrine and juxtaposed manners whereas Wg secreted by Golgi transport forms gradient and acts in the long-range signaling, and different organs differentially utilize these two types of Wg signaling for their own development.

195C *Drosophila* clock cells use multiple mechanisms to transmit time-of-day signals in the brain

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While the mechanisms of the circadian clock have been well-studied, how daily environmental cues and internal drives are coordinated to regulate behavior is poorly understood. The integration of circadian information with other sensory cues occurs downstream of the core clock cells in the brain, at the intersection of multiple behavioral circuits which signal using both classical fast neurotransmitters and neuropeptides. The *Drosophila pars intercerebralis* (PI), an analog of the mammalian hypothalamus, is a peptidergic center that receives both circadian and nutritional state information. We demonstrate that the *pars intercerebralis* (PI), previously identified as a target of the morning cells in the clock network, also receives input from evening cells. We determined that morning and evening clock neurons have time of day dependent connectivity to the PI, which is regulated by specific peptides as well as by fast neurotransmitters. Interestingly, PI cells that secrete the peptide DH44, and control rest:activity rhythms, are inhibited by clock inputs while insulin-producing cells are activated, indicating that the same clock cells can use different mechanisms to drive cycling in output neurons. Inputs of morning cells to the *DILP2*+ neurons are relevant for the circadian rhythm of feeding, reinforcing the role of the PI as a circadian relay that controls multiple behavioral outputs. Preliminary RNAseq of PI neuron populations identified additional neuropeptide receptors that may contribute to clock-to-PI signaling. Our findings provide mechanisms by which clock neurons signal to non-clock cells to drive rhythms of behavior.

196A Plasticity in the circadian circuit mediated by the reproductive state in females of Drosophila melanogaster

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Rest-activity cycles are common to both males and females of Drosophila melanogaster. However, there are some important sex differences related to the timing of the resting period during daylight hours. Until very recently, the majority of the circadian studies focused on males, probably because of the lower complexity of their behavior. As a consequence, the temporal organization of female locomotor activity has received much less attention. After mating, female physiology undergoes several important changes, which are reflected in their activity patterns. To explore the hypothesis that mating could alter circadian clock function and thus modify the temporal organization of the behavior, we performed a high resolution analysis of the locomotor activity using a custom-made video tracking method. By comparing the rest-activity patterns on virgin and mated females as well as on males, we observed that, in contrast to males and virgins, mated females loose their ability to anticipate the night-day transition when locomotor activity is analyzed under light:dark conditions. Our results show

that this postmating response is mediated by the action of the sex peptide on ppk neurons, since silencing the SP receptor in these neurons restores the ability to anticipate the light/dark transition in mated females. Using the anterograde trans-synaptic tracing tool trans-Tango we identified the small lateral ventral neurons as new postsynaptic targets of ppk+ SP sensory neurons in mated females. Our results are consistent with a model whereby mating-triggered signaling is delivered onto the clock network to modulate changes in the temporal organization of female behavior.

197B Sex differences in the effects of insulin signaling on food consumption in adult *Drosophila melanogaster*

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The core functions of insulin signaling are in nutrient sensing and growth. Insulin signaling can also play a role in control and development of sexually dimorphic traits. Interactions between insulin signaling and the sex determination hierarchy are known to contribute to regulating sexual dimorphism in body size, locomotor activity and mating behaviors. Insulin signaling is also known to affect feeding behavior in adult and larval stages – however the effects have varied across studies. In addition, few studies have examined the role of insulin signaling in feeding in both sexes. In this study we used the gene-switch system to reduce insulin signaling in adult males and females of *Drosophila melanogaster*. Feeding was compared using a qPCR oligo-based assay between drug-treated InRDN expressing (reduced insulin signaling) animals, matched genotype vehicle-only animals (no drug), and drug-treated wild type controls (effect of drug only). Females ate more than males across all conditions, as expected. Males and females both showed increased feeding with reduced insulin signaling, but females increased feeding significantly more than males. These results indicate that insulin signaling may have a larger effect on feeding in females relative to males.

198C Characterization of circadian Rhythms in a DNA repair mutant

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The *Drosophila* gene, *glaikit (gkt)*, is orthologous to human TDP1 which plays a role in DNA repair by removing DNA protein crosslinks. *gkt* mutants show phenotypes commonly associated with TDP1 mutations such as decreased motor abilities, shorter lifespan, and sensitivity to DNA damage reagents. Recent studies have demonstrated that excess night-time light, from sources like shiftwork and photopollution, alters circadian rhythms and may enhance levels of oxidative stress, leading to DNA damage. In order to better understand the interaction between DNA repair and circadian rhythms, *gkt* mutants and wild-type (w^{1118}) flies were placed under different lighting schedules in order to assess changes in circadian and locomotor behavior. When comparing *gkt* mutants to w^{1118} under different lighting conditions, we see significant changes in behavior. These findings suggest that circadian rhythmicity may be altered in *gkt* mutants. Assessing circadian change in *gkt* mutants will allow for a better understanding of the relationship between circadian rhythmicity, TDP1, DNA repair, and cancer.

199A Role of *dTRPA1*$^{+}$ and PDF^{+} neurons in modulating rhythmic activity in flies experiencing constant warm temperature

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Stressful conditions like prolonged durations of warmth can cause an organism's body to overheat or desiccate. To mitigate such harsh effects of extreme environmental conditions, organisms can modulate their behaviours, for example, by being more active during the night, when the conditions are likely to be less harsh, than the day. Previous work from our laboratory has shown that a mutant for a thermosensitive ion channel *Drosophila* Transient Receptor Potential-A1 (dTRPA1) does not shift its locomotor activity into the night when ambient temperatures are warm. We also know that a few core clock neurons in the brain express *dTRPA1.* I asked what the relative contributions of the clock- and non-clock *dTRPA1*$^{+}$ neurons are in modulating rhythmic activity under constant warm ambient temperature. I also explored how information from the *dTRPA1*$^{+}$ neurons is communicated to downstream neurons by screening across some neurotransmitters, and I will discuss the possible role of one neurotransmitter in the poster.
While the role of the circadian neuropeptide Pigment Dispersing Factor (PDF) and the PDF^{+} clock neurons has been extensively investigated in determining phase of locomotor activity under relatively cool ambient conditions, thus far, there have been no reports of their functions in phasing of activity under a relatively warm context. I observed that flies that lack PDF or the PDF^{+} neurons are unable to suppress activity at certain times through the night, suggesting that these neurons are important for the flies to appropriately distribute (or phase) activity, under warm ambient conditions.

200B Differential expression of *miR-210* in bees as an agent of maternal care

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The European honeybee, *Apis Mellifera*, experiences a division of labor system where young adults assume a nurse role and maintain provide childcare for the brood, while older adults become foragers and venture outside the hive to gather resources. This drastic division of labor is likely orchestrated by a host of genomic features, including microRNAs. Because miRNAs are highly conserved across species and can be easily turned on and off, it was hypothesized that they have a role in regulating bee division of labor. Brain microRNA samples were extracted from honeybees (age-dependent division of labor), bumblebees (a size-dependent division of labor) and solitary bees (solitary bee) and were sequenced and characterized with differential gene expression analysis. To isolate factors unrelated to age and isolate miRNA's related to nurse/forager behaviors, precocious young foragers and old nurses were generated in a single cohort system. It was revealed that nurses and foragers do not contain a distinct microRNA signature. Less than fifty percent of the variation in microRNA expression could be explained along two-component axes. Nurses and Foragers did not share microRNA expression profiles across the groups. Of the honeybee and bumblebee species, ame-miR-210 was the only gene that was significantly upregulated in nurses. Because miR-210 is a highly conserved microRNA, future studies seek to characterize how nursing and maternal type behaviors are affected by miR-210 in *Drosophila* by using an oviposition preference behavioral assay. Previous research has found miR-210 expression in the ocelli, mushroom bodies, and retinal tissue of *Drosophila*. Funded by NIH and NSF grants to YB.

201C How the Fly Decides: a New Assay to Study Decision Making

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Decision making is a vitally important, but poorly understood, part of animal and human life. Humans with Parkinson's disease, Conduct Disorder, and Alcohol Use Disorder show defects in decision making. To study decision making, I chose to use the fruit fly. Drosophila offers an opportunity to identify genes and neurons that contribute to accurate decision making. Previous assays have identified candidates that affect sensory detection thresholds or contextual states (such as hunger or mating drive) but it is not yet know whether there are central or general mechanisms that contribute to decision making in many different contexts.

I developed a new assay to present pairs of competing stimuli where flies chose between grooming, courting, and feeding. I established balanced drive conditions where wild-type flies choose equally between behaviors and am now testing candidate genetic mutants and neurons. I plan to screen FoxP, Leucokinin Receptor, and NPF mutants as well as mushroom body and central complex neurons. I will then expand out to a targeted screen based on these pilot results.

202A Developmental exposure to Bisphenol F impairs courtship behavior and causes developmental lethality

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Bisphenols are pervasive environmental chemicals used to produce polycarbonate plastics and epoxy resins. The most well-known bisphenol—bisphenol A (BPA)—is a well-established endocrine disrupting chemical. More recent studies have also linked BPA exposure to abnormal neurodevelopment and nervous system function in several model organisms. Due to its established harmful effects, BPA has been removed from some commercial products; however, manufacturers often replace BPA with chemical analogues, like bisphenol F (BPF). BPF has undergone far less toxicology testing than BPA. Due to its structural similarity with BPA, BPF may have similar harmful effects. Our study aims to investigate the developmental consequences of BPF exposure causes on its own and in combination with a genetic risk factor for neurodevelopmental factors—*fragile X mental retardation 1 (fmr1)*. *fmr1* is the functionally conserved fruit fly ortholog of human *FMR1*. Loss of *fmr1* disrupts a number of behavioral and neuronal processes. We exposed *Canton S*, *w1118* and *fmr1* null mutant flies to 1 millimolar (mM) and 2 mM BPF during embryonic and larval development and then used the naïve courtship assay to compare the resulting courtship index (CI) of adults. The CI reflects the total time spent participating in courtship behaviors for the duration of the assay. Exposure to 2mM significantly reduced the CI of *w1118*, but not Canton S flies. This result conveys the importance of performing toxicological assessment in organisms with different genetic backgrounds. *Fmr1* mutant flies exhibit a significantly reduced CI compared to *w1118* and Canton S flies, consistent with previous studies. We also found that exposure to BPF increased developmental lethality of both *w1118* and *Canton S* flies. We are currently completing the analysis of *fmr1* flies exposed to BPF for both the courtship and lethality studies, as well as investigating how BPF impacts axon guidance in the adult mushroom body in all three genetic strains.

203B Behavioral modification in response to auditory stress in *Drosophila melanogaster*

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Veterans are approximately 30% more likely than non-veterans to suffer from severe hearing impairment, with males being more affected than females. Tinnitus, or ringing in the ears, which is increasingly common among military service men and women, has been linked to significant cognitive and psychological impairment and can be worsened by the same sounds that trigger post-traumatic stress disorder (PTSD). In fact, tinnitus and PTSD often present as comorbidities and recent studies suggest these two disorders may share a common neurological pathway. Additional studies are required to better understand the connection between hearing loss impaired cognitive function such as that observed in with PTSD. Here, we use the fruit fly, *Drosophila melanogaster*, to explore the relationship between hearing loss and cognitive function. Negative geotaxis climbing assays and courtship behavior analysis were used to examine neurobehavioral changes induced by prolonged, intense auditory stimulation. Preliminary results suggest that exposure to loud noise for an extended period of significantly affect *Drosophila* behavior, with males being more sensitive than females. Based on our results, there appears to be a potential connection between noise exposure and behavior, further suggesting that *Drosophila* could be an effective model to study the link between hearing loss and PTSD.

204C The psychedelic drug psilocybin has long lasting antidepressant-like effects in male Drosophila

Charles Nichols[1], Meghan Hibicke[1] 1) LSU Health Sciences Center.

Introduction: The forced swim test (FST) is a measure of depressive-like behavior in rodents recently adapted for *Drosophila*. The fly FST is potentially a high-throughput screening method for evaluating the neurocircuitry underlying antidepressant effect. In this study we first pharmacologically evaluated the predictive validity of the fly FST using psychostimulant methamphetamine (METH), functional sedative DL-α-methyltyrosine (αMT), SSRI antidepressant citalopram (CIT), and experimental antidepressant psilocybin (PSI), a serotonergic psychedelic currently in human clinical trials. Differences in the FST were compared with locomotor activity changes in the Drosophila Activity Monitoring System (DAMS), and the pharmacological mechanisms by which PSI may have affected DAMS activity were investigated using 5-HT_{1A} antagonist WAY 100635 (WAY), and 5-HT_2 antagonist ketanserin (KET).
Methods: Groups of one-day old, non-entrained flies were fed for five days (5x) on 1% agarose, 10% sucrose medium containing METH (5.0 mM), αMT (3.0 mM), CIT (0.3, 1.0, or 2.5 mM), PSI (0.03 mM), WAY (1.0 mM), or KET (1.0 mM), or for one day (1x) on CIT (1.0 mM) or PSI (0.03 or 3.5 mM) then vehicle medium until testing. Control flies were fed vehicle medium for five days. Flies were tested in the FST for time spent immobile, latency to first immobility, and number of bouts of immobility (*n*=7(16)/group) five days after first treatment, and in the DAMS (*n*=16/group) continually and fifth-day activity compared.
Results: Males were consistently less active than females in both assays. METH increased and αMT reduced DAMS activity in both sexes, but did not significantly affect FST behavior. Only CIT (2.5 mM) reduced DAMS activity, and only in females. CIT (1.0 mM) 5x, but not 1x, reduced immobility in the FST in males, and increased latency to first immobility, and reduced bouts of immobility in both sexes. PSI (3.5 mM) 1x reduced DAMS activity in females, but both doses increased DAMS activity in males. However, PSI (0.03 mM) 5x had no effect in females and reduced DAMS activity in males. KET 5x reduced DAMS activity in both sexes, while WAY 5x reduced DAMS activity only in males. In the FST, PSI (3.5 and 0.03 mM) 1x reduced immobility and bouts of immobility, and increased latency to first immobility in males.
Conclusions: FST behaviors are decoupled from overall locomotor activity and are serotonergically mediated. In males, both high and low doses of psilocybin in the food for only 24 hours led to significantly reduced immobility in the FST 5 day later, indicating a single exposure leads to a long lasting antidepressant-like effect. The fly may therefore serve as a useful model to study the molecular and/or genetic mechanisms underlying the observed long-lasting antidepressant effects in humans.

205A Sexual experience does not affect the strength of male mate choice for high quality females

Alison Pischedda[1], Chelsea Sinclair[1] 1) Barnard College, Columbia University.

Although females are traditionally thought of as the choosy sex, there is increasing evidence in a number of species that males will preferentially court or mate with certain females over others when given the choice. In *Drosophila melanogaster*, males discriminate between potential mating partners based on a number of female traits, including species, mating history, age and condition. Males also show courtship and mating preferences for large females over small females, likely because larger females have higher fecundity. Interestingly, many of these male preferences are affected by the male's previous sexual experience, such that males increase courtship toward females they have previously mated with and decrease courtship toward females that have previously rejected them. It is unclear, however, whether male mate choice based on female body size shows similar plasticity. Here, we manipulate the past sexual experience of *D. melanogaster* males and test whether this has any effect on the strength of male mate choice for large females. We find that sexually inexperienced males show a robust courtship preference for large females, and that this preference is unaffected by previous mating or rejection experience with

small or large females.

206B

Monitoring circadian behavior in DNA repair-deficient Drosophila *Shahida Qazi*[1], Elyse Bolterstein[1] 1) Northeastern Illinois University.

Mus109 is one of 58 mutagen-sensitive genes found in Drosophila melanogaster. This recessive gene has been postulated to play a role in DNA repair due to sensitivity of *mus109* mutant flies to different DNA damaging reagents such as methyl methanesulfonate, which instigate base adducts; nitrogen mustard, which cause base adducts and interstrand crosslinks; and ionizing radiation, which result in damaged bases and DNA strand breaks. The concept of circadian rhythms has been recently been connected to DNA repair by recent findings that the NER mechanism is directly regulated by the circadian clock and has shown to be heightened in the evening. Similarly, our study explores the relationship between DNA repair and circadian rhythms in D. melanogaster by assessing their behavioral patterns of *mus109* mutants. We used Drosophila Activity Monitors to measure the activity of $mus109^{l5}/mus109^{D1}$, $mus109^{l5}/mus109^{D2}$, and w^{1118} females under a normal light-dark cycle and free-running (dark-dark) cycle. Preliminary findings suggest that *mus109* mutants have altered daily activity levels in comparison to the wildtype flies during their normal light-dark cycle as well as the free-running dark-dark cycle. Hence, this study further defines the function of *mus109* as it supports the relationship between circadian rhythms and DNA repair. Future research plans include analysis of other circadian endpoints such as period length, as well as investigating circadian behavior in *mus109* mutants that are treated with DNA damaging reagents.

207C Parasitoid-induced reproductive modifications in *Drosophila*

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In response to a selection pressure imposed by the parasitoid wasps, adult fruit flies have developed various behavioral adaptations that reduce the risk of infection in their progeny. Here we studied parasitoid-induced innate reproductive adaptations in *Drosophila* females in terms of sensory modalities, neural circuits, and germline modifications involved in the behavior. To address whether the observed alteration in reproductive behavior is a general stress response or a parasitoid-selective behavior, we first examined the egg-lay responses of wildtype flies to different wasp species. Upon exposure to larval parasitoids that infects the developing larvae, the female flies reduce their total egg-lay. However, when exposed to pupal parasitoids that specifically infects fruit fly pupae, the adult females increase their mean egg-lay. This parasitoid-selective alteration in reproductive behavior necessitates both olfactory and visual perception of the parasitoid. In the absence of either parasitoid-selective olfactory or visual cues, *Drosophila* females fail to display behavioral modifications that further supports the observation that adult flies can recognize and distinguish between different species of wasps.

While larval parasitoid-induced egg-lay depression is associated with transient retention of matured eggs and increased apoptosis of the developing oocytes in the wasp-exposed ovaries, the increased number of oocytes due to germ cell proliferation accounts for enlarged ovaries observed in pupal parasitoid-exposed females. We further delineate the function of neuroendocrine signaling involving neuropeptide F and its cognate receptor, NPFR, in larval parasitoid-induced behavioral and germline modifications. Thus, by providing evidence for innate recognition of predatory threat and the mechanisms that underlie an ecologically relevant form of behavioral adaptation, we discuss our understanding of how parasitoid-selective signals modify the female germline physiology in *Drosophila*.

208A Traumatic brain injury coupled with tau expression promote *Drosophila* inter-male aggression

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Traumatic brain injury (TBI) is a disruption of normal brain function that results from mild to severe impacts to the head, and can affect memory, as well as behaviors such as anxiety, aggression or depression. In addition, TBI is correlated with the development and progression of neurodegenerative disease. One major hallmark of TBI is the presence of hyperphosphorylated tau in neurological tissues. In a healthy brain, tau is a microtubule-associated protein that controls microtubule-based processes, but upon hyperphosphorylation, tau forms aggregates called neurofibrillary tangles that contribute to neurodegeneration. The sequence of molecular events that causes tau to oligomerize into tangles, and how these tangles contribute to a wide range of brain dysfunctions and behavioral changes are open questions. Prior studies have found that pan-neuronal expression of human 2N4R tau in Drosophila induces age-related neurodegeneration in the absence

of neurofibrillary tangle formation. Recently, a high impact method to inflict TBI in flies has shown that flies display similar post-trauma responses to humans, including incapacitation, ataxia and neurodegeneration. We have utilized this method to subject young flies expressing 2N4R tau to closed head TBI in an effort to study the contribution of tau to neurodegeneration on the molecular, cellular and behavioral levels. At 24 hours after inflicting TBI on 7– 9 day old virgin males expressing the tau 2N4R isoform via the pan-neuronal driver, *elav*-Gal4, we recorded flies in groups containing two virgin males and one virgin female to observe courtship and aggression. We found that pan-neuronal expression of tau caused an increase in inter-male aggression in flies subjected to TBI, both in terms of the amount of time engaged in aggressive acts and in the total number of aggressive acts. These flies also exhibited a delay in copulation latency, likely due to increased time engaged in aggression. In order to identify which neuronal circuits are responsible for these altered behaviors, we have screened a variety of specific drivers for neuronal types known to be involved in mating and aggressive behaviors. In addition to behavioral studies, we are analyzing the localization and aggregation of tau using immunohistochemistry and biochemical techniques. Ultimately, we hope to elucidate how different molecular states of tau contribute to neurodegeneration and lead to alterations in brain function and behaviors.

209B Altered gravity reveals female preference for symmetric mates in *Drosophila*

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Investigating whether, why and how, females choose symmetric mates is important conceptually for sexual selection, but this has been rather challenging to empirically examine as individuals in nature are very symmetric. We found that in *Drosophila melanogaster*, perturbing gravitational cues during development introduces random noise that increases random variation in bilateral symmetry (Fluctuating asymmetry or FA). We exploit this tool to study female preference for symmetry, and show that indeed females preferentially choose symmetric males, when rival males court her in a mate-choice assay. We further demonstrate that this preference for symmetry is mediated through irregularities in auditory cues (courtship song), and reinforced by chemosensory cues (male pheromones), that females perceive during courtship. Our study clearly illustrates how symmetric males have an edge over rival males, in ways that can boost their reproductive fitness. These findings shed light on how developmentally instable individuals suffer discrimination through sexual selection, and provides a robust model system to test the developmental instability-sexual selection hypothesis.

210C Designing FISH Oligopaint probes for a highly repetitive Y chromosome

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One of the main obstacles in studies with the Y chromosome lies in its heterochromatic state, resulting in a lack of assembled scaffolds and contigs to the Y chromosome for most species. From the estimated 41 Mb of highly repetitive sequences for the *Drosophila melanogaster* Y chromosome, only 10% is assembled by the most recent official genome release (dm6). In parallel, the protocol for designing oligopaint probes does not include such repeat-rich sequences to avoid unwanted background signals in microscopy analysis. Therefore, the resulting amount of 1381 available probes for the *D. melanogaster* Y chromosome, at least ten times smaller than for its other chromosomes, is not sufficient for further cytogenetic applications. Our main goal was to design more probes for this chromosome in an efficient process. Here we combined two straightforwardly reproducible techniques, one to infer and improve the number of exclusive sequences for the Y chromosome (YGS), despite their repetitiveness, and another for designing probes by using a series of parameters that may influence the wet lab experiment of FISH Oligopaint hybridization (OligoMiner). We also added other filtering steps, such as a final alignment to female reads, minimizing the chance of off-target hybridization. By exploring different sets of parameters within these two techniques, we tested possible combinations that produce the most efficient set of probes. Our pipeline only requires a genome assembly, male and female short reads of the species of interest. For testing purposes, we run it with different *D. melanogaster* genome assemblies, including both the official release dm6 and a Sanger assembly, for instance. This resulted in an increase in the number of candidate probes for the Y chromosome to almost 13K, which is close to ten times greater than those currently available. Our results suggest that this methodology is reproducible for designing probes to other species' Y chromosomes, solving a problem for cytogeneticists and other scientists that are working with similar chromosomal structures by allowing them to visualize in more detail biological processes in which the Y chromosome is involved.

211A Progress towards functional understanding of the gene repertoire of *Drosophila*.

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Gene function studies show a knowledge bias towards 'already-known proteins'. The majority of human research is focused on just 10% of genes and, similarly, we estimate that over half of research in flies is focused on just 8% of genes. Thus, despite rich sequence knowledge, there is poor functional coverage of the coding genome. Two decades after the initial publication of the *Drosophila* genome sequence and subsequent increase in functional studies, we examine the extent of gene product characterization. Using the Gene Ontology (GO), we analyse the depth and coverage of GO annotation for the 13,969 protein coding genes in terms molecular mechanism, biological role and cellular location. In parallel with other well-studied eukaryotic genomes, we find that approximately a quarter of protein coding genes have no functional annotation. We further examine how much of this knowledge is supported by direct experimental investigation and how much is derived from sequence-based prediction and use this to indicate areas of research bias in flies.
There is an intrinsic assumption that genes of unknown function are not important, but this was challenged when the J. Craig Venter Institute published the JCV-syn3.0 minimal bacterial genome, in which genes of unknown function made up 17% of the minimal complement. Thus, there is value in providing lists of 'unknowns' to seed research and here we provide such a gene set for flies. Using the cohort of fly genes without GO annotation, we examine these for other functional information by the analyses of additional data curated by FlyBase to produce a set of 'unknown' and 'understudied' genes. Of these genes, 25% have predicted orthologs in humans and many have domain features that indicate that they are associated with cellular membranes.
We hope this list of 'unknowns' will stimulate future work so that a complete functional characterization of the protein coding genome can be achieved.

212B Building bioinformatics resources at DRSC: 2021 update

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The Drosophila RNAi Screening Center (DRSC) bioinformatics group has implemented a suite of informatics tools useful for Drosophila research (https://fgr.hms.harvard.edu/tools). These include gene-centric resources that facilitate ortholog mapping and mining of information about orthologs in common genetic model species; reagent-centric resources that help researchers identify RNAi and CRISPR sgRNA reagents or designs; and data-centric resources that facilitate visualization and mining of transcriptomics data, protein modification data, protein interactions, and other data types. Our established resources are actively updated and improved based on user feedback. For example, we have launched a new version of the Find CRISPRs sgRNA design tool in which we added domain annotations, SNP annotations, and new efficiency prediction scores based on a genome-scale CRISPR knockout screen data from Drosophila cell lines, as well as an enhanced user interface that combines a genome browser view with a table of all relevant sgRNA designs. In addition, we have launched new resources, including Paralog Explorer, which is useful for identifying paralogs and associated expression or interaction data; DRscDB, which is useful for mining and comparison of single-cell RNAseq data across species, and 'transcription factor to target gene' or TF2TG, which is useful to explore potential transcriptional regulatory networks based on motif binding predictions, chromatin immunoprecipitation (ChIP)-seq data, and high-throughput chromosome conformation capture (Hi-C) data. Altogether, our established and new resources support Drosophila research at all stages of the research pipeline, from development of candidate gene lists and identification of reagents to data analysis, visualization, and integration.

213C Modulation of V-ATPase subunits prevents tumor growth and restores autophagy in a *Drosophila* model of glioma

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Autophagy is a conserved catabolic process that maintains homeostasis by degradation of cellular components in lysosomes. How autophagy acts in tumorigenesis is currently debated and is the focus of an intense investigation. To understand *in vivo* the role of autophagy in the development of glioblastoma, an aggressive and incurable tumor of the central nervous system, we used *Drosophila* as a genetic model system. Most glioblastomas result from constitutive activation of the epidermal growth factor receptor (EGFR) and the phosphoinositide 3-kinase (PI3K) pathways, which are known regulators of cell growth. In the *Drosophila* model, the overexpression in glial cells of a constitutively active form of the *human EGFR* and *dp110* result in an increased size of the larval brain, due to an expansion of the glial compartment, marked by Repo. Interestingly, we found that downregulation of the components of the vacuolar-ATPase (V-ATPase), which is required for lysosomal acidification, reduces tumor growth and prevents glial expansion. Additionally, we observed that the hyperplastic tissue displays high levels of the autophagy adapter ref(2)P, the *Drosophila* p62/SQSTM1, and found that this is due to inhibited induction of autophagy. Consistent with this, we observed that upon downregulation of V-ATPase subunits, the tumoral accumulation of ref(2)P is

limited and autophagy flux restored. Collectively, our data suggest that V-ATPase inhibitors could represent new therapeutic targets for the treatment of glioblastoma.

214A Tep1 regulates Yki activity in Neural Stem Cells in Drosophila Glioma Model

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Glioblastoma Multiforme (GBM) is the most common form of malignant brain tumor with poor prognosis. Amplification of the Epidermal Growth Factor Receptor (EGFR), and mutations leading to activation of Phosphatidyl-Inositol-3 Kinase (PI3K) pathway are commonly associated with GBM. Using a previously published Drosophila glioma model generated by coactivation of PI3K and EGFR pathways [by downregulation of Pten
and overexpression of oncogenic Ras] in glial cells, we showed that the Drosophila Tep1 gene (ortholog of human CD109) regulates Yki (the Drosophila ortholog of human YAP/TAZ) via an evolutionarily conserved mechanism. Oncogenic signaling by the YAP/TAZ pathway occurs in cells that acquire CD109 expression in response to the inflammatory environment induced by radiation in clinically relevant models. Further,
the downregulation of Tep1 caused a reduction in Yki activity and reduced glioma growth. A key function of Yki in larval CNS is stem cell renewal and formation of neuroblasts. Other reports suggest different upstream regulators of Yki activity in the optic lobe versus the central brain regions of the larval CNS. We hypothesized that Tep1 interacts with the Hippo pathway effector Yki to regulate neuroblast numbers. We tested if Tep1 acts through Yki to affect glioma growth and if in normal cells Tep1 affects neuroblast number and proliferation. Our data suggest that Tep1 affects Yki mediated stem cell renewal in glioma, as reduction of Tep significantly decreases the number of neuroblasts in glioma. Thus, we identify Tep1-Yki interaction in the larval CNS that plays a key role in glioma growth and progression.

215B Using early pupal stages as a system to study circulatory tumor cell movement *in vivo*

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Drosophila melanogaster is increasingly used as a model organism with which to study various aspects of tumour cell dynamics. We describe a live imaging time-lapse technique to study the dynamic of attaching, moving and reattaching of tumour cells in developing pupal legs. We generated *RasV12* and *Scribble-1* clones using the MARCM system, previously shown to result in tumour production. Live imaging of these growing tumors between 10 to 12 hours after pupal formation revealed not only growing tumours attached to legs but also many tumour cells alone or in small clusters moving around the legs, while others seemed to de-attach and re-attach to new leg regions. From time-lapse studies we deduced that although the size and number of tumour cell relocation events varied their direction remained the same, from proximal to distal regions. These findings present a new *in vivo* system for investigating the conditions under which tumour cells move from potentially primary sites to secondary locations, as well as the mechanisms involved.

216C Tumors Overcome the Action of the Wasting Factor ImpL2 by Locally Elevating Wnt/Wingless

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Tumors often secrete wasting factors associated with atrophy and degeneration of host tissues. If tumors were to be affected by the wasting factors, mechanisms allowing tumors to evade the adverse effects of the wasting factors must exist and impairing such mechanisms may attenuate tumors. We use *Drosophila* midgut tumor models to show that tumors upregulate Wingless (Wg) to oppose the growth-impeding effects caused by the wasting factor, ImpL2 (Insulin-like growth factor binding protein (IGFBP)-related protein). Growth of Yorkie (Yki)-induced tumors is dependent on Wg while either elimination of *ImpL2* or elevation of Insulin/IGF signaling in tumors revokes this dependency. Notably, Wg augmentation could be a general mechanism for supporting the growth of tumors with elevated ImpL2 and exploited to attenuate muscle degeneration during wasting. Our study elucidates the mechanism by which tumors negate the action of ImpL2 to uphold their growth during cachexia-like wasting and implies that targeting the Wnt/Wg pathway might be an efficient treatment strategy for cancers with elevated IGFBPs.

217A Phosphorylation of a conserved amino acid in WASH has a critical function in tumor suppressive cell competition

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Cell communication between adjacent polarity-deficient cells and polarized epithelial cells employs the apical transmembrane

ligand Sas and its receptor tyrosine phosphatase Ptp10D to control tumor-suppressive cell competition. RNAi knock down of *Ptp10D* in polarity defective *scrib*$^{-/-}$ cells, elevates EGFR signaling, activates Yki and converts the *scrib* "loser cells" to "winners" via switching the JNK "pro-apoptotic" to a "pro-proliferative" outcome. In the trachea, the PTP/Btk29A/WASH circuit controls the initiation of luminal endocytosis and airway clearance by balancing endosomal F-actin assembly and cortical actin bundle distraction. *Ptp10D* antagonizes *Btk29A* and Btk29A promotes WASH activity by phosphorylating the conserved Y^{273}. A phosphomimetic WASH^{Y273D} construct induces endosomal actin patches via the Arp2/3 complex and promotes apical endocytosis.
Here, we show that the Ptp10D/Btk29A/WASH circuit functions in the *scrib*$^{-/-}$ model of cell competition. Inactivation of either Btk or WASH inhibits the aberrant growth of *scrib*$^{-/-}$Ptp10D^{RNAi}. WASH^{Y273D} overexpression upregulates JNK signaling, suppresses apoptosis, elevates EGFR signaling and activates Yki, thus inducing over-proliferation of *scrib*$^{-/-}$ cell clones. This indicates that activation of endocytic trafficking is sufficient to induce tumorous growth in *scrib*$^{-/-}$ cells. WASH promotes actin nucleation via Arp2/3 complex to control endosomal fission. We found that Arp2/3-RNAi suppressed the overgrowth of *scrib*$^{-/-}$Ptp10D^{RNAi} and *scrib*$^{-/-}$WASH^{Y273D}, suggesting that the endosomal function of WASH is the critical target of PTP during cell competition.
Ptp10D targeting to the interface of *Scrib*$^{-/-}$cells does not rely on Clathrin-mediated endocytosis but is maintained there by retromer and WASH function. Disruption of Ptp10D targeting to the clone interface via inactivation of components of the retromer complex (Vps29 and Vps26) leads to elevated EGFR signaling and overgrowth of *Scrib*$^{-/-}$ cells. However, in *scrib*$^{-/-}$ WASH^{Y273D} cells, PTP is targeted to the interphase but EGFR accumulates in the plasma membranes suggesting a bifurcation in the recycling routes of EGFR and PTP. Overall, our data argue that WASH phosphorylation controls the levels of endosomal EGFR signaling via Vps29 mediated recycling and maintenance of Ptp10D in the interface of *Scrib*$^{-/-}$ with wild-type cells.

218B Methionine restriction breaks obligatory coupling of cell proliferation and death by an oncogene Src in Drosophila

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Oncogenes often promote cell death as well as proliferation. How oncogenes drive these diametrically opposed phenomena remains to be solved. A key question is whether cell death occurs as a response to aberrant proliferation signals or through a proliferation-independent mechanism. Here, we reveal that Src, the first identified oncogene, simultaneously drives cell proliferation and death in an obligatorily coupled manner through parallel MAPK pathways. The two MAPK pathways diverge from a lynchpin protein Slpr. A MAPK p38 drives proliferation whereas another MAPK JNK drives apoptosis independently of proliferation signals. Src-p38-induced proliferation is regulated by methionine-mediated Tor signaling. Reduction of dietary methionine uncouples the obligatory coupling of cell proliferation and death, suppressing tumorigenesis and tumor-induced lethality. Our findings provide an insight into how cells evolved to have a fail-safe mechanism that thwarts tumorigenesis by the oncogene Src. We also exemplify a diet-based approach to circumvent oncogenesis by exploiting the fail-safe mechanism.

219C Measuring the Response of WRNexo-Deficient Drosophila to Metabolic Stress

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Aging is a natural and necessary component of the human body's lifespan. However, many diseases, including cancer, are directly proportional in severity relative to a person's age. Cells are continually exposed to both exogenous and endogenous stress, which can exacerbate the body's reaction throughout the aging process. Aging has been associated with the accumulation of reactive oxygen species (ROS) that are produced through deterioration of mitochondria during normal cellular metabolism. Oxidative stress occurs when an accumulation of ROS overwhelms antioxidant defenses, which can lead to cancer-causing alterations in DNA sequence and structure. Cells remedy ROS-induced DNA damage base excision repair (BER) pathway. The protein WRN plays an integral role in BER by stimulating enzymes that recognize and remove damaged bases. Furthermore, mutations in WRN in humans cause the disease Werner syndrome (WS), characterized by accelerated aging (e.g. shortened lifespans, a lack of growth spurt, and muscle atrophy). Additionally, many WS patients experience metabolic dysfunction and live with diseases such as insulin-resistant diabetes. We can model WS using *Drosophila*, which have an ortholog to human WRN, *WRNexo*. Previous research in our lab showed that like human WS patients, *WRNexo* mutants (*WRNexo_delta*) are sensitive to replication stress and show signs of accelerated aging, including low body fat in *WRNexo_delta* larvae. However, *WRNexo's* role in oxidative stress is still unknown.
We are investigating how WRN exonuclease responds to metabolic stress by testing sensitivity of *WRNexo_delta* to starvation and oxidative stress during different stages of the fly life cycle. Preliminary results show altered activity levels in *WRNexo_delta* females compared to age-matched *w*^{1118} controls, suggesting that *WRNexo* is required for normal metabolism. We did not

see a difference in activity between *WRNexo_delta* versus w^{1118} males, which suggests that *WRNexo*'s role in metabolism is sex-specific. Future work will investigate the impact of external oxidative stressors on *WRNexo_delta* activity. These findings will help further our knowledge of the connection between aging, DNA repair mechanisms, and metabolism.

220A The model for selective elimination of epithelial tumor clones by AdoR mutation

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Homozygosity for the *Adenosine receptor* (*AdoR*) mutation selectively and cell-autonomously eliminates tumor mosaic clones (reduces their frequency for more than an order of magnitude), with no or minor effects on non-tumor marker clones, like *yellow*[1], *singed*[3], or *UAS-GFP* (in a MARCM setting, used as a dominant repressible marker). The eliminated mosaic tumor clones are homozygous for mutations in the different cell proliferation regulating pathways, including the gene *warts*[X1] *(wts)* in the Salvador-Warts-Hippo (SWH) pathway, the gene *scribble*[1] *(scrib)* in the Scrib-Dlg-Lgl pathway, and *discs overgrown*[3] (*dco*) in the Double-Time/CKIε pathway. This may point to some universal mechanism of tumor clones elimination by mutant AdoR. The effect was independent of the applied mechanism of the heterozygosity loss (LOH): spontaneous, various chemical mutagen-induced, or hs>FLP - FRT dependent one.

Analysis of cell-autonomous clone-specific MARCM expression in the tumors of *UAS-AdoR*$^{+}$, *UAS-Ent2*$^{+}$ (the *Equilibrative nucleoside transporter 2*), or *UAS-AdoR*^{RNAi}, *UAS-Ent2*^{RNAi}, or combined *UAS-Ent2*$^{+}$ with mutant *AdoR* revealed that the increase of the tumor cell supply with *AdoR*$^{+}$ or *Ent2*$^{+}$ product benefits tumor frequency while depleting them of the functional AdoR or Ent2 dramatically manifold eliminates tumor frequency. The most deleterious was the combination of the *UAS-Ent2*$^{+}$ overexpression with the absence of the functional AdoR. After we reduced extracellular adenosine concentration in the posterior compartment of the wing by ectopically expressing there a secreted *UAS-ADGFA*$^{+}$ deaminase, frequency of the AdoR^{-} wts tumors in it was restored order of magnitude back to its AdoR-WT level, compared to the anterior compartment. In the series of tumor clones scrib — dco — scrib wts — wts, the intensity of their elimination by the mutant AdoR was increasing accordingly, following the increase in the competitiveness of the tumor clone.

According to our model, tumor clones grow destructively, either by invasiveness or cell competition, killing nearby WT cells and releasing adenosine and ATP (convertible to adenosine) from them, utilizing the excess extracellular adenosine for the growth of the tumor, and being protected from its uptake to a dangerous intracellular concentration by the functional AdoR receptor. With the lack of protective AdoR, extracellular adenosine released in their destructive growth kills them. On the other hand, non-tumor color-marked clones do not destruct the neighbors facing any increased concentration of adenosine around them. And thus non-tumor clones continue growing, tolerating the AdoR mutation devoiding them from protection by the functional AdoR receptor.

221B *CG33993*, a new SH2 domain containing protein acting as a negative feedback loop regulator of EGFR/Ras-driven tissue hyperplasia

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Activation of Ras signalling occurs in ~30% of human cancers. However, activated Ras alone is insufficient to produce malignancy. Thus, the discovery of cooperating cancer-relevant genes is imperative in our fight against cancer. In this work, we have identified a novel EGFR interacting protein, CG33993, which cooperates with oncogenic Ras in the Drosophila wing imaginal disc. We find that CG33993 is expressed at high levels in some regions of maximal activity of the pathway, such as the presumptive wing margin. In addition, while elimination of CG33993 function results in a mild reduction of EGFR activity and no effect in morphogenesis, it enhances the overgrowth driven by excessive activation of the EGFR/Ras pathway. Furthermore, CG33993 gain of function conditions leads to phenotypes associated with strong inhibition of EGFR signalling. Finally, CG33993 expression is upregulated upon constitutive activation of the EGFR/Ras pathway. Based on these results, we propose that CG33993 constitutes a component of a novel negative feedback loop that tunes down excessive EGFR activity, buffering cells against potentially harmful EGFR oncogenic signalling. The identification of more factors behaving like CG33993 can be very helpful in our search for targets for cancer therapy, as they hold the potential to prevent the growth of cancer cells while sparing normal tissues.

222C T-cell lymphoma: mimicking a commonly found PLC-γ activating mutation in Drosophila

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Activating mutations of PLC-γ are commonly found in human primary cutaneous T-cell lymphomas. Although many mutations of the Drosophila PLC-γ homolog, encoded by *small wing* (*sl*) have been described, all are either hypomorphic or loss of function alleles. To generate a reagent useful in understanding the role of increased PLC-γ signaling in human tumors we have initiated a search for activating *sl* alleles, using either EMS mutagenesis or CRISPR. To date our EMS screens have been unsuccessful, but one of two CRISPR-induced, single amino acid changes shows promise. Flies homozygous for an S349F edited X-chromosome (equivalent to a PLC-γ mutation that occurs repeatedly in T-cell lymphoma) show normal eye development but, in a background also carrying wild-type copies of *sl* on a genomic transgene, ommatidia in two independent lines show occasional extra outer photoreceptors adjacent to R3 and/or R4. Experiments are underway to determine the identity of these additional cells, as well as the pathway that is disturbed by the presence of the S349F mutant form.

223A The cell junction protein Polychaetoid/ZO-1 ensures junction robustness during morphogenetic movements of *Drosophila* embryogenesis

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Adherens junctions play a major role in assuring cell-cell adhesion, and though their linkage to the cytoskeleton, mediate dramatic shape changes and rearrangements during embryonic morphogenesis. To do this, junctions need to be strong yet also dynamic, to allow cell rearrangement without tissue rupture. We want to understand how junction-associated proteins, like the multi-domain scaffold Polychaetoid (Pyd; fly homolog of ZO-1), work together to allow junctions to react dynamically to cell shape change and force generation. We hypothesize that the junctional complex and its linkage to the cytoskeleton involves multivalent connections among different junction-associated proteins, creating a junctional substructure that mediates binding to the cytoskeleton. I am using structured illumination microscopy (SIM) to explore this. My preliminary data reveal a segmented pattern of the junction-associated proteins Canoe (Cno) and Pyd along the zonula adherens of cell-cell borders in the elongating germ band of *Drosophila* embryos, with alternating clusters with little overlap in some places. I am now broadening my analysis to include other junction-associated proteins like Bazooka (Par-3) and Armadillo (β-Catenin) and Non-muscle myosin II. Furthermore, I will analyze how these patterns change in *pyd* mutants. In parallel, I am exploring the functional role of Pyd as one player in this robust network. While Pyd is not absolutely essential for embryonic viability, mutants display defects in cell rearrangements during germband extension. I observe elongated cells, an elevated number of rosettes and small holes within the epithelium but these occur without major tissue rupture, suggesting defects in responding to elevated forces on cell-cell junctions. We are now expanding our analysis to include other players in this protein network, including Canoe/Afadin, the tricellular junction protein Sidekick, and Canoe's regulators Rap1 and the GEF Dizzy.

224B α-Catenin mechanosensing cooperates with Ajuba, Vinculin, and Canoe to support embryonic morphogenesis

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Cell-cell contacts known as adherens junctions (AJs) must form both robust yet dynamic interactions in order for cells to remain tightly bound to each other during morphogenesis. Adhesion and epithelial integrity depend on the physical link between F-actin and the AJs mediated by α-Catenin, a core component of the cadherin-catenin complex. α-Catenin is thought to act as a mechanosensor which translates actomyosin generated tension into downstream biochemical signals. Tension stretches the α-Catenin M-region into an open conformation, revealing cryptic binding sites for actin binding proteins such as Vinculin. This interaction is thought to reinforce adhesion in the face of increased actomyosin forces. However, Vinculin null mutants, along with mutants for several other α-Catenin binding partners such as α-Actinin and Ajuba, have only subtle or no developmental defects. It is therefore unclear what role α-Catenin mechanosensing plays *in vivo* in epithelial cell adhesion. Here, we have used live imaging of *Drosophila* mesoderm invagination and germband extension to investigate the role of α-Catenin mechanosensing in adhesion during morphogenesis. We found that the α-Catenin M-region is essential for epithelial integrity during morphogenesis. During gastrulation, the M-region counteracts the removal of E-cadherin from cell edges under higher tension, supporting adhesion. The M-region contains three domains, M1, M2, and M3. M2 is required for the enrichment of Ajuba to high tension edges and contributes the most to adhesion. Surprisingly, deletion of M2 and M3, which exposes the Vinculin binding M1 domain rescues α-Catenin knockdown embryos more poorly than removal of the entire M-region. The M1 domain appears to have an inhibitory, Vinculin independent effect on E-cadherin junctional stability, possibly through its negative effect on Ajuba recruitment. Furthermore, we found a striking genetic interaction between the M-region and the AJ protein Canoe, implying that Canoe and the M-region act in parallel. Our work provides *in vivo* evidence for the hypothesis that the α-Catenin M-region is required for mechanosensitive recruitment of binding partners Ajuba and

Vinculin, reinforcing AJs against forces produced by actomyosin contractility. This function cooperates with other cell adhesion factors, such as Canoe, in a redundant system to maintain epithelial integrity during morphogenesis.

225C The Endocycle in Development and Cancer

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The endocycle is a G/S cell cycle which skips mitosis to generate large, polyploid cells. Specific tissues of *Drosophila* and many other eukaryotes, including humans, utilize the endocycle as a normal growth program. In addition to these developmental endocycling cells (devECs), evidence shows that conditional signals can result in induced endocycling cells (iECs), such as during wound healing or in response to stress. We have previously shown that devECs and iECs repress the apoptotic response to DNA damage. Our lab has also shown that transient iECs in both flies and humans can return to an error-prone mitosis (RTM). These data are consistent with mounting evidence that cancer cells can transiently enter the endocycle in response to genotoxic chemotherapeutics and switch back to error-prone, polyploid mitoses. However, much remains unknown about both the switch into and out of the endocycle, and the interaction between pro-growth/oncogenic signals and transient endocycles. We will report our progress using *Drosophila* as an *in vivo* model system to decipher how endocycles contribute to normal and abnormal tissue growth. Using genetic and transcriptomic approaches, we have recently reported that downregulation of a Cyclin A/CDK1 – Myb-MuvB – Aurora B (AurB) pathway promotes the switch to endocycles in both devECs and iECs. We will present evidence that repression of this pathway is common to devECs and iECs in a variety of tissues and developmental contexts. Additionally, we will discuss our ongoing efforts to determine how iECs synergize with pro-growth pathways that are often mis-regulated in human cancers.

226A Structured illumination microscopy reveals the replication initiation dynamics in *Drosophila* polytene chromosomes

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At the level of extended chromatin domains, replication timing is accurately reproduced in subsequent cell generations. However, at the intra-domain level, the organization of replication is still unknown. At the end of 3d instar *Drosophila* polytene chromosomes contain 2x1024 carefully aligned DNA strands. Thus, they provide a unique opportunity to analyze the probabilistic nature of replication initiation occurring at the same time on the same chromosome. To analyze replication in polytene chromosomes at the ultrastructural level we applied spatial super-resolution structured illumination microscopy (3D-SIM). S phase induction allowed studying the dynamics of replication at the very beginning of the S phase. We confirmed the previous model, that the spatio-temporal organization of replication is in general closely related to the two main chromatin domain types present in polytene chromosomes: the rb-bands (the most compact bands) and the intervals in between of them (INTs, corresponding to alternating zones of loose bands and interbands). INTs correspond to early replication initiation zones. The activation of replication in these zones occurs stochastically in time and space, and each zone is characterized by its origin activation speed. There are sites of highly effective early replication initiation, but they may correspond to several potential replication origins.

227B The checkpoint gene Bub3 moonlights as a metabolic regulator

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Cells adapt their growth and metabolism to their needs and the extracellular signals they receive. For instance, nutrients or stress, are not only proliferative but also metabolic stimuli, suggesting that the cell cycle and the cellular metabolism communicate. To ensure that the cell cycle is a fine-tuned process, cell cycle regulators respond to extracellular signals, control the progression through the cell cycle, and ensure the different quality control checkpoints for cell duplication. One such checkpoint is the mitotic spindle assembly checkpoint (SAC). The SAC delays the metaphase to anaphase transition in the presence of unattached or unaligned chromosomes. Mitotic progression will only then be allowed when all the chromosomes are correctly attached to microtubules via protein structures called the kinetochores and correctly aligned at the metaphase plate. A key protein essential for the mitotic checkpoint signalling is Bub3. The Drosophila Bub3 has been previously shown to function as a tumour suppressor gene albeit by an unknown mechanism independent of its SAC function. Data will be presented on the transcriptional analysis of a Bub3 tumour and an enhancer/ suppressor screen in Drosophila, looking for the candidate genes that modulate the Bub3 tumour growth. Several candidates in the glycogen and lipid metabolism were found to enhance the hyperproliferation caused by the Bub3 knock-down. Further implications of these findings will be discussed.

228C The Krüppel-like-factor Cabut has cell cycle regulatory properties similar to E2F1

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Using a gain-of-function screen in *Drosophila* we identified the Krüppel-like factor Cabut (Cbt) as a positive regulator of cell cycle gene expression and cell proliferation. Enforced *cbt* expression is sufficient to induce an extra cell division in the differentiating fly wing or eye, and also promotes intestinal stem cell (ISC) divisions in the adult gut. Although inappropriate cell proliferation also results from forced expression of the *E2f1* transcription factor or its target, *Cyclin E*, Cbt does not increase E2F1 or Cyclin E activity. Instead, Cbt regulates a large set of E2F1 target genes independently of E2F1, and our data suggest Cbt acts via distinct binding sites in target gene promoters. Although Cbt was not required for cell proliferation during wing or eye development, Cbt is required for normal ISC divisions in the midgut, which expresses E2F1 at relatively low levels. The E2F1-like functions of Cbt identify a distinct mechanism for cell cycle regulation that may be important in certain normal cell cycles, or in cells that cycle inappropriately, such as cancer cells.

229A Deciphering Mechanisms of *Egfr*-Mediated Cell Survival in the *Drosophila* Eye Using Single-Cell Omics

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The *Drosophila* eye is one of the premier models for deciphering basic mechanisms of cellular differentiation and survival. Previously, the *Epidermal growth factor receptor* (*Egfr*) was shown to be required for the survival of photoreceptor cells in the *Drosophila* eye. However, the precise cellular events underlying cell survival are not known. One hypothesis is that the loss of *Egfr* leads to the inactivation of genes necessary for survival, which in turn triggers the activation of caspases. Alternatively, *Egfr* may actively inhibit pro-apoptotic genes and loss of *Egfr* leads to their activation, thereby causing cells to undergo apoptosis. To identify the molecular mechanisms necessary for *Egfr*-mediated cell survival, we performed single cell RNA sequencing (scRNA-seq) and single cell Assay for Transposase Accessible Chromatin sequencing (scATAC-seq) on wild-type *Drosophila* eye discs at the late larval stage. Our analyses show that all expected cell types are well-represented and form distinct clusters in our datasets. We also conducted chromatin immunoprecipitation sequencing (ChIP-seq) for the Egfr nuclear effector Pointed (Pnt), using late larval eye discs. By intersecting our scRNA-seq and Pnt-ChIP-seq data, we identified several putative direct Pnt target genes, which are expressed in differentiating photoreceptors (PRs) and may be required for their survival. *Diap1*, a gene that was previously shown to be a Pnt-target and required for the survival of PRs also appeared in our dataset. We are currently integrating our scATAC-seq and Pnt-ChIP seq data to identify Pnt-bound loci with scATAC-seq peaks overlapping with Pnt-ChIP-seq peaks in PR clusters. Furthermore, we will be performing motif analyses to identify known binding motifs that are enriched in these putative regulatory regions. We also performed scRNA-seq on eye discs expressing a dominant negative form of Egfr in subsets of PRs after the onset of differentiation. Intersection of lists of differentially expressed genes derived from a comparison of scRNA-seq data from wild-type and mutant tissue with scATAC-seq and Pnt ChIP-seq profiles is likely to reveal key mediators of the cell survival/death pathway regulated by *Egfr*. These data will provide one of the first genome-wide studies of *Egfr*-dependent cell survival in the *Drosophila* eye and are likely to have broad implications for fundamental mechanisms of cell survival in all higher eukaryotes.

230B Identifying the Secretome and Transmembrane Proteins of Non-Professional Phagocytes

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Apoptosis and the engulfment of dead cells are essential for 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. Defects in clearance coincide with persistent corpses, pro-inflammatory signal activity, a compromised immune system and inflammatory disease states. 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 untargeted exploratory approaches have not been used to determine the signaling molecules of follicle cells that promote cell clearance. There has been increasing evidence that suggests crosstalk between phagocytes and dying cells for death progression and we hypothesize that distinct signaling pathways are activated by phagocytes to promote programmed cell death. To identify secreted and transmembrane proteins that promote programmed cell death and phagocytosis in the ovary, we have taken an unbiased, high-throughput molecular approach using a horseradish peroxidase (HRP) catalyzed reaction that allows us to proximally biotinylate endogenous proteins within the endoplasmic reticulum (ER) *in vivo*. We generated flies carrying an HRP-KDEL construct under UAS control, and then expressed it in desired follicle cell populations. The KDEL sequence serves as

an ER retention signal to localize HRP to the ER. We found that HRP-KDEL localizes around the follicle cell nucleus, suggesting it is expressed appropriately in the ER. We confirmed that the HRP was functional and biotinylated proteins in vivo using immunocytochemistry and western blot. Biotinylated proteins have been purified from the ovary and analyzed via mass-spec to identify enriched secreted and transmembrane proteins. We have identified promising transmembrane and secreted protein candidates with potential functions including ion-binding and solute transport as well as structural proteins that reinforce the vitelline membrane, and analysis of their function in the ovary is underway. These novel drivers of cell death and phagocytosis will provide insight to the cell death and clearance field on universal crosstalk mechanisms to induce cell death.

231C Selective activation of a pro-death transcriptional program controls neuroblast apoptosis

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Stem cells can exhibit multiple behaviors *in vivo*, including cell division, differentiation and death. Our lab uses the *Drosophila* embryonic nervous system to study the regulation of programmed cell death, or apoptosis, in abdominal neuroblasts. The vast majority of abdominal neuroblasts are eliminated by a wave of cell death during embryogenesis, but a subset of neuroblasts are protected and do not undergo apoptosis. We sought to examine the regulation of the cell death pathway in these two populations of cells, doomed and surviving neuroblasts, to determine what level of the cell death pathway is blocked in surviving neuroblasts. Using genetic tools to track individual neuroblasts *in vivo* during embryonic development, we find that expression of the pro-death Hox gene, *abdominal-A (abd-A)*, is a distinguishing feature of doomed neuroblasts. We examine the regulatory landscape of *abd-A* and two other apoptotic regulators, *grainyhead (grh)* and *Dichaete (D)*, whose role in regulating the cell death pathway is described for the first time here. We find significant evidence of cross-regulation between *abd-A, grh* and *D,* and determine that *D* acts downstream of the pro-apoptotic activities of *abd-A* and *grh*. We also show that *D* acts through an intergenic cell death enhancer, enh1, that is required for neuroblast apoptosis. To determine the mechanism through which transcription factors such as *abd-A, grh* and *D* act to regulate cell death gene expression, we have begun to examine the chromatin architecture of the cell death locus. Our findings from this study have informed our understanding of how stem cells make and execute life or death decisions within the *Drosophila* nervous system.

232A Molecular Regulation of Clearance by Nonprofessional Phagocytes in the *Drosophila* Ovary

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Billions of cells die every day to maintain homeostasis within the human body. As these cells die, resultant cell corpses are quickly removed to avoid the release of pro-inflammatory contents into the extracellular milieu. Two cell classes are responsible for clearing away dying cells – professional and nonprofessional phagocytes.

For professional phagocytes, such as macrophages, clearance is their primary function. Nonprofessional phagocytes, however, have a separate primary function that is supplanted when they interact with cell corpses. Although nonprofessional phagocytes are less efficient at clearance than professionals, their large numbers and close proximity may make them more accessible to dying cells, especially in immunoprivileged tissues from which professionals are excluded. The aim of our study is to identify genes that are differentially regulated in nonprofessional phagocytes to increase their clearance capacity.

The ovary of *Drosophila melanogaster* is an ideal model for studying nonprofessional phagocytes. This immunoprivileged tissue contains three visually distinct cell types – a developing oocyte, 15 nurse cells, and follicle cells that transition from a protective epithelial layer to phagocytes in conjunction with death in the germline. During the final stages of oogenesis, nurse cells rapidly dump their contents into the developing oocyte and subsequently die. In anticipation of this process, a small subset of follicle cells transition into phagocytic stretch follicle cells to regulate nurse cell death and corpse removal. Our study seeks to characterize the molecular changes that regulate this transition.

We obtained a global view of these changes by analyzing the translatomes of epithelial follicle cells and phagocytic stretch follicle cells. Translatomes were isolated by immunoprecipitating GFP-tagged ribosomes and sequencing the bound mRNA. Computational analysis revealed 1128 statistically significant differentially expressed genes, with 13 demonstrating a greater than 5-fold expression change. Pathway analysis indicated expression changes in genes encoding trans-membrane and secreted proteins, including classes with functions regulating ion-channels, integrin binding, and kinase activity. Progress on the characterization of this transition will be presented.

233B Characterization of the nucleolar protein Noc1 in apoptosis induced proliferation (AiP)

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Ribosome biogenesis is a complex biological process that takes place in the nucleolus, an intranuclear compartment organized around rRNA genes. The synthesis of ribosomes requires the intervention of several proteins and ribonucleotide particles; among these, the members of the Noc family (Noc1, Noc2 and Noc3) are fundamental for the intranuclear transport of rRNAs during the maturation of the 60S ribosome subunit in yeast.
In Drosophila, the Noc genes are conserved at the nucleotide and aminoacidic levels, but their function has not yet been characterized. Here, we show that Noc proteins are necessary for Drosophila development. In addition, reduction of Noc1 in cells of the wing imaginal disc results in activation of the apoptotic pathway and to non-autonomous proliferation of nearby cells - a mechanism known as apoptosis induced proliferation (AiP), that involves also to the upregulation of p53. Further analysis is currently ongoing to better understand the role of Noc proteins in ribosomal biogenesis and in AiP, and to highlight possible novel mechanisms of p53 upregulation that would link Noc(s) function to ribosomopathies and tumors.

234C Sequencing analysis of the E.3.2 and N.1.2 mutants identified in a Flp/FRT screen for regulators of cell growth in *Drosophila melanogaster*

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A genetic screen was carried out in *Drosophila melanogaster* to identify novel regulators of cell growth utilizing a Flp/FRT based screen in the eye. This EMS based screen was conducted in an apoptotic null background and resulted in the identification of over one hundred mutants. The identified mutants are mapped by undergraduate students in genetics classrooms across the country as part of the Fly-CURE, a Course-based Undergraduate Research Experience (CURE).
Recent work by undergraduates at multiple institutions mapped two of the mutants, E.3.2 and N.1.2, to the *schnurri* (shn) and *hippo* (*hpo*) gene loci, respectively. In order to further characterize these mutants at the nucleotide level, primers flanking each of the coding exons were designed, exons were PCR amplified using a high-fidelity polymerase, and Sanger sequencing was performed. Sequencing reads for each exon were then compared to wild-type genetic sequence. Standard BLAST analysis of the *Shn*E.3.2 mutant sequence to the wild-type *schnurri* gene sequence identified silent point mutations in exons 3, 8 and 9, a conservative missense mutation in exon 9, and a nonconservative missense mutation in exon seven resulting in a change from an alanine to a threonine. Sequencing analysis of *hippo*N.1.2 exons were unable to produce reliable results due to poor sequencing reads, with the exception of a confirmed transition mutation in the 5' untranslated region of exon 1. Future work will involve confirmatory sequencing of the exon seven mutation identified in the *Shn*E.3.2 locus and additional PCR amplification and sequencing of the *hippoN.1.2* exons.

235A *Bfc*, a novel *Serpent* co-factor for the expression of *Croquemort*, regulates efferocytosis in *Drosophila melanogaster*

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Clearance of apoptotic cells (efferocytosis) is the process by which phagocytes recognize, engulf, and digest apoptotic cells during development. Impaired efferocytosis is associated with developmental defects and autoimmune disease.
In *Drosophila melanogaster,* apoptotic cell recognition requires phagocyte surface receptors, including the scavenge receptor CD36-related protein, Croquemort (Crq), which is up-regulated by the presence of apoptotic cells and by excessive levels of apoptosis. Here, we identified a novel gene, *Bfc* (Booster for Croquemort), that participates in efferocytosis by specifically regulating *crq* transcriptional expression. We found that Bfc interacts with the zinc-finger domain of the GATA transcription factor Serpent (Srp) to enhance its direct binding to the *crq* promoter, and thus genetically interacts with *Srp* in the regulation of *crq* expression and efferocytosis. *Bfc* therefore serves as a *Srp* co-factor to increase transcription of the *crq* engulfment receptor to boost macrophage efferocytosis activity in response to excessive apoptosis. This work contributes to clarifying how phagocytes integrate apoptotic cell signals to mediate efferocytosis.

236B What's size got to do with it? Understanding the role of sibling cell size asymmetry.

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An important and highly conserved developmental process used to create cellular diversity is asymmetric cell division (ACD). ACD can take many forms to give rise to two daughter cells with different cell fates. One form is manifested physically in which the sibling cells differ in size; here referred to as sibling cell size asymmetry. Defects in ACD may result in developmental defects, disease, or cancer. Currently, the physiological role of sibling cell size asymmetry remains elusive. A great model to study sibling cell size asymmetry is neural stem cells in the developing *Drosophila* brain. During mitosis, these neural stem

cells undergo shifts in Non-muscle Myosin II, hereafter Myosin, dynamics resulting in unequal sibling cell size. Our objective was to begin examining how permanent changes to sibling cell size asymmetry influence cell fate. To manipulate sibling cell size asymmetry in developing *Drosophila* brains, we permanently trapped active Myosin to the apical cortex using an anti-GFP nanobody (vhhGFP). In agreement with published data, we have shown that manipulating the localization of Myosin affects sibling cells size resulting in cases of inverted sibling cell size as well as symmetric sibling cells. We also examined if altering physical asymmetry, by establishing symmetry or inverting asymmetry, lead to differences in sibling cell fate indicated by alterations in cell cycle timing. Our results show sibling cell size is not correlated with cell cycle timing. Overall, these results underscore the importance of examining other factors which may be required for proper sibling cell size in *Drosophila* neural stem cells. These attributes may be conserved in a variety of organisms which undergo physical ACD. Future work will determine if changes to sibling cell size affect the fates of their progenitors which may result in abnormal neurogenesis.

237C The post-transcriptional regulations of centrosomal *plp* mRNA in *Drosophila*

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Centrosomes, functioning as microtubule organizing centers, are composed of a proteinaceous matrix of pericentriolar material (PCM) that surrounds a pair of centrioles. *Drosophila* Pericentrin (Pcnt)-like protein (PLP) is a key component of the centrosome that serves as a scaffold for PCM assembly. The disruption of *plp* in *Drosophila* results in embryonic lethality, while the deregulation of Pcnt in humans is associated with MOPD II and Trisomy 21.
We recently found *plp* mRNA localizes to *Drosophila* embryonic centrosomes. While RNA is known to associate with centrosomes in diverse cell types, the elements required for *plp* mRNA localization to centrosomes remain completely unknown. Additionally, how *plp* translation is regulated to accommodate rapid cell divisions during early embryogenesis is unclear. RNA localization coupled with translational control is a conserved mechanism that functions in diverse cellular processes. Control of mRNA localization and translation is mediated by RNA-binding proteins (RBPs). We find PLP protein expression is specifically promoted by an RNA-binding protein, Orb, during embryogenesis; moreover, *plp* mRNA interacts with Orb. Importantly, we find overexpression of full-length PLP can rescue cell division defects and eggs hatching failures caused by Orb depletion. We aim to uncover the mechanisms underlying embryonic *plp* mRNA localization and how Orb activates *plp* translation.

238A Spindle Orientation: What role does Dlg play?

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Orientated cell divisions are critical to animal development; they promote cell diversity and help organize and expand tissues. The orientation of the mitotic spindle at metaphase determines the placement of the newly formed daughter cell; which may impact tissue architecture formation in symmetric cell divisions and daughter cell fate in asymmetric divisions. Spindle orientation in animals typically relies on a highly conserved biological machine comprised of at least four proteins: in flies, these are called Partner of Inscuteable (Pins), Gαi, Mushroom body defective (Mud), and Dynein. According to the canonical model, Pins acts as a cortical anchor for Mud and Dynein, which exert a pulling force on astral microtubules that reels the spindle into alignment. Previous work across a variety of systems has identified the apical-basal polarity protein Discs large (Dlg) as an important co-factor for the spindle machinery, but its role remains unclear. Two models have been proposed, and both rely on the physical interaction between Dlg and phosphorylated Pins. In one model, Dlg helps recruit and/or anchor Pins to the mitotic cell cortex. In another, Dlg provides a link between Pins and an accessory microtubule capturing activity. I am using state of the art biochemical and imaging tools to distinguish between these models in Drosophila epithelial tissues. This work will serve to expand current understanding of this highly conserved and fundamental process.

239B Identification of a non-LTR retrotransposon at *Drosophila* centromeres.

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During cell division, chromosomes must be accurately segregated to ensure the faithful inheritance of genetic information. Errors in this process are detrimental for the viability of cells and organisms and are associated with cancer in humans. Chromosome segregation is mediated by a specific chromosomal locus, called the centromere, that acts as a platform for the formation of the kinetochore, which attaches to spindle microtubules that ultimately drive chromosomes to daughter cells. Centromeres are characterized by a specialized chromatin marked by the histone H3 variant CENP-A, which is both necessary and sufficient for centromere and kinetochore formation. Although the importance of CENP-A in specifying the centromere locus is well known, the function of the underlying DNA remains unclear. We recently identified the organization and linear

sequence composition of all endogenous *Drosophila melanogaster* centromeres. We discovered that the centromeres consist of islands of complex DNA enriched in retroelements flanked by arrays of simple satellites. While each centromere has a different assortment of several DNA elements, all of which are also present elsewhere in the genome, all centromeres contain copies of the non-long terminal repeat (non-LTR) retroelement *G2/Jockey-3*, which is also the most highly enriched repeat in CENP-A chromatin immunoprecipitations. While centromeric DNA satellites are highly divergent even between closely related species, we found that *G2/Jockey-3* is also enriched at the centromeres of three species in the *simulans* clade, suggesting that this element may play a conserved role at the centromere. Retroelements have been found at the centromeres across taxa, but the functional significance of this association is unclear. To test if retroelements are transcribed, we performed PRO-seq on embryos and found nascent transcripts mapping to *G2/Jockey-3.* To determine if at least some transcripts are associated with the centromere, we performed single-molecule RNA FISH and found that *G2/Jockey-3* transcripts localize to centromeres in 3rd instar larvae brains squashes in both *D. melanogaster* and *D. simulans*. Collectively, our data suggest a role for *G2/Jockey-3* transcription or *G2/Jockey*-3 non-coding RNAs in centromere function. Ongoing experiments using a short hairpin to knock-down *G2/Jockey-3* will determine if these transcripts are required for centromere function.

240C The nuclear envelope ESCRTs lagging chromosomes into daughter nuclei

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Cells dividing with late-segregating chromosomes must incorporate those lagging chromosomes into telophase daughter nuclei or risk excluding part of their genome as damage-prone micronuclei. While recent research has demonstrated how micronuclei formation can cause aneuploidy, the mechanisms that allow lagging chromosomes to rejoin daughter nuclei and maintain euploidy remain poorly understood. We address this knowledge gap by modeling lagging chromosome integration into telophase daughter nuclei in *Drosophila* neuroblast divisions. We find that lagging chromosomes join nuclei by passing through large channels in the nascent nuclear envelopes surrounding daughter nuclei. Failure to form these channels results in lagging chromosomes that are unable to enter daughter nuclei and instead form micronuclei. Additionally, effective passage of lagging chromosomes through nuclear envelope channels requires membrane fusion proteins, including components of the ESCRT-III complex, that are important for nuclear membrane sealing. Thus, nuclear envelope reassembly processes both inhibit and promote lagging chromosome integration into daughter nuclei. Taken together, our data suggest a model in which the modification of nuclear envelope reassembly on daughter nuclei enables lagging chromosome integration and maintenance of genome integrity.

241A Function of the RhoGEF Cysts in imaginal disc morphogenesis and regulation of tissue growth

Ming Cao[1], Milena Pellikka[1], Ulrich Tepass[1] 1) University of Toronto.

Rho GTPases such as Rho1, Rac and Cdc42 are small signaling G proteins that regulate a number of cellular processes including cytoskeletal organization, cell movements, cell polarity, and vesicle trafficking. The Drosophila genome encodes 9 Rho GTPases, 27 Rho guanine nucleotide-exchange factors (GEFs), and 22 Rho GTPase-activating proteins (GAPs), the latter two being the main regulators of Rho protein activities. To date, it remains largely unclear how GEFs and GAPs regulate the spatiotemporal activity of Rho proteins during development. Our lab has recently characterized the RhoGEF Cysts, the Drosophila homolog of four mammalian GEF proteins including p114RhoGEF. Cysts activates the Rho1 – Rok – myosin II pathway to enhance actomyosin at epithelial adherens junctions and is required to maintain epithelial integrity during embryonic development. Here, we address the role of Cysts in the development of the wing disc epithelium. We found that Cysts localizes to the apical adherens junctions in the wing disc. Loss of Cysts function led to excessive epithelial foldings, and partial loss of epithelial cell polarity when cell death was inhibited with P35 protein of baculoviruses. The loss of epithelial integrity was associated with an upregulation of Hippo/Yorkie pathway targets suggesting that Cysts is required for the normal regulation of tissue growth. The hinge region appeared to be particularly sensitive to the loss of Cysts. Furthermore, defects, including excessive tissue foldings and neoplastic tissue overgrowth were enhanced by the loss of both Cysts and RhoGEF2. Our data suggest that Cysts is an important regulator of junctional Rho1 activity in the wing disc, and that Cysts and RhoGEF2 cooperate in controlling Rho1 activity.

242B Analysis of the Drosophila Tribbles pseudokinase reveals functional features of a divergent C-terminal tail that mediates target degradation via the cullin-RING E3 ubiquitin ligase (CRL) complex

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The conserved pseudokinase Tribbles (Trbl) regulates cell growth, division and differentiation by binding and mediating the degradation of the key growth regulators, including Akt kinase, String-Cdc25 phosphatase and the transcription factor Slbo-C/

EBP. Protein sequence alignment of Trib family members reveals a conserved central kinase-like domain (KLD) required for target protein binding flanked by a variable N-lobe and an extended C-terminal tail, which includes a conserved COP1 binding site required for E3 ligase binding and proteosomal degradation via the cullin-RING E3 ubiquitin ligase (CRL) complex. Structural studies of human Trib1 reveal that in the absence of substrate the C-tail is bound to the N-lobe and upon degron binding, an allosteric change in shape occurs leading step-wise to a flipping of the conserved SLE loop in the KLD from an "in" to and "out" position, a twisting of an alpha helix in the N-lobe and the release of the C-tail. In most metazoan, the liberated C-tail binds COP1 E3 ligase but in Drosophila no good COP1 binding site can be detected in the fly C-tail, and what's more, no COP1 E3 ligase homolog can be detected in the Drosophila genome. To identify the functional subdomains in the Trbl KLD and C-tail, we conducted a site-directed mutagenesis and identified conserved motifs required for (1) protein instability, (2) degron binding strength, (3) subcellular distribution linked to lipid binding, and (3) substrate turnover. Our data suggest (1) that the SLE motif directs the subcellular distribution of Trbl to regulate compartment-specific interactions with targets and (2) a PEST motif in the C-tail conserved among Drosophilids abrogates protein turnover. Preliminary data shows that this PEST motif functionally interacts with Cul3, a core component of the CRL complex, suggesting that the divergent Trbl tail bypasses the COP1 E3 ligase adaptor by binding directly to a central subunit of the proteosomal complex.

243C Mapping of the O.2.2 mutation, a regulator of cell growth, in *Drosophila melanogaster*.

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The Fly-CURE is a Course-based Undergraduate Research Experience (CURE) that utilizes the model organism *Drosophila melanogaster* in undergraduate classrooms across the country. A Flp/FRT screen conducted in the eye and in a background of blocked apoptosis was employed to identify mutations that affect cell growth and developmental patterning. This work characterizes and maps the O.2.2 mutation, located on chromosome 2R. Genetic crosses conducted between ;FRT42D,Dark^{82},O.2.2/CyO mutant flies and ;FRT,Dark^{82};Ey>Flp flies resulted in pupal lethality due to the mosaic eye. Subsequent complementation mapping to over eighty chromosome 2R deficiency stocks narrowed down the location of the O.2.2 mutant to cytological bands 50C6-50C9. This narrowed down genetic region possesses nineteen protein coding genes, including eleven genes of unknown function. Future work will involve further complementation analysis of single gene mutants in the defined region to complete mapping of the O.2.2 mutant.

244A Spatiotemporal expression of regulatory kinases directs the transition from mitotic growth to cellular morphogenesis

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Embryogenesis depends on a tightly regulated balance between mitotic growth, differentiation, and morphogenesis. Understanding how the embryo uses a relatively small number of proteins to transition between growth and morphogenesis is a central question of developmental biology, but the mechanisms controlling mitosis and differentiation are considered to be fundamentally distinct. Here we show the mitotic kinase Polo, which regulates all steps of mitosis from mitotic entry to cytokinesis, also directs cellular morphogenesis after cell cycle exit. In mitotic cells, Aurora B (AurB) activates Polo to control a cytoskeletal regulatory module that directs cytokinesis. In the post-mitotic mesoderm of late stage embryos, the control of Polo activation transitions to the uncharacterized kinase Back Seat Driver (Bsd), where Bsd activates Polo to direct muscle morphogenesis. The transition between mitotic growth and morphogenesis is accomplished through the spatiotemporal transcriptional regulation of AurB and Bsd. The functions of Bsd and Polo are conserved, arguing that regulating kinase expression to activate cytoskeletal regulatory modules is a widely used strategy to direct cellular morphogenesis.

245B Regulation and Effects of Ferritin on ovarian cell migration in Drosophila Melanogaster

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Collective cell migration is important in the study of disease progression, wound healing, and animal development. In Drosophila melanogaster there are clusters of cells in the female ovaries, known as border cell clusters, that migrate collectively at a certain time in development. Border cell clusters are useful in studying collective cell migration because we can use genetic and imaging tools to investigate cell migration at a molecular level.
Our lab and others have found that Janus Kinase/Signal Transducer and Activator of Transcription (Jak-STAT) and steroid hormone signaling pathways are important for the regulation of border cell migration. Flies have a single steroid hormone called ecdysone which binds to its receptor - a heterodimer of Ecdysone receptor (EcR) and Ultraspiracle. Based on previous microarray analysis, we hypothesize that the ferritin complex plays an important role downstream of the ecdysone signaling

pathway. Ferritin is an iron storage molecular complex made of heavy and light chains that are encoded by 3 different genes in Drosophila: fer1hch, fer2lch, and fer3hch. Our preliminary results indicate that varying the expression of ferritin genes affects the migration of border cell clusters. We have found that Fer1HCH protein is enriched in follicle cells.
In this project, we are working to characterize the normal spatiotemporal expression of the ferritin genes, and predict how the ferritin gene cluster in border cells is genetically regulated. To do this, we are mining genomic data for transcription factor binding site information and regulatory sequence information from genetic databases. We identified regulatory sites for STAT and EcR amongst other transcription factors within the gene regions as well as upstream and downstream of the gene regions. Moving forward, we would like to characterize the roles of each ferritin subunit and how it affects collective cell migration and oogenesis. This work may suggest important, conserved roles for ferritin in migratory cell types across different species.

246C Influence of Ecdysone Receptor Signaling on Border Cell Migration Kinetics

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Migratory cells play a significant role in spatiotemporally-regulated physiological processes such as normal embryonic development and wound healing. Dysregulation of these cells has severe implications in birth defects and diseases such as cancer. Border cell migration, during *Drosophila* oogenesis, serves as a useful model to study collective cell migration *in vivo.* Derived from the anterior epithelium of the egg chamber, the border cell cluster is composed of 6-8 cells that migrate together toward the posteriorly-located oocyte. The directional translocation of the cluster, in response to chemoattractants, requires the extension of protrusions that adhere to other cells in the migratory path. The availability of sophisticated genetic tools and imaging techniques enables the manipulation and *in vivo* observation of border cell migration. Developmental timing in flies is largely controlled by the sole steroid hormone ecdysone, which binds to a heterodimeric receptor complex and regulates transcription of its targets. Our work focuses on understanding how signaling by the ecdysone receptor (EcR), a conserved nuclear hormone receptor, affects border cell migration specifically in the egg chamber. Downregulation of ecdysone signaling results in delayed migration likely as a result of delayed detachment from the epithelium, or slower migration, or both. Using a combination of live imaging video analysis and immunofluorescence characterization, we are studying protrusion activity and variations in the levels and localization of E-cadherin, an adhesion molecule, in border cells with reduced ecdysone signaling. Interestingly, an EcR transcriptional reporter is specifically activated in the cluster despite ubiquitous expression and apparent availability of the signalling components throughout the egg chamber. To this effect, we are studying a possible role of the recently discovered Ecdysone Importer in the cluster. Additionally, we are assaying alternative reasons for the specificity of the response such as potential interactions between chromatin regulators and EcR. Lastly, we are assaying binding sites of EcR within migration-specific target gene loci using bioinformatic and chromatin immunoprecipitation analyses. Elucidating the role of EcR in cell migration kinetics will be a useful guide to improve our understanding of nuclear hormone receptors in development and disease.

247A ArfGAP1 regulates collective cell migration *in vivo*.

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Collective cell migration plays important roles in morphogenesis and embryonic development and is a main feature of the formation of metastases in several cancers. Unlike single cell migration, collective cell migration is characterized by cell-cell adhesion and cell-cell communication. We have previously demonstrated that vesicular trafficking plays a critical role in cell guidance and cell-cell communication during collective cell migration. A recent screen aimed at identifying new regulators of vesicular trafficking involved in collective migration identified ArfGAP1 as a regulator of border cell migration in the *Drosophila* ovary.
Between stages 9 and 10 of the *Drosophila* egg chamber development, the so-called border cells form a cluster that is attracted by the oocyte through the secretion of ligands to receptor tyrosine kinases (RTKs). We found that the depletion of ArfGAP1 specifically in border cells induces migration defects. Clusters devoid of ArfGAP1 are able to initiate their migration, but loose directionality. We are currently investigating the cause of this phenotype by analyzing various determinants of border cell migration. Our initial results indicate that the depletion of ArfGAP1 reduces the level of active RTKs at the plasma membrane, as they accumulate in a late endosomal compartment. Our results suggest that ArfGAP1 is necessary for the proper sorting of active RTKs in endosome and possibly their recycling to the plasma membrane.
We identified ArfGAP1 as a new regulator of border cell migration that might acting through vesicular trafficking to maintain of active receptor tyrosine kinases at the plasma membrane. This study could potentially reveal a new important mechanism in collective cell migration, and by extent in cancer dissemination.

248B Analysis of the *Drosophila* border cell gene expresson profile reveals stage-specific changes during migration

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Collective cell migration underlies many processes essential for normal development, adult homeostasis, and metastasis in cancer. *Drosophila* border cells, which migrate during oogenesis, are a genetically tractable model in which to study genetic and biochemical drivers behind collective cell migration. The 6 to 10 border cells are specified from the follicle cell epithelium that surrounds each egg chamber. During stage 9 of oogenesis, this cluster of cells must navigate the surrounding tissue environment – at first, moving rapidly by extending protrusions from a leading cell then finally, tumbling more slowly before stopping at the oocyte by stage 10. Previous research identified key signaling pathways, such as ecdysone steroid hormone, JAK/STAT, EGFR/PVR, Jun Kinase, and Hippo/Warts, that together control the initiation and directed migration of border cells. Detailed characterization of the many expected downstream targets of these pathways that promote continued border cell migration through the tissue is still lacking. The extent to which select genes change expression during migration and whether this could facilitate continued collective cell migration is also poorly understood. To address these questions, we performed RNA-sequencing of border cells during three stages of development. We labeled border cells with *slbo*-mCD8:GFP and hand-selected egg chambers in which border cells were at the pre-, mid-, or late-stages of migration. The mCD8:GFP tag allowed us to isolate dissociated GFP-expressing border cells using magnetic beads. These cells were further processed for RNA-sequencing. Bioinformatics analyses confirmed the presence of known border cell-enriched genes in our sorted cell population. We report here that subsets of genes either increase or decrease their expression levels from early to late stages of migration. Among genes in these groups, genes involved in cell adhesion, regulation of transcription, and regulation of the cytoskeleton are overrepresented. Thus, we have identified a small number of differentially expressed genes and are currently testing their functions in border cells. Bioinformatics analyses and functional testing of these genes will allows us to further investigate downstream pathway components that help drive the migration of border cells during all stages of their development.

249C A role for the conserved PP1 regulatory subunit PPP1R15 in collective cell migration in vivo

Yujun Chen[1], Jocelyn McDonald[1] 1) Kansas State University.

Collective cell migration is essential in many developmental and pathological processes. Despite extensive research conducted in a variety of models, the mechanisms underlying collective cell migration especially within intact tissues and organs are still poorly understood. Drosophila border cells travel as a cohesive cluster during oogenesis and provide an excellent genetic model for identifying how cell collectives move inside tissues. While roles for several serine-threonine kinases and their target substrates have been established, much less is known about serine-threonine phosphatases. Previously we found that Protein Phosphatase 1 (Pp1) catalytic subunits are critical molecular regulators of border cell collective versus single cell behaviors. Here we identify a critical role for the conserved Pp1 regulatory subunit PPP1R15 (also known as GADD34) in border cell migration. We found that overexpression of PPP1R15 causes border cells to round up and completely dissociate from the cluster during migration. These defects are fully rescued by overexpressing Pp1 catalytic subunits (Pp1c). Moreover, overexpressing PPP1R15 mutants that abolish interaction with Pp1c, suppresses the migration and cluster-dissociation phenotypes. These data together suggest that PPP1R15 functions through Pp1c. RNAi-mediated knockdown of PPP1R15 prevents border cell delamination from the follicular epithelium and increases the number of border cells in the cluster. The major known role for PPP1R15 is to dephosphorylate eIF2alpha during stress-induced protein synthesis. We find that PPP1R15-RNAi promotes eIF2alpha phosphorylation and elevates expression of the known downstream transcription factor, ATF4, in border cells. We are currently constructing phosphomimetic and dominant-negative eIF2alpha to further study the function of this pathway in border cell migration. Overall, our work identifies PPP1R15 as a key molecular regulator of collective cell behaviors in addition to its classical function in endoplasmic reticulum (ER) stress. Given the high degree of conservation of Pp1 complexes, our work provides new perspective on the function of PPP1R15 during development.

250A Septins are Required for Collective Cell Migration in the *Drosophila* Ovary

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Collective cell migration is essential to processes such as embryo development, wound healing, and cancer metastasis. The border cell cluster in the *Drosophila* ovary models collective cell migration, with the advantages of genetic analysis and live imaging. Septins, considered the fourth element of the cytoskeleton, have diverse functions such as curving membranes and bending and bundling actin. Through microarray analysis, my lab showed that septins are enriched in migratory cells in the *Drosophila* ovary. The role of septins in border cell migration is unknown. I have found that septins are required for border cell migration. Septins are expressed throughout migration and this expression is specifically required in the migratory border

cells. I also found that septins show co-dependence of expression in the posterior follicle cells of the *Drosophila* egg chamber and in border cell clones, suggesting interactions between septins. These findings will help us understand the role of septins in collective cell migration.

251B The role of the Rho family of GTPases in germ cell migration

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The formation of the gonads in *Drosophila melanogaster* requires the migration of primordial germ cells (PGCs) from the posterior end of the embryo towards the somatic gonadal precursor cells (SGPs) that reside in the mesoderm. Migration of primordial germ cells during this process involves cell polarization, protrusion formation, and cell adhesion. The Rho family of GTPases is known to be involved in these cellular processes. Specifically, Rho1 is thought to be involved in the assembly of focal adhesion processes that allow for communication between the cell and the extracellular environment. Rho1 is normally expressed towards the rear of migrating cells. On the other hand, Cdc42 promotes the formation of filopodia, which are involved with cell motility and is expressed at the leading edge of migrating cells. It has previously been shown that Rho1 and Cdc42 are required for proper germ cell migration. In this study, we are examining the roles of Rho1 and Cdc42 in more detail, examining more closely the stage of germ cell migration affected and looking at cell shape changes and actin localization changes in both loss- and gain-of-function mutants of Rho1 and Cdc42. Through this study, we hope to gain more insight into the role of these GTPases in gonad morphogenesis.

252C A targeted RNAi screen identifies conserved cell junction genes required for collective cell migration and invasion

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Tumor cells are highly plastic and can use different modes of invasion, including as single cells or as cell collectives. Collectively migrating cells are frequently observed in different cancer types and are efficient in invading deeper into tissues. Collectives have a higher survival probability and can also influence treatment strategies by enhancing tumor resistance to known therapies. Very little is known about the molecular mechanisms employed by collectives to promote tumor progression. The relatively simple border cells from the *Drosophila* ovary are an excellent genetic model for studying conserved regulators of collective cell migration and invasion including cancer. 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 at the Cleveland clinic, we demonstrated that patient-derived glioblastoma cancer stem cells can undergo collective cell invasion. Cell-cell adhesions signal the cells in the cluster to stay together in this highly dynamic process of collective cell migration. Whether collective cell invasion requires specific cell junction proteins is still unknown. To understand better how adhesions and adhesion regulatory proteins contribute to invasion, we performed an RNAi screen in the border cell system. We targeted conserved cell adhesion genes that were associated with glioblastoma patient survival. Through this screen, we identified five candidate genes – Dachsous, Symplekin, Lachesin, Roughest, Wnt4 – whose downregulation caused consistent migration defects of the border cells in the ovary. We are now testing these genes in the wing epithelia and the larval tumor model. We anticipate that expressing RNAi of these genes in these models will enhance the invasive phenotype of these tumors. This approach will provide insight into conserved mechanisms that drive collective cancer cell invasion.

253A RhoGEF2 regulation of amoeboid migration

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Individual cells can utilize rapid, contraction dependent amoeboid propulsion to traverse complex, varied environments during development, homeostasis, and disease. Amoeboid migration is largely dependent upon posterior contractility driven by local activation of the small Rho GTPase, RhoA, and downstream activation of myosin II, but the upstream mechanisms governing this polarized activity remain unclear. Using the developmental migration of primordial germ cells (PGCs), we show here that a RhoA specific guanine nucleotide exchange factor, RhoGEF2, is necessary for efficient amoeboid migration. RhoGEF2 is the sole Drosophila RGS homology (RH) containing RhoGEF and is an EB1 dependent microtubule plus-end tracking protein which is canonically activated and displaced from EB1 downstream of G protein coupled receptors (GPCRs) by Gα12/13 proteins. RhoGEF2 is enriched at the rear of PGCs during all stages of migration and remains polarized but misoriented in PGCs lacking Tre1, a GPCR we have recently shown to regulate the directed migration dependent dispersal of PGC clusters. Maternal depletion of RhoGEF2 delays the separation of PGC clusters, while PGC specific overexpression of constitutively active Gα12/13

augments RhoA and myosin II polarity and enhances migration speed, but ultimately impairs pathfinding. Interestingly, Gα12/13 is not required for PGC migration and we provide evidence for alternative phosphorylation dependent disassociation of RhoGEF2 from EB1 at serine residues proximal to an EB1 binding SxIP motif in cell culture. Lastly, RhoGEF2 polarity is not dependent on its PDZ domain and requires conserved hydrophobic residues in its PH domain, purported to be involved in binding active RhoA and forming a positive feedback loop. We propose a model where positive feedback driven RhoGEF2 polarization generates effective amoeboid PGC migration.

254B Defining the role of individual prostaglandins in collective cell migration

Samuel Mellentine[1], Emily Fox[1], Maureen Lamb[1], Tina Tootle[1] 1) University of Iowa Carver College of Medicine.

Collective cell migration – the coordinated movement of associated cells – is important for both normal development and tumor invasion. While prostaglandins (PGs), short-range lipid signals, regulate cell migration, and are up regulated in many cancers, their mechanisms of action are poorly understood in collective migration. To address this, we use *Drosophila* border cell migration during Stage 9 of oogenesis. During border cell migration a cluster of border cells delaminates from the outer epithelium of the follicle and migrate collectively and invasively between the nurse cells. Pxt is the Drosophila cyclooxygenase-like enzyme responsible for all PG synthesis. We find that PGs produced in the somatic cells regulate on-time border cell migration, whereas PGs produced in the germline cells regulates cluster cohesion. One downstream effector of the PGs during border cell migration is Fascin, as heterozygosity for both a *fascin* and *pxt* mutant results in delayed border cell migration and elongated border cells clusters.
Contributing to these phenotypes is altered integrin localization, as integrin-based adhesions are essential for correctly timed border cell migration. Integrins are enriched on the border cell membranes, and this enrichment is lost when PGs or Fascin are lost, and when PGs and Fascin are co-reduced. Intriguingly, we find that PGs produced in the border cells control integrin-based adhesions and cluster elongation. These data lead to the model that PG signaling in the border cells regulates Fascin to control on-time migration, whereas PG signaling in the nurse cells regulates Fascin to control integrin-based adhesions and cluster elongation. As Pxt is upstream of all PG synthesis, we are currently determining which PG acts in the border cells, versus the nurse cells. Based on the literature, we hypothesize that PGE_2 is produced and acts in the border cells to promote on time border cell migration. Such studies are critical to understanding the specific functions of individual PG signaling cascades in the migratory cells versus their substrate, and thus increase our understanding of both developmental cell migrations and pathological migrations including cancer metastasis.

255C The Role of Rap1 in Building the Migratory Border Cell Cluster

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Collective cell migration is critical during normal tissue development and maintenance, but also serves as a mode of tumor invasion. During late oogenesis, border cells migrate as a cohesive multicellular group. Four to six anterior follicle cells are specified to become border cells and surround the two central polar cells. This border cell cluster then migrates through a densely packed tissue microenvironment composed of the large germline-derived nurse cells. Proper cluster assembly is critical to this process, as optimal cluster size results in better migration efficiency. Previous studies have shown that larger clusters have a migration advantage up to a general size threshold. The small GTPase Rap1 has been identified as critical during border cell migration, contributing to both actin-rich protrusions and maintenance of the cell-cell adhesion marker E-cadherin. Here we report that Rap1 regulates border cell cluster assembly prior to their collective migration. Dominant negative- (DN-) Rap1, or Rap1 RNAi, expression results in smaller clusters that are significantly reduced in cell number. In contrast, constitutively active Rap1 generates larger clusters. Although JAK/STAT is required for border cell cluster specification and size, we find that a reporter for JAK/STAT signaling is unaltered by DN-Rap1 compared to controls. We have preliminary evidence that expression of baculoviral p35, a known inhibitor of apoptosis, abrogates the cluster assembly defects caused by dominant negative Rap1, thus restoring proper cluster size. These results suggest an important role for Rap1 in cell survival coupled to cluster assembly that may be independent of canonical cell fate specification mechanisms. Our current focus is on disentangling the relationships between cell health, fate specification, cell recruitment to the cluster, and how cluster size relates to migration efficiency in this context.

256A Cactin is required for collective border cell migration in *Drosophila*

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Collective cell migration is a fundamental process in normal development and cancer. Our lab uses border cells in *Drosophila* ovary as the model system to study how collect cell migration is regulated. Border cells are specified in late stage

8 egg chambers from the follicle cell population, then they round up and delaminate from the anterior side of the egg chamber and migrate toward the oocyte in stage 9. In a RNAi screen, we identify a gene named cactin. In cactin-RNAi egg chambers, border cells fail to detach from the anterior side. Our data suggest cactin regulates aPKC localization and Rac1 activity, thus is required for border cells organization and delamination.

257B

Regulation of Misshapen during Border Cell Migration *Gabriela Molinari Roberto*[1], Gregory Emery[1] 1) Institute for Research in Immunology and Cancer, UdeM, Montreal, CA.

Introduction: Collective cell migration is an important process during development, wound repair and metastasis. Border cells migration in *Drosophila* is a powerful model to study collective cell migration. During this process, the kinase Misshapen (Msn) coordinates protrusion formation with contractile forces through the group of cells. However, it is still unknown how Msn is regulated in border cells. Msn has two described domains: a kinase domain (KD) and a CNH domain. Msn's KD can be phosphorylated by the kinase Tao, while CNH domains are described to be bind to small GTPases. Therefore, our hypothesis is that Tao and small GTPases regulate Msn activity during border cell migration.
Methods and Results: First, we characterized Tao's function in border cells by RNAi depletion through the UAS/Gal4 system. Border cell clusters depleted for Tao or Msn do not detach and present disoriented ectopic protrusions. Interestingly, Msn phosphomimetic mutant restores the migration defect of Tao depleted clusters. This result indicates that the main function of Tao in border cells is to phosphorylate and activate Msn. Using a knock-in GFP-Msn fly, we observed an enrichment of Msn at the periphery of the clusters. Then, we tested whether Rho1, Cdc42 and Rap1 GTPase regulates Msn's localization. We discovered that constitutively active Rho1, as well as dominant negative Rho1, Rap1 and Cdc42 decrease significantly Msn enrichment at the cluster periphery. As a next step, we will perform Co-IP to determine if Msn interacts with its potential regulators.
Conclusion and Relevance: Our first data indicate that Tao regulates border cell migration as an upstream activator of Msn, while Rho1, Cdc42 and Rap1 activity regulates Msn's localization in border cell clusters. Data from our lab indicates that the human Msn ortholog, MAP4K4, regulates the collective cell migration of epidermoid carcinoma cells. Therefore, on the long run, we want to determine if the regulatory mechanisms of Msn are conserved in mammals.

258C Investigating Targets of Jak-STAT and Ecdysone Signaling in Border Cell Migration by Binding Motif Analysis

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Within *Drosophila melanogaster* egg chambers, a group of 6-8 cells called a border cell cluster engages in anterior to posterior migration towards the oocyte. This process requires strict spatiotemporal coordination through Janus Kinase and Signal Transducer and Activator of Transcription (Jak-STAT) and Ecdysone (steroid hormone) signaling. Both Jak-STAT and ecdysone signaling are extensive pathways that are important in many different contexts of development and disease. Genetic reduction of either pathway yields incomplete border cell migration. Ecdysone Receptor (EcR) bound to Ultraspiracle (USP) and activated STAT are conserved transcription factors that bind directly to DNA to regulate transcription of their targets. Thus, we are interested in understanding which targets of these pathways are specific to migration and if the two pathways co-regulate any of those targets. Putative target genes, including *mind bomb 2 (mib2)*, have been identified by our lab and others previously. The target gene loci contain binding motifs to which transcription factors can bind to modulate transcription. Additionally, these signaling pathways and their targets are also subject to regulation by microRNAs (miRs). We used FIMO on the Galaxy Project public server and TargetScanFly v.7 to predict binding sites of transcription factors, such as STAT92E or EcR, and miRs. DNA sequences of putative target gene regions were obtained from FlyBase and transcription factor binding sites were sourced from JASPAR. *mib2*, as expected, had binding sites for both EcR and STAT92E, indicating a potential for co-regulation of the gene during border cell migration. Additionally, data analysis has shown apparent conserved binding sites for miRs. High probability sites will be validated in vivo and/or with chromatin immunoprecipitation. Notably, our work suggests that *miR-8* contributes to border cell regulation through a role in a STAT signaling feedback loop. This research could help identify key migration specific targets in disease including metastatic cancers.

259A Investigating functions of axonemal dynein assembly factors in *Drosophila* motile ciliated cells

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The important role of motile cilia in human health and disease has come to the forefront of research in recent years. Defective ciliary motility can result in Primary Ciliary Dyskinesia (PCD), an autosomal-recessive disorder characterised by chronic respiratory infections, *situs inversus* and infertility. PCD is a genetically heterogeneous disease, with 50 causative genes having

already been identified, indicating the complexity of the motile cilia assembly process.

The dynein motor complexes that drive ciliary motility require cytoplasmic pre-assembly. This process involves dedicated 'axonemal dynein assembly factors' (DNAAFs), a group of genes that have been associated with PCD. Some of these DNAAFs have been proposed to form novel 'R2TP-like' complexes, by analogy with the well-known multifunctional HSP90-associated co-chaperone, R2TP. How DNAAFs interact with molecular chaperones to control dynein assembly and the involvement of R2TP-like complexes in dynein assembly remains unclear.

Drosophila provides a convenient model for the identification and functional analysis of DNAAFs: the machinery of ciliary motility is highly conserved and is required for only two types of motile ciliated cells: spermatozoa and the auditory/ proprioceptive chordotonal sensory neurons.

Here we investigate the function two DNAAFs: DYX1C1 and PIH1D3, which are hypothesised to form part of an R2TP-like complex. Through the use of co-immunoprecipitation, we have shown the interaction between these proteins to be conserved in *Drosophila*. We show also that the expression of both proteins is exclusive to the cytoplasm of motile ciliated cells.

We show that CRISPR/Cas9-generated null mutants flies have typical 'fly PCD' phenotypes using male fertility and behavioural assays. With mass spectrometry of testes, we have found depletions in both outer and inner dynein arm proteins. This result is supported through examining the dynein structures using both confocal and electron microscopy. Future work includes further examining the interactions of these DNAAFs through techniques including GFP-trap, to elucidate the precise role of this R2TP-like complex during the dynein assembly process.

260B Fat body HIF-1α promotes organismal hypoxia tolerance by restraining excess cytokine and immune signaling

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Our cells and organs need oxygen from the air we breathe to survive and function. However, in certain disorders such as stroke, heart disease and cancer, tissues are often deprived of oxygen. This lack of oxygen, known as hypoxia, leads to the tissue damage and deregulation that are characteristic of these diseases. Understanding how tissues and organs respond to low oxygen is therefore an important question in biological research. In their natural ecology, Drosophila larvae have evolved to grow on rotting, fermenting food rich in microorganisms – an environment characterized by low ambient oxygen. Hence, they provide a good genetic model system to study how hypoxia influences physiology and development. As in other model organisms, the conserved hypoxia-inducible factor-1 alpha (HIF-1α) transcription factor – known as sima in Drosophila – is needed for cellular and organismal adaptive responses to hypoxia in flies. Here we describe a key role for fat body sima in regulating whole body hypoxia tolerance. We find that knockdown of sima in the adult fat body leads to reduced survival when animals are exposed to hypoxia. In addition, we find that fat body sima knockdown animals show defective regulation of lipid and glycogen stores upon hypoxia exposure. Similarly, we see that sima knockdown in the larval fat body leads to reduced larval hypoxia survival, while fat body overexpression of sima in larvae mimics the effects of hypoxia, leading to reduced growth, delayed development and decreased viability. Our data suggest that these non-autonomous effects of fat body sima on whole-body hypoxia tolerance may be mediated via modulation of immune and cytokine signaling. We see that adult hypoxia exposure leads to a rapid increase in expression of the innate immune transcription factor relish/ NF-κB and its target genes. We also see that hypoxia induces a rapid and pronounce induction of the secreted cytokines Upd2 and Upd3, and an increase in their JAK/STAT-dependent target genes. Interestingly, these inductions in both relish and Upd/JAK/STAT signaling are markedly exacerbated in both ubiquitous and fat body-specific sima knockdown animals. Moreover, we find that when we mimic these effects of sima knockdown by just overexpressing Upd3 in adult animals, we see a decrease in hypoxia survival. These data suggest that upon exposure to hypoxia, fat body sima is needed to promote hypoxia tolerance, in part by restraining excess immune and cytokine signaling.

261C Using natural genetic variation in *Drosophila* to characterize the underlying mechanisms of stress preconditioning

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Cellular stresses rarely occur in isolation, but often in the context of other stresses. It is not well understood how stress responses are altered in the presence of other stresses. The accumulation of misfolded proteins results in a number of cellular stresses, including endoplasmic reticulum (ER) stress. The ER is a large, eukaryotic organelle responsible for protein folding, lipid synthesis, and calcium storage. When misfolded proteins accumulate in the ER, they initiate a large transcriptional

response that upregulates genes whose protein products help to refold or degrade misfolded proteins or initiate apoptosis. An effective ER stress response is critical for healthy development and aging. While the ER stress response is one of the most characterized stress responses, much of what is known comes from studies that examine it in isolation. In reality, ER stress occurs in a complex milieu of ongoing and previous stresses that likely impact how the cell responds. **This project aims to use natural genetic variation to understand the impact of stress preconditioning on the ER stress response, whereby conditioning with low levels of stress alters the ability to withstand subsequent ER stress.** To do this, we measured survival in approximately 200 strains of Drosophila Genetic Reference Panel (DGRP), a collection of wild-derived strains, which were subjected to heat shock (or no heat shock control), allowed to recover for 4 hours, and then placed on tunicamycin to induce ER stress. Different genetic backgrounds led to a striking range in phenotypic responses to preconditioning, ranging from dying 2 times slower to 4.5 times faster on ER stress after heat shock as compared to no heat shock preconditioning. This phenotypic range allowed us to run a GWAS to investigate underlying pathways and modifiers of preconditioning. GWAS revealed a potential role of histone methylation and the JAK-STAT cascade in preconditioning. RNAseq was also performed immediately after heat shock for the 5 DGRP strains that had the most beneficial and most detrimental effects of preconditioning. Long non-coding RNAs and components of the innate immune system were most differentially expressed, suggesting a role for both pathways in stress preconditioning. Understanding how previous stress events and genetic background influence the ER stress response will provide insight in a more physiological context and have important implications for how we approach therapeutic development.

262A The Transcription Factor Xrp1 is required for PERK-mediated Unfolded Protein Response in *Drosophila*

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Eukaryotic cells adapt to misfolded protein overload in the endoplasmic reticulum (ER) by inducing a stress-responsive gene expression program known as the Unfolded Protein Response (UPR). One of the ER transmembrane stress sensors, PERK, induces UPR target gene expression most notably through the transcription factor ATF4. Possible PERK effectors other than ATF4 remain poorly understood. Here, we report that Xrp1 is a mediator of an ATF4-independent PERK signaling response. Through cell type-specific gene expression profiling in *Drosophila* eye discs, we found that delta-family glutathione-S-transferases (*gstD*) are among the transcripts most prominently induced by the UPR-activating transgene $Rh1^{G69D}$. Consistently, $Rh1^{G69D}$ expression induced a *gstD-GFP* reporter, and such response required *Perk.* Surprisingly, such *gstD-GFP* induction by $Rh\text{-}1^{G69D}$ occurred independently *ATF4*, but rather required another bZIP transcription factor, *Xrp1*. *Perk* overexpression was sufficient to induce *gstD-GFP*, which was abolished in *Xrp1* mutants. Mutation of putative Xrp1 binding sites in the *gstD-GFP* reporter also abolished reporter induction in response to $Rh\text{-}1^{G69D}$. These results indicate that the most prominently induced UPR target in the *Drosophila* eye disc is regulated by a previously unrecognized UPR axis consisting of PERK and Xrp1.

263B Defining the role of nuclear actin in the nucleolar stress response

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Cells respond to stressors in a variety of ways: overcoming the stress and regaining homeostasis, inducing cell death, or adopting an altered state to adapt to the stressor. One key meditator of the cellular stress response is the nucleolus. During periods of cellular stress, the nucleolus undergoes rapid structural reorganization which inhibits normal cellular functions such as ribosome biogenesis and cell growth. While these data suggest a direct relationship between the structure and the function of the nucleolus, the mechanisms controlling nucleolar structure and functions, and their relationship to the cellular stress response remains unclear. The process of Drosophila oogenesis is ideal for uncovering the mechanisms regulating the nucleolar stress response. If cellular stress is sensed, oogenesis arrests and cellular stress response pathways are activated. If homeostasis fails to be achieved, follicles undergo cell death, leading to a sharp decrease in female fertility. Using *Drosophila* oogenesis, we have identified nuclear actin as a critical regulator of the nucleolus. Our findings show nuclear actin is present in three distinct pools during oogenesis that all localize to the nucleolus, suggesting nuclear actin has conserved, but poorly understood functions in the nucleolus. In order to explore the relationship between the nucleolus and nuclear actin, we will perturb nuclear actin and assessed nucleolar functions under normal and stress conditions. These studies will advance the understanding of how nuclear actin contributes to the functions of the nucleolus to respond to cellular stress and maintain cellular homeostasis.

264C The H3.3K27M oncohistone antagonizes reprogramming in Drosophila

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Development proceeds by the activation of genes by transcription factors and the inactivation of others by chromatin-mediated gene silencing. In some cases development can be reversed or redirected by mis-expression of master regulator transcription factors. This must involve the activation of previously silenced genes, and such developmental aberrations are thought to underlie a variety of cancers. Here, we express the wing-specific Vestigial master regulator to reprogram the developing eye, and test the role of silencing in reprogramming using an H3.3K27M oncohistone mutation that dominantly inhibits histone H3K27 trimethylation. We find that expression of the oncohistone blocks eye-to-wing reprogramming. CUT&Tag chromatin profiling of mutant tissues shows that H3K27me3 domains are globally reduced with oncohistone expression, suggesting that previous developmental programs must be silenced for effective transformation. Strikingly, mis-expressed Vg and H3.3K27M synergize to stimulate overgrowth of eye tissue, a phenotype that resembles that of mutations in Polycomb Repressive Complex 1 components. Our results imply that growth dysregulation can result from the simple combination of crippled silencing and transcription factor mis-expression, an effect that may explain the origins of oncohistone-bearing cancers.

265A Silencing and position-effect variegation in a dual-reporter transposition mutagenesis screen

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Highly repetitive DNA sequences are often associated with invasive or pathogenic DNA, and can also be found in gene-poor regions of eukaryotic genomes like centromeres and telomeres. Genomes often defensively package repeats in dense, transcriptionally refractory, stably heritable heterochromatin which plays a crucial role in the regulation of gene expression through transcriptional silencing. How these sequences trigger the establishment and maintenance of heterochromatin remains poorly understood, but repeats appear to serve as a signal of foreignness to host genomes and can activate the siRNA, miRNA, or piRNA silencing pathways. Silencing of repetitive DNA is implicated in the formation and function of centromeres and telomeres, in defense against transposons and viruses, and in human trinucleotide repeat expansion diseases like Friedrichs Ataxia and Fragile X syndrome. To investigate the mechanism and regulation of repeat-triggered silencing, undergraduate students participating in a classroom research experience (CURE) carried out a transposition mutagenesis to investigate the effects of large tracts of repetitive DNA on gene expression and chromatin state. Students mobilized a p-element containing a 256-copy tandem array of the E. coli lac operator (LacO), flanked by white and yellow reporter genes. After setting up mobilization crosses, students screened F2 progeny for changes in eye or body color indicating expression of either or both reporter genes (287/14137 or 2% of all male F2 screened). Of transgene-expressing flies, wild type expression of yellow and white comprised 82% of all mutants and indicate presumed euchromatic insertions resulting in high reporter gene expression. The white gene alone was silenced in 11% of identified mutants; the yellow gene alone was silenced in 4%, demonstrating a surprising decoupling of reporter gene silencing. 3% of mutants showed variegation of the white gene and full expression of the yellow gene, but the converse was not observed, nor was variegation of body color. Molecular mapping of insertion sites with inverse PCR suggest that LacO repeat insertion can trigger silencing even in gene-rich euchromatic locations.

266B Towards understanding the cytological and biochemical bases of symbiont-induced cytoplasmic incompatibility

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Wolbachia are gram-negative, obligate intracellular bacteria that primarily reside in and modify the reproductive tissues of their arthropod hosts. *Wolbachia*-mediated cytoplasmic incompatibility (CI) has attracted considerable attention due to its utility in suppressing viral transmission from mosquitoes to humans and its consequences on arthropod speciation. CI occurs in a cross between an infected male and an uninfected female or an infected female with a different *Wolbachia* strain, resulting in catastrophic mitotic defects and embryonic lethality. CI is caused by two genes, *cifA* and *cifB,* located in prophage WO of *Wolbachia*. Dual transgenic expression of *cifA* and *cifB* in the male germline of uninfected *D. melanogaster* recapitulates CI and individual expression of *cifA* in the female germline rescues it. However, the contributions of the *cif* gene products to the cytological and biochemical bases of CI remain largely unknown. Using a TUNEL-based sperm genomic integrity assay, we found that transgenic *cif* expression causes sperm DNA fragmentation. We also detected various types of morphological

defects in the sperm head structure that impede the normal development of sperms, leading to a reduced number of sperm bundles as well as mature sperm count in *Wolbachia*-infected as well as dual *cif*-expressing flies. Our data demonstrate previously unknown roles for the *cif* genes and corroborate the Host Modification Model of CI whereby *cifs* alter sperm nuclei development and morphology to impair spermatogenesis and ultimately cause embryonic death.

267C Dissecting the mechanism of X recognition in *Drosophila melanogaster*

Reem Makki[1], Victoria Meller[1] 1) Wayne State University.

Drosophila melanogaster males have one X chromosome while females have two. This creates an imbalance in X to autosome gene dosage between the sexes. To maintain an appropriate ratio of X gene expression, male fruit flies increase transcription from X-linked genes approximately two-fold. This involves the Male Specific Lethal (MSL) complex, which is recruited to transcribed X-linked genes and modifies chromatin to increase expression. The MSL complex is thought to assemble at Chromatin Entry Sites (CES), which contain the MSL recognition Element (MRE), and then spread *in cis* to active genes in the vicinity. Since MRE sequences are present on autosomes, it is unclear how the MSL complex recognizes X-chromatin. We found that repetitive sequences that are strikingly enriched in X euchromatin, the 1.688^{x} satellite repeats, promote recruitment of the MSL complex to nearby genes. The 1.688^{x} repeats do not contain MREs. Unlike CES, the 1.688^{x} repeats do not recruit the MSL complex directly. To facilitate dissection of the mechanism of recruitment, we developed a dual-luciferase reporter to measure the ability of DNA sequences to recruit compensation. Firefly luciferase is placed on an autosome near a transgene containing the recruiting element (1.688^{x} repeats or CES). The Firefly transgene also contains sequences that limit luciferase expression to males. Recombination mediated by Hybrid Element Insertion (HEI) was used to introduce a closely linked landing site. A distant *Renilla* luciferase far from recruiting elements is used for normalization. The constructed Firefly luciferase reporter is functional and luciferase expression was limited to males, the sex in which dosage compensation occurs. Immunostaining showed the MSL complex is recruited to the autosomal site and luciferase expression increases in the presence of recruiting elements. Measuring recruitment on an autosome avoids the confounding effects of redundant X-linked elements. This reporter will be used in an RNAi screen to identify genes necessary for recruitment of compensation by the 1.688^{x} repeats or CES. We expect to be able to differentiate the recruiting pathways used by different types of DNA sequences. As 1.688^{x} sequences appear to recruit through a different mechanism than the CES, we expect to identify genes not previously known to participate in X recognition.

268A Mapping R-loops during Drosophila development reveals new paradigms for R-loop formation and genome stability

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R-loops are a three-stranded nucleic acid structure formed when RNA re-anneals to the template strand during transcription. They are involved in the establishment of histone modifications, gene regulation and modulation of the chromatin environment. R-loops are often described as a 'double-edged sword' because while they play essential roles in chromatin regulation, conflicts between R-loops and replicating DNA can lead to DNA damage. Whether R-loop abundance and location is actively regulated during animal development is unknown.

Drosophila melanogaster provides a unparalled tool to determine if developmental programs regulate R-loop levels. During early embryogenesis, cell cycles occur very rapidly and largely in the absence of transcription. At a precise time in development, the maternal to zygotic transition (MZT), zygotic transcription is activated but S phase still occurs quickly (~1 hour). Furthermore, replication-transcription conflicts likely activate the S phase checkpoint at the MZT. Therefore, R-loops have the potential to drive genome instability at the MZT. Additionally, the majority of histone modifications are absent pre-MZT. Therefore, the Drosophila embryogenesis system provides a powerful system to understand the causes and consequences of R-loop formation during development.

We have mapped R-loops genome-wide at nuclear cleavage cycle 14, the first major cycle of the MZT, and later in embryonic development using ssDRIP-seq (single-strand DNA:RNA immunoprecipitation sequencing). Our analysis shows that R-loop levels and positions change across development, with a significant decrease in R-loop abundance late in embryogenesis. Furthermore, while R loops may impact genome stability at the MZT, they are not the sole driver of genome instability at this time point. We have been able to combine our ssDRIP-seq data sets with time-matched modENCODE datasets to identify chromatin associated factors that correlate with R loop formation during Drosphila development. Our analysis has revealed new features of R-loop biology. For example, unlike in other species, R-loops form at genes that lack polyadenylation. Additionally, satellite regions form extensive R-loops specifically at the MZT that aren't found in later embryogenesis or in S2

cells. We hypothesize that this may be due to the permissive chromatin environment of the early embryo. Our findings reveal new characteristics of R-loops specific to development and embryogenesis.

269B New insights into the mechanism of transcriptional silencing by piRNAs

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Histone modifications are essential for the control of gene expression and chromosome architecture in eukaryotes. Regulated recruitment of histone- and DNA- modifying 'writer' complexes to specific genomic regions is crucial for precise control of gene activity and formation of eu- and heterochromatin. Small non-coding RNAs and Argonaute proteins play an important role in chromatin regulation in various eukaryotic systems. In animals, the best established small RNA pathway involved in chromatin regulation is the piRNA pathway. A dedicated clade of Argonaute proteins, Piwi-s, and piwi-interacting RNAs (piRNAs) are associated with the formation of repressive chromatin and transcriptional silencing of transposable elements. The current model suggests that the piRNA/Piwi complex recognizes complementary nascent RNAs and induces the deposition of silencing chromatin marks such as histone 3 lysine 9 trimethylation (H3K9me3) and in some species, DNA methylation, at target genomic loci. However, the molecular mechanism that connects the Piwi/piRNA target recognition complex to the silencing effector is not well understood.

We found a novel and essential role of the SUMO pathway in piRNA-mediated transcriptional repression that links the piRNA-guided target recognition complex to the silencing effector in Drosophila. Our work showed that the SUMO E3 ligase Su(var)2-10 interacts with and acts downstream of the piRNA/Piwi complex to induce SUMOylation of proteins at target chromatin, which in turn leads to SUMO-dependent recruitment of the SetDB1/Wde histone methyltransferase that deposits the repressive H3K9me3 mark and transcriptional silencing. Parallels with the mechanism of transposon silencing by DNA-binding silencing factors in other contexts suggest that the piRNA pathway has co-opted a conserved mechanism of SUMO-dependent recruitment of the chromatin modifier complex to confer repression of genomic parasites. Furthermore, we found that SUMOylation is widely required for H3K9me3 deposition across the genome of female germ cells, and SUMO-dependent H3K9me3 silences dozens of host genes independently of piRNAs. Together, our findings identify the SUMO pathway as a key novel player in chromatin regulation by small non-coding RNAs and beyond in animals.

270C Repeat-binding proteins participate in *D. melanogaster* dosage compensation

Maggie Sneideman[1], Victoria Meller[1] 1) Wayne State University.

Drosophila melanogaster males carry one X and one Y chromosome, but females have two X chromosomes. To equalize expression of the X-linked genes between the sexes, males increase the transcription of X-linked genes approximately two-fold. This is mediated by the Male-Specific Lethal (MSL) complex, which modifies chromatin to elevate expression. The MSL complex first binds at Chromatin Entry Sites (CES) on the X, and then spreads into nearby active genes. CES contain a short motif that is bound by the adapter protein CLAMP. CLAMP is necessary to attract the MSL complex to the CES. However, these motifs are also found on the autosomes. These autosomal sites bind CLAMP but fail to recruit the MSL complex. Another factor must therefore distinguish the X from the autosomes. Observations in several species suggest that higher order chromosome organization, or location in the nucleus, contributes to X chromosome dosage compensation. Non-histone proteins that regulate these processes might thus participate in compensation. The fly X chromosome is strikingly enriched for chromosome-specific repeats. One of these, the AT-rich 1.688^{x} satellite repeats, plays a role in identifying the X. To determine if proteins that anchor chromatin, bind AT-rich regions or satellite DNA participate in X recognition I tested candidate genes for X-specific or male-specific effects by RNAi knock down. I also performed knock down in a background compromised for dosage compensation to detect genetic interactions with compensation. No knock downs produced a male-specific phenotype. As a proof of concept I found that knock down of the nucleosome remodeler *Imitation switch* (*ISWI*), a treatment that disrupts the compensated male X chromosome, displayed a strong genetic interaction. Knock down of the satellite-binding protein *D1* and *Scaffold Attachment Factor A* (*SAF-A*) also enhanced lethality in males with compromised dosage compensation. In the future I will use ChIP to determine if these proteins localize to the 1.688^{x} repeats and use a dual luciferase reporter assay (see poster by Makki and Meller) to determine if these genes participate in recruitment of compensation by the 1.688^{x} repeats or CES. I expect these studies to contribute to our understanding of how flies identify the X chromosome for compensation.

271A Replication in Context: Understanding replication through higher ordered chromatin

Reyhaneh Tirgar[1], DongSheng Han[1] 1) Vanderbilt University.

Thousands of molecular machines termed 'replisomes' work in an orchestrated manner to replicate our genome every cell division. Remarkably,1.8-meters of DNA (>6 billion base pairs) is packaged inside a nucleus with a diameter of 5-10µm. To make this 20,000-fold compaction possible, DNA is tightly wrapped around nucleosomes and packaged with additional proteins into chromatin. To access DNA every time the genome is duplicated, the replication machinery must traverse through a heterogenous chromatin environment throughout the genome. One potentially challenging form of chromatin that replisomes encounter is the highly condensed and repetitive heterochromatin, which can constitute ~20% of our genomes. Heterochromatin coalesces into a nuclear structure known as the chromocenter and does not visibly decondense during replication. Further, heterochromatin domains are phase separated which deem them less accessible to the replication machinery. The molecular mechanisms and protein factors utilized to stably progress through densely packaged chromatin is largely unknown. The most promising data has linked chromatin remodelers Mi-2 and ISWI in overcoming heterochromatin structure during S phase. Preliminary data from our lab has shown ISWI and Mi-2 are associated with the replisome. Given these observations, I hypothesize that 1) heterochromatin is a barrier to replisome progression and 2) chromatin remodelers facilitate replisome progression through heterochromatin to overcome its compact structure . To study my hypothesis, I am exploiting the powerful developmental system of Drosophila melanogaster embryogenesis. During the first 13 cell cycles of the early embryo, replication proceeds in the absence of visible heterochromatin. Then, at the mid-blastula transition, replication slows, and heterochromatin is formed. I am establishing FIBER-FISH, a single-molecule DNA combing technique combined with fluorescence in-situ hybridization to allow for direct measurement of replisome progression within heterochromatin regions. Using FIBER-FISH, I will measure rates of replication fork progression in the presence and absence of heterochromatin to learn how replisomes stably replicate through heterochromatin. By depleting chromatin remodelers during embryogenesis, this system will allow me to determine if chromatin remodelers have a differential impact on fork progression within heterochromatin and euchromatin.

272B The expanded *Drosophila ananassae* Muller F element: expanded genes, pseudogenes, a *mael* retrogene, and NUMTs

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The *Drosophila melanogaster* Muller F Element is primarily heterochromatic, exhibiting properties such as enrichment of HP1a and H3K9me3. However, the distal 1.4 Mb of the F element contains ~80 protein-coding genes with a range of expression levels similar to genes in euchromatin. In *D. ananassae*, the F element is greatly expanded (>19.7Mb) compared to *D. melanogaster* (5.2Mb) due to an increase in the density of transposons and simple repeats. To assess the impact of this chromosome expansion on gene characteristics, Genomics Education Partnership (GEP) students constructed gene models for the *D. ananassae* F element (based on multiple lines of computational and experimental evidence), which we reconciled using *Apollo*. Comparison of the *D. ananassae* reconciled gene models with their *D. melanogaster* orthologs showed that F element expansion is associated with an increase in coding span sizes due to an increase in intron sizes. The median coding span increased 6.3-fold in *D. ananassae* (42,301bp vs. 6,736bp). The median intron size increased 6.2-fold (1,774bp vs. 287bp), while the median coding exon size remained similar. Further, the F element harbors unusual features. We identified a novel retrogene derived from the processed mRNA of the Muller D element gene *mael*, as well as a higher frequency of horizontal gene transfer events and pseudogenes. There are at least three nuclear mitochondrial DNA (NUMT) segments in the nuclear genome, two of which reside in the F element. One of these F element insertions contained the entire mitochondrial genome. Furthermore, half of the 30 regions within the nuclear genome that have similarity to mitochondrial proteins reside on the F element. Comparison of the *D. ananassae* orthologs of *vir, CG14395,* and *msk* genes against the *D. ananassae* genome identified at least ten instances where pseudogenized versions of these genes were found within 1kb of each other in a specific order (*vir – CG14395 – msk*). Five of these pseudogene clusters are found on the *D. ananassae* F element, indicating enrichment. Multiple sequence alignments using Clustal Omega revealed regions of greater conservation within the individual pseudogenes, suggesting that these pseudogene clusters may contain regions that are under selection. Continuing analyses will provide more insights into the factors that contribute to chromosome expansion and its impact on gene characteristics. Supported by Washington University in St. Louis and by NIH R25GM130517.

273C Genome annotation of Drosophila ananassae dot chromosome contig 33

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Introduction: We participated in the Genomics Education Partnership (GEP), a project to train undergraduate students to produce high quality annotations for the dot chromosomes/Muller F and D elements of different Drosophila species.

Chromatin structure is important to determine expression of genes. Heterochromatin is condensed chromatin structure inactive for transcription, while euchromatin has loose chromatin structure and is active for transcription. The dot chromosomes across Drosophila species exhibit many heterochromatic features, but can have also have properties of euchromatin. A comparative genomic analysis of the Muller elements can identify key features of heterochromatic domains across Drosophila. These findings are important to provide insights into the nature of heterochromatin formation and over 40 million years of divergence time for evolutionary patterns.
Methods: We annotated contig 33 of the dot chromosome March 2020 assembly for the *Drosophila ananassae* Muller D element. We used the GEP UCSC Genome Browser mirror to analyze tracks that could provide supporting evidence for determining exact exon/intron boundaries and transcriptional activity for all the genes on contig 33. We used the BLAST algorithm to identify conserved regions between *D. ananassae* and *D. melanogaster*. We used a GEP Gene Model Checker to generate gene models for the identified annotations.
Results: We identified three genes, Abp1 (Actin binding protein-1), Tgi (Tondu-domain-containing Growth Inhibitor), and stv (Starvin) in contig 33 and identified one, two, and seven isoforms for Abp1, Tgi, and stv, respectively. We annotated the exact locations of exon/intron boundaries by searching for compatible splice donor and acceptor sites by visual inspection of the genome browser and checked for their accuracy using the GEP gene model checker. The gene model generated a dot plot used to compare the *D. ananassae* sequence to *D. melanogaster*'s. Our proposed gene model dot plots indicate that the *D. ananassae* Abp1 gene has considerably more similarity than the Tgi gene isoforms do to *D. melanogaster*.
Conclusion: Our findings will be submitted and compared/reconciled with other students' to compile the data and produce a final annotated sequence verified by the Genomics Education Partnership to provide student-curated and refined annotations of all Drosophila genomes.

274A Comprehensive phylogenomic of *Lactobacillus plantarum* reveals genome signals involved in host-bacteria interactions

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The microbiome of *Drosophila melanogaster* is emerging as a valuable model to study gut colonization, due to the host genetics, simple and cultural microbiome, and robust and quantitative colonization phenotypes, particularly by *Lactobacillus plantarum*, a Gram-positive bacteria intimately associated with the foregut tissue of *D. melanogaster*. Multiple molecular mechanisms are involved in this interaction, including fly production and maintenance of the colonization habitat in the foregut and *L. plantarum*'s ability to recognize, bind to, and proliferate in the foregut habitat.

Little is known about the mechanisms of specificity for bacteria commensal species at the genomic level, such as genes, operons, and genomic islands. Phylogenomic methods are a new approach that takes advantage of evolutionary paths to predict the function of novel genes and proteins. This method is based on the reconstruction of ancestral genome states, providing a historical context for the study of co-adaptations between organisms.

In this study, we sequenced 13 *L. plantarum* strains isolated from wild *D. melanogaster* flies and utilized an additional 323 publicly-available *L. plantarum* genomes from diverse environments including humans, pigs, rodents, other arthropods, and food fermentations. We first identified the core proteome of 628 proteins and used these sequences to reconstruct an *L. plantarum* phylogeny. We then used the same methods on monophyletic lineages within the complete phylogeny to determine environment-specific genomic regions, yielding a set of genes uniquely present in *L. plantarum*-derived *D. melanogaster*. Our study complements previous work from Martino *et al* 2016, and we are currently taking a candidate approach to identify *L. plantarum* genes involved in colonization and maintenance of the *D. melanogaster* gut.

275B Annotation of Genes in the Insulin Signaling Pathway Across Drosophila Species

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Signaling pathways are composed of a sequence of gene regulatory and enzymatic reactions which result in an important biological outcome. Despite the fact that signaling pathways are conserved over millions of years of evolution in order to achieve the same outcome, their structures are evolving, with gene duplications or the introduction of new regulators and the loss of others. This project focused specifically on the Insulin Signaling pathway, which is critical for growth regulation, the regulation of glucose uptake into cells, and cross-talk between other signaling pathways. The annotation of genes in well-characterized signaling and metabolic pathways allows for understanding of the relationship between gene evolution and their position within a network. More specifically, the number of differences in nucleotide sequences between two species correlates to the rate of molecular evolution. *Drosophila* has proven to be an excellent model for studying the

evolution of gene expression regulatory mechanisms, as genes in this pathway are well conserved, and *Drosophila* species have undergone many duplications and losses over time. This project focused on the annotation of coding spans (CDS) of the Target of Rapamycin gene and the annotation of the genomic neighborhood across *Drosophila* species. Target of Rapamycin is an important gene to study as it contributes to chromatin DNA binding, protein binding, protein kinase activity, and protein self-association. Our annotations show that while *Drosophila melanogaster* contains two isoforms, Tor-PA and Tor-PB, only one of these isoforms, Tor-PA, exists in species *Drosophila takahashii* and *Drosophila serrata*. Additionally, Tor-PA only contains 5 exons in *D. serrata*, while the gene contains 7 exons in *D. melanogaster* and *D. serrata*. This annotation is essential since it allows for comparative analyses to further understand the evolution of a system. In our specific analysis, it is revealed that not all isoforms are conserved and gene structure has evolved over time.

276C Timeor: a web-based tool to uncover temporal regulatory mechanisms from multi-omics data

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Uncovering how transcription factors (tfs) regulate their targets at the dna, rna and protein levels over time is critical to define gene regulatory networks (grns) in normal and diseased states. rna-seq has become a standard method to measure gene regulation using an established set of analysis steps. However, none of the currently available pipeline methods for interpreting ordered genomic data (in time or space) use time series models to assign cause and effect relationships within grns, are adaptive to diverse experimental designs, or enable user interpretation through a web-based platform. Furthermore, methods which integrate ordered rna-seq data with transcription factor binding data are urgently needed. Here, we present Timeor (Trajectory Inference and Mechanism Exploration with Omics data in R), the first web-based and adaptive time series multi-omics pipeline method which infers the relationship between gene regulatory events across time. Timeor addresses the critical need for methods to predict causal regulatory mechanism networks between tfs from time series multi-omics data. Furthermore, Timeor can disentangle both the cooperative and independent temporal roles of multiple transcription factors simultaneously. We used Timeor to identify a new link between insulin stimulation and the circadian rhythm cycle. Timeor is available at https://github.com/ashleymaeconard/Timeor.git.

277A Deciphering developmental robustness with machine learning

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For years, *Drosophila* researchers, especially those studying development, have relied on image/video data to understand the effects of genetic and pharmacological perturbations. Over the past few years, this data has grown dramatically more complex. However, the sophistication of our methods for analyzing this data have not grown at the same rate. Many researchers still qualitatively analyze mutant phenotypes to identify when anomalies first present themselves. As researchers begin to analyze more subtle phenotypes using images that contain an increasing number of features, the odds that a researcher correctly identifies the initial phenotype decreases. Here, we applied a machine learning framework to analyze when and where anomalies first appear in developing mutant embryos. Using a pipeline that includes transfer learning, PCA, and topological dimensionality reduction, we have created a model that can cluster images from movies of developing *Drosophila* based on their genotype. Additionally, early images from mutant embryos cluster with wildtype while later images diverge from the wildtype cluster. This suggests that the model can identify when phenotypic perturbations are first visible thereby making it a viable option for phenotypic identification. The model takes less than an hour to run, requires minimal image pre-processing, and is robust to imaging conditions, which will enable its use other contexts.

278B Mapping of high-throughput datasets reveals Max and E93 cluster at the histone locus

Mellisa Xie[1], Heather Skye Comstra[1], Casey Schmidt[1], Lauren Hodkinson[1], Leila Rieder[1] 1) Emory University.

Nuclear bodies are membraneless structures containing concentrated genetic regulatory factors that coordinate gene expression. The histone locus body (HLB) is a discrete nuclear body that is the main site of histone mRNA production, which forms at the histone locus. The model organism *Drosophila melanogaster* has a histone locus that contains tandem arrays of the five histone genes. While some HLB components of *D. melanogaster* are known, there are many uncharacterized factors that target the histone locus and have unknown relationships to the HLB. Based on the established protein-protein interaction

between known HLB component Myc and the protein Max, and functional similarities between known HLB component Zelda and the protein E93, I predicted that Max and E93 are novel HLB members. After mapping high-throughput sequencing datasets of Max and E93 to the histone locus, I determined that there is Max signal at the HLB but not E93 signal. Through this quantitative approach, the Max signal indicates interactions of an additional protein that clusters at the locus. Further wet-lab experiments will determine if the proteins interact at the locus or promote histone RNA production.

279C

Type II Phosphatidylinositol 4-kinases as Regulators of the Actin Cytoskeleton *Joseph Albanesi*[1], Suprabha Pulipparacharuvil[1], Gwanghyun Jung[1] 1) University of Texas Southwestern Medical Center, Dallas TX.

Reorganization of the actin cytoskeleton is critical for cell division, morphogenesis, and motility, and is also important for intracellular trafficking in the secretory and endocytic pathways. Phosphatidylinositol phosphates, particularly PI(4,5) P_2 and PI(3,4,5)P_3, regulate the activities and subcellular distributions of a wide range of proteins that control actin dynamics. The major substrate in the synthesis of these lipids, PI4P, is generated by two families of PI kinases, PI4KII and PI4KIII. At present, there is little known regarding the contribution of these enzymes to actin remodeling. Here we address this issue by investigating the consequences of interfering with PI4KII expression on the actin cytoskeletons of Drosophila S2 cells. Depletion of Drosophila PI4KII (dPI4KII) from S2 cells induced a serrate/stellate morphological phenotype, similar to that which was previously reported upon inactivation of the Rac/SCAR/Arp2/3 actin nucleation pathway in S2 cells. Although the effect of its depletion on cell morphology suggests that it regulates the cortical actin cytoskeleton, dPI4KII does not localize to the periphery of S2 cells. Instead, we observed strong colocalization with three endoplasmic reticulum (ER) markers, KDEL, atlastin, and Boca, but almost no colocalization with the Golgi marker p115. This result was unexpected, given the abundance of mammalian PI4KIIs on the Golgi and a previous finding from the Brill lab that dPI4KII is adjacent to many Golgi puncta in Drosophila salivary glands.

280A Autocrine insulin pathway signaling regulates actin dynamics in cell wound repair

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Cells undergo daily injuries that often damage the plasma membrane and underlying cytoskeleton. These injuries require a rapid repair program to avert further damage, prevent infection and restore normal function. Using the early syncytial *Drosophila* embryo as a genetically amenable model, we are investigating the mechanisms of single cell wound repair, exploiting transcriptomic analysis to identify new candidate genes. We performed microarray analyses and drug inhibition assays and find that the initiation of cell wound repair is dependent on translation, while transcription is necessary for later steps in the repair process. From the microarray analysis, we identified 80 upregulated genes and 173 downregulated genes, totaling 253 genes whose expression was changed in response to laser wounding. We validated the top 15 up- and down-regulated genes respectively using RNAi knockdown and found that the wounded knockdown embryos exhibited disruptions at various post-initiation steps of the cell wound repair process, including wound over-expansion, delayed/altered rates of wound contraction, aberrant actin dynamics (premature actin ring disassembly, failure of actin ring disassembly, and/or accumulation of actin inside the wound), and/or remodeling defects. Interestingly, two of the top up-regulated genes are in the *Drosophila* Insulin-like signaling pathway. We find that disruptions of genes throughout the canonical insulin signaling pathway also lead to abnormal wound repair. We have identified two downstream regulators - Girdin and Chickadee (profilin) - that control actin dynamics during the repair process. Our findings add to the understanding of how cells repair wounds, as well as provide new insights for wound repair in disease states.

281B Flies as a cell biology platform to study T3SS-secreted early effectors of the intracellular pathogen *Chlamydia trachomatis*

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Chlamydia trachomatis (*Ct*) infection is the most frequently reported sexually transmitted infection in the United States. *Ct*, an obligate intracellular bacterial pathogen, injects multiple protein effectors into the host cell via the type III secretion system (T3SS) to induce its entry. One of the first effectors to translocate to the host cell is a protein called Tarp, which is important for efficient invasion of the host cell. Tarp promotes localized F-actin accumulation at the site of entry through its actin-nucleating and F-actin bundling activities. Studies of the cell biological impact of Tarp on host cells have been limited to work in cell culture. Here, we utilize the genetic and experimental tractability of *Drosophila* to further understand the impact of Tarp on host cells *in vivo* in the context of an intact organism. Tarp expression in different tissues give rise to various cell biological

defects that are consistent with its ability to alter host actin. We found that Tarp can: 1) cause microvilli overgrowth in the follicle cell epithelium of the egg chamber; 2) cause morphological changes in the mechanosensory bristles; and 3) impact the collective migration of border cells in the egg chamber. Tarp is just one of many early effectors secreted into the host cell during *Ct* invasion. We aim to express multiple *Ct* effectors to determine functional cooperativity between effectors in manipulating host actin dynamics. Moreover, we will use genetic tools to screen for novel molecular pathways impacted by the presence of *Ct* effectors. Thus, Drosophila provides a novel *in vivo* cell biological platform to study *Ct* effector influence on host cells.

282C Dunk regulates cortical localization of myosin II during *Drosophila* cellularization through interaction with the scaffolding protein anillin

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Cleavage is a common step of early embryonic development, generating a monolayer of epithelial cells at the surface of the embryo called "blastoderm". In *Drosophila*, this process is achieved by cellularization, a special form of cytokinesis that partition the peripherally localized syncytial nuclei into individual cells. Similar to typical animal cytokinesis, cellularization is initiated by recruiting non-muscle myosin II ("myosin") to the cleavage furrows, which involves a cortical flow of myosin towards the leading edge of the newly formed furrows. We have previously identified a gene *dunk* that is required for cortical localization of myosin during this process, but the underlying mechanism is unclear. Through a genome-wide yeast two-hybrid screen, we identified anillin (Scraps in *Drosophila*), a conserved scaffolding protein involved in 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 and $PI(4,5)P_2$. Anillin has been reported to regulate cytokinetic rings during mid-late cellularization, but its role in early cellularization is less clear. We found that anillin colocalizes with myosin when myosin flows towards the nascent furrows at the onset of cellularization. Embryos maternally mutant for *anillin* showed a cortical myosin loss phenotype specifically during early cellularization, closely resembling the *dunk* mutant phenotype. Several lines of evidence further support that anillin and Dunk function together to regulate myosin cortical localization in early cellularization. First, in *dunk* mutant embryos, the localization of anillin at the cleavage furrows is severely disrupted. Second, there is a genetic interaction between *anillin* and *dunk* as revealed by embryos doubly heterozygous for the two genes. Finally, *dunk* and *anillin* mutants showed similar synthetic effects with mutations in *bottleneck*, which encodes for an actin cross-linking protein restraining actomyosin ring formation during cellularization. Taken together, our data suggest that Dunk regulates cortical myosin during early cellularization by interacting with anillin and regulating its cortical localization.

283A *Drosophila* Wash and the Wash regulatory complex function in nuclear envelope budding

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Nuclear envelope (NE) budding is a phenomenon wherein large macromolecular complexes, which are too large to be exported through nuclear pores, are packaged and expelled through the nuclear membranes. Although a general outline of the cellular events occurring during endogenous NE budding can be inferred from the proteins known to be involved and visualization of the process, very little is yet known about the molecular machinery and mechanisms underlying the physical aspects of NE bud formation. Using genetics, biochemistry, and super-resolution imaging, we identify Wash, the Wash regulatory complex (SHRC), capping protein, and Arp2/3 as novel molecular components involved in the physical aspects of NE bud formation. Interestingly, depletion of WASH in salivary gland nuclei causes wrinkled nuclei and loss of nuclear buds, whereas knockdown of SHRC results only in the loss of nuclear buds. Using double immunofluorescent staining and point mutations we find that Wash affects NE budding in two ways: 1) indirectly through general nuclear lamina disruption via an SHRC-independent interaction with Lamin B leading to inefficient NE bud formation, and 2) directly by blocking NE bud formation along with its SHRC. We also show that Wash requires Arp2/3 and capping protein for NE bud formation, suggesting Wash's ability to form new branched actin networks may be needed. By mass spec and native PAGE, we find that Wash acts as part of multiple, separable nuclear complexes to affect its diverse set of nuclear properties/events. We are currently investigating the specific function of the protein components of each of these different nuclear complexes to further elucidate Wash's mechanistic role in NE budding and other nuclear processes. We are also using live imaging techniques to examine NE budding dynamics. NE budding is emerging as an important endogenous nuclear process, as well as sharing many similarities with herpesvirus nuclear egress mechanisms, opening up potential avenues for exploration in both normal and disease biology.

284B Actin bundles play a dofferent role in shaping scales compared to bristles in the mosquito *Aedes aegypti*

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The vast majority of the epithelial cells that cover insects contain cellular extensions; namely bristles, hairs, and scales. These cellular extensions are homologous structures that differ in their morphology and function. They contain actin bundles that dictate their cellular morphology. Most of our knowledge on the role of actin in bristle development are from studies in *Drosophila.* While the organization, function, and identity of the major actin-bundling proteins in bristles and hairs are known, this information on scales is unknown. In this study, we characterized the development of scales and the role of actin bundles in the mosquito, *Aedes aegypti*. We show that scales undergo drastic morphological changes during development, from a cylindrical to fat shape with longer membrane invagination. Scale actin-bundle distribution changes from the symmetrical organization of actin bundles to an asymmetrical organization. By chemically inhibiting actin polymerization and by knocking out the *forked* gene in the mosquito (*Ae-Forked*; a known actin-bundling protein) by CRISPR-Cas9 gene editing, we showed that actin bundles are required for shaping bristle, hair, and scale morphology. We demonstrated that actin bundles are required for bristle elongation, but not for that of scales. In scales, actin bundles are required for width formation. In summary, our results reveal, for the first time, the developmental process of mosquito scale formation and also the role of actin bundles and actin-bundle proteins in scale morphogenesis. Moreover, our results reveal that although scale and bristle are thought to be homologous structures, actin bundles have a differential requirement in shaping mosquito scales compared to bristles.

285C Pericentrin-like-protein and Kinesin-1 drive centriole motility for proper subcellular positioning in *Drosophila*.

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Centrosomes are a key microtubule organizing center in the cell. They comprise a pair of centrioles surrounded by a matrix of proteins termed the pericentriolar material. Through microtubule nucleation they organize the mitotic spindle, cilia and flagella. To fulfill these functions, centrosomes must be motile to achieve proper positioning within the cell. Little is understood about the different mechanisms of centrosome motility. Typically, motility is thought to be governed by the activity of microtubule motors, pushing or pulling on the microtubules anchored at the centrosome. In some cell types, centrioles lack PCM and microtubules, and are referred to as inactive centrioles. Inactive centrioles must be motile as their intracellular positioning is critical for asymmetric cell division. Despite this, the mechanisms of inactive centriole movement are not well understood. *Drosophila* are an ideal model to understand centriole motility as many interphase cell populations have inactive and motile centrioles. High resolution live imaging of the wing disc peripodial squamous epithelium revealed that centrioles are microtubule cargo, appearing to move bidirectionally along the microtubule network in a manner involving Kinesin-1. Importantly, super resolution imaging demonstrated that Kinesin-1 localizes to the outside of the centriole in interphase *Drosophila* cells. To identify centriole components which could perform as an adaptor for motor transport, we performed a targeted RNAi screen to knock down centriole components and then visualize movement. Pericentrin-Like-Protein (Plp) was identified as essential for interphase centriole movement. Through yeast-2-hybrid and an *in vivo* interaction assay we found that Plp interacts with the C-terminal cargo binding region of the Kinesin-1 heavy chain. Next, we investigated this motility in vitro and found that Plp and Kinesin-1 can walk together on microtubules. By random mutagenesis, we have now generated a series of mutations in Plp which ablate interaction with Kinesin-1. Our data support a model where Plp acts as a novel adaptor that links the centriole to the microtubule transport machinery, facilitating movement. In this work we propose the first detailed mechanism of how centrioles can move independently of their role as an MTOC, in the context of developing tissue. Further understanding of inactive centriole motility has far-reaching implications in studies of asymmetric cell division and sensory ciliogenesis.

286A Recapitulating bristle-like actin module organization by the actin-binding proteins, Forked, Fascin, and Javelin in *Drosophila* oocyte

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The generation of F-actin bundles is tightly controlled by the sequential action of multiple actin-binding proteins.

In *Drosophila* bristle development, two major actin-bundling proteins (ABPs) – Forked and Fascin, were identified but still, the molecular mechanism by which these ABPs and other proteins generate bristle actin bundles is unknown. Previously, we demonstrated that Forked generated a distinct ectopic network of actin bundles in the oocyte. Thus, in this study, we developed a method to recapitulate bristle actin module organization using the *Drosophila* ovary. We study the additive effect of two other actin-associated proteins: Fascin and Javelin, on Forked-dependent actin bundles formation in the oocyte. Using a combination of confocal microscopy, super-resolution structured illumination microscopy (SR-SIM), and Correlative light electron microscope analysis, we demonstrated that co-expression of Fascin and Forked generate tightly packed actin filament bundles that resemble bristle-like actin bundles in their geometric organization. We also revealed that Forked but not Javelin was highly co-localized with the actin bundles. Next, the addition of Javelin to Fascin and Forked proteins led to a dramatic increase in actin bundle density (number of actin bundles). Thus, the combination of Fascin and Forked alone is sufficient to generate geometrically similar bristle actin bundles in the oocyte. The effect of Javelin on the ectopic actin network and its structure-function analysis suggest that Javelin may have a role in spatial actin bundles organization. To conclude, our study suggests that the oocyte could be used to study actin-bundling activity as the combination of ectopically expressing Forked, Javelin, and Fascin generates a unique actin bundle network.

287B Short stop is a gatekeeper at the ring canals of *Drosophila* ovary

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Microtubules and actin filaments are two major cytoskeletal components essential for a variety of cellular functions. Spectraplakins are a family of large cytoskeletal proteins cross-linking microtubules and actin filaments among other components. In this study, we examine the role of Short stop (Shot), the single *Drosophila* spectraplakin, in the directed transport of material from nurse cells into the oocyte in *Drosophila* ovary. *Drosophila* oocyte is transcriptionally silent throughout oogenesis and its growth is completely dependent on the acquisition of organelles, mRNA, and proteins from the interconnected sister cells (nurse cells), through ring canals, cytoplasmic bridges that remained open after incomplete separation of germline cells. Knockdown of *shot* causes striking oocyte growth defects, resulting in small oocytes that fail to grow over time. Both actin-binding and microtubule-interacting functions of Shot are required for the oocyte growth, while the central spectrin repeat domain is dispensable for this function. The small oocyte phenotype is explained by the fact that Shot controls the directionality of flow of material from the nurse cells towards the oocyte. In agreement with this flow-directing function of Shot, we found that it is localized at the asymmetric actin fibers adjacent to the ring canals at the nurse cell side and controls the polarity of microtubules located in the ring canals connecting the nurse cells and the oocyte. Together, we propose that Shot functions as a gatekeeper directing the flow of material from the nurse cells to the oocyte, likely via organization of microtubule tracks.

288C Rapid diversification of Arp2 specialized for roles in *Drosophila* sperm development

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The actin cytoskeleton often forms branched networks that are critical in many fundamental cellular processes, including cell motility, cell division and vesical movement. Branched actin networks are generated by the Arp2/3 complex, a 7-membered protein complex including actin-related proteins (Arps) 2 and 3. Similar to actin and most Arps, Arp2 is evolutionarily ancient among eukaryotes and under stringent sequence conservation, yet we surprisingly discovered two clade-specific gene duplications of Arp2 in *Drosophila*: *Arp2D* in the *obscura* clade and *Arp2D2* in the *montium* clade. Our targeted sequencing and phylogenetic analyses indicate that these duplicates have evolved independently and arose 13-14 million years ago, suggesting they have been evolutionarily retained for a function. Intriguingly, we found both *Arp2D* and *Arp2D2* are testis-specific in expression, whereas canonical *Arp2* is ubiquitously expressed. Why would evolution recurrently select for a divergent Arp2 for roles in fly sperm development? To explore their functions, we localized them in the testis by generating transgenic flies expressing Arp2D-GFP (in its native species *D. pseudoobscura*) and Arp2D2-GFP (in the non-native species *D. melanogaster*). We found both localize to post-meiotic actin structures known as actin cones, which are critical in germ cell development. *Drosophila* germ cells remain interconnected within a cyst, and during the last step of sperm maturation, actin cones separate the sperm by encasing each sperm in its own membrane and pushing out excess cytoplasm. The duplicates Arp2D and Arp2D2 concentrate at the front of actin cones, where branched actin networks actively extrude excess membrane and propel cones down the sperm tail. Based on the localization of the divergent Arp2 proteins, we hypothesize recurrent diversification of *Drosophila* Arp2 has specialized actin polymerization for the construction of actin cones.

289A Mechanisms of localized actin network assembly during actin cap formation in the Drosophila embryo

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Cells undergo shape change to facilitate biological processes. Actin within the cytoskeleton cortex is a dynamic network of proteins that allow cell shape change. During early *Drosophila* embryogenesis, an actin cap forms above the nucleus, and the growth of this structure creates a dome that houses the mitotic apparatus. The centrosomes and the Rac-GEF Sponge promote actin cap formation and growth, but it is unclear if centrosomes and Sponge have a specific relationship. Using spinning disk confocal microscopy, I imaged the localization of Spg and Rac-GTP and perturbed specific proteins using RNAi. My experiments show that centrosomes have a close spatial relationship with Sponge and Rac-GTP. Sponge and Rac-GTP do not localize to the centrosomes themselves, but when a new actin cap starts to form at anaphase, there is an enrichment of Sponge and Rac-GTP at the cortex above the centrosomes. Dual live imaging with a plasma membrane marker showed that the plasma membrane is highly folded across the cortex, and that Sponge and Rac-GTP were enriched at a sub-set of plasma membrane folds above the centrosomes. Moreover, Sponge and Rac-GTP localized to tubule-like plasma membrane structures that emanated below the folds and towards the centrosomes. To perturb centrosome integrity and/or signaling, Aurora A kinase was targeted by RNAi, and the cortical Sponge recruitment was diminished compared to control RNAi. The data suggest that the centrosomes recruit Sponge to promote cap growth and that the plasma membrane might be pulled towards the centrosomes to facilitate the signal transduction. The actin elongation factor, Dia, has been shown to promote bundled actin along the folds. With *dia* RNAi, the local level of Rac-GTP recruitment was not affected, but Rac-GTP localized to more wavy structures, suggesting that Dia promotes the structural integrity of the plasma membrane folds but not the induction of Rac-GTP at the folds. We propose that the centrosome promotes the local accumulation of Sponge at plasma membrane folds, where Sponge induces Rac-GTP and subsequent assembly of Arp2/3 networks that work in conjunction with Dia-based actin bundles for growth of the cap into a dome-like compartment.

290B Determining how the Misshapen kinase regulates the size of the germline ring canals in the developing egg chamber

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Our lab has recently demonstrated that the Misshapen (Msn) kinase is required to regulate the size of the intercellular bridges that connect germ cells in the developing egg chamber. Altering the levels or localization of Msn leads to changes in ring canal size and defects in anchoring and stability. Even though Msn plays an essential role in the size regulation of ring canals, the molecular targets of this kinase are not known. Moesin, which links actin filaments to the membrane, has been identified as a target of Msn in other contexts. In the germline, moesin has been shown to localize to microvilli structures that surround the ring canals, which suggests that it could play a role in ring canal anchoring and/or size control. To test this model, we have used a combination of genetics, fluorescence microscopy, and biochemistry to determine whether Msn phosphorylates moesin in the germline to control ring canal size and/or stability. Msn could also impact ring canal size by regulating adherens junctions in the germline. When Msn levels were reduced, we observed an accumulation of the adherens junction protein, E- cadherin, in punctate structures around the ring canals, suggesting that loss of Msn affects normal E-cadherin targeting or turnover. To further characterize this phenotype, we are examining the normal delivery and turnover of E-cadherin in the germline and assessing how Msn impacts those processes. Future studies will further characterize the potential roles for Msn in regulating moesin activity and/or E-cadherin trafficking.

291C Toxicological Study and Genetic Basis of BTEX Susceptibility in Drosophila melanogaster

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Petroleum exploitative and exploratory activities over the past 50 years have led to the environmental release of benzene, toluene, ethylbenzene and xylene, also known as BTEX compounds. This release has caused harm to humans and other environmental components, impacting both disease incidence and pollution. Testing the effects of these chemicals and understanding the underlying toxicogenomic mechanisms requires an inexpensive protocol that has been established in *Drosophila melanogaster*. In this study, the toxicological profile of benzene, toluene, ethylbenzene, p-xylene, m-xylene, and o-xylene in *Drosophila melanogaster* was evaluated. Adult animals were monitored for acute toxicity effects. Similarly, the impact of fixed concentrations of benzene and xylene on apoptosis and mitosis were investigated in adult progenitor tissues found in third instar larvae. In addition, Genome Wide Association Screening of the *Drosophila* Genetic Resource Panel (DGRP) was conducted to identify genes that are critical for toxicological responses in *Drosophila melanogaster for p-xylene.* Toluene is the most toxic to adult flies with an LC_{50} of 0.166 mM, while a significant and dose-dependent decrease in fly eclosion was observed with benzene, p-xylene, and o-xylene. An increase in apoptosis and mitosis was also observed in animals exposed

to benzene and p-xylene. The genome-wide analyses revealed 38 regions of *Drosophila melanogaster* genome as critical for responses to p-xylene. Further, the study revealed 16 genes whose human homologues have been linked to certain human diseases. This study reveals the strength of *Drosophila melanogaster* genetics as an accessible approach to study BTEX compounds.

292A The septate junction protein Macroglobulin complement-related plays an essential role in *Drosophila melanogaster* oogenesis

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Components of the invertebrate occluding junction – known as the septate junction (SJ) – are required for morphogenetic developmental events during embryogenesis. To determine whether SJ proteins are similarly required for morphogenesis beyond the embryonic stages, we examined the localization and requirement of the SJ protein Macroglobulin complement-related (Mcr) during oogenesis. A *Drosophila* egg consists of germline cells surrounded by a layer of follicular epithelium (FE). We found that Mcr is expressed in the FE throughout oogenesis. During early and mid-oogenesis, Mcr localizes along the lateral membrane of the follicle cells. By stage 10B, Mcr becomes enriched at the apical-lateral domain (the presumptive SJ). This re-localization event requires Rab5 and Rab11, in a manner similar to SJ biogenesis in the embryo. RNAi-mediated knockdown of Mcr in the FE throughout oogenesis results in egg elongation and dorsal appendages formation defects. Further analysis reveals that the requirement of Mcr in egg morphogenesis is late in oogenesis. In stage 12 egg chambers, Mcr is required for maintaining actin cytoskeleton organization and FE integrity. Moreover, Mcr is required for eggshell formation, as evident via egg permeability and lack of follicle cell imprints of stage 14 egg chambers expressing *Mcr-RNAi* in the FE. Together, these results demonstrate an essential requirement for Mcr during egg development, suggesting conserved functions for SJ genes in epithelial tissue morphogenesis across developmental stages.

293B The role of organismal physiology in the regulation of cell competition

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Cell competition results from cellular interactions in which normally viable, but less “fit”, cells are eliminated from a growing tissue due to their interactions with relatively more fit cells in the tissue. As such, it is typically viewed as a local interaction between the competing cell populations. Utilizing a model of cell competition in the wing disc, we find that competitive cell-cell interactions in the wing disc are also systemically regulated. Our preliminary data indicate that a neuroendocrine axis that includes the secreted *Drosophila* insulin-like peptide 8 (Dilp8) and its receptor, leucine-rich repeat-containing G-protein coupled receptor 3 (Lgr3), contribute to control of cell competition. *dilp8* is expressed in many larval tissues, including the wing disc, whereas *lgr3* expression is limited to neurons in the ventral nerve chord and central larval brain. Developmental timing and tissue growth are regulated by Dilp8/Lgr3 via a subset of *lgr3*-expressing neurons that modulate synthesis of the steroid hormone ecdysone. We are interrogating the mechanism by which signaling through the Dilp8-Lgr3 axis reaches peripheral tissues such as the wing disc to regulate cell competition. We will discuss our ongoing work into how organismal physiology affects cell competition.

294C PlexinA mediates medulla layer formation and photoreceptor targeting in *Drosophila*

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Neurodevelopment in both vertebrates and invertebrates is marked by the establishment of a complex network of connections between neurites that allow for the processing of external stimuli by the brain. The development of the *Drosophila melanogaster* visual system alone requires more than 100,000 neurons and glia to form a highly sophisticated neural circuit that sends visual cues from the retina to distinct regions of the visual processing center of the optic lobe. One of these cell types - the R7 photoreceptor cells - senses ultraviolet light and sends axon projections from the retina to the M6 layer of the medulla. The distinct layers of the medulla organize regions of synaptic connectivity, thereby decreasing the complexity of the region through which neurites must navigate to form connections with partner cells. We show that a loss of *plexin A* - the gene that encodes the transmembrane protein Plexin A (PlexA) - results in changes to the gross morphology of the medulla, and a loss of distinction between medulla layers. These changes to the shape and layered architecture of the medulla lead to a shortening of R7 projections into the medulla, indicating that the local environment surrounding R7 neurites is a critical mediator of R7 growth cone targeting and synapse formation. Members of the Plexin family of proteins have been shown to interact with Semaphorin partners through a defined Sema domain and mediate various cell behaviors such as axon

guidance and defasciculation in both invertebrate and vertebrate models. We show that the N-terminal Sema domain of Plexin A is required for normal medulla development, and loss of the Sema domain alone phenocopies the morphological changes observed in *plexin A* mutants, suggesting that PlexA may require Semaphorin partners to mediate medulla development and layer formation. Further investigation into the PlexA mechanism of action will distinguish whether PlexA acts as a ligand produced by tangential neurons that affects the patterning of other neural processes, or as a receptor that controls the projection of tangential neurons, which then establish the layered structure of the medulla. These results will help to elucidate the cellular and molecular mechanisms underlying the formation of medulla layers during *Drosophila* neurodevelopment.

295A Distinct spatial signalling requirements for patterning of the *Drosophila* embryo termini by Torso

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Patterning of *Drosophila* early embryo termini requires activation of the receptor tyrosine kinase Torso (Tor) by its putative ligand Trunk (Trk). This event is restricted to the anterior and posterior poles of the developing embryos by the localized factor Torsolike (Tsl). Tsl is the only member of the membrane attack-complex/perforin-like protein superfamily in *Drosophila*, and it is thought to be required for the interaction between Trk and Tor. Here we show that this can take place in a *tsl* deficient mutant background when Trk is expressed at high-levels, as indicated by the development of correctly positioned posterior structures in these embryos. This finding suggests that Tsl is required to promote the Trk/Tor signalling and that localized signalling may be only required for the development of the anterior structures. To test this idea we also performed a low-level ubiquitous activation of Tor signalling using optogenetics in *tsl* mutant background and found out that this can rescue the formation of only posterior but not anterior structures in agreement with the previous findings. In summary, we report that the anterior and posterior termini are subject to different Tor signalling requirements and that Tsl is not a core component of this pathway, but instead performs a novel regulatory role.

296B The haplolethality paradox of the *wupA* gene in *Drosophila*

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Most species contain two copies of their genetic endowment, each received from each progenitor. If one of the duplicated genes is non-functional, due to null mutations or genomic loss, the remaining gene copy may supply enough product as to cover the requirements for normal function or, alternatively, may reflect the insufficiency through a visible phenotype. In rare occasions, however, mutation in one copy is so deleterious that causes lethality, usually at early stages of development. These so called “haplolethal regions”, exist across species and represent an evolutionary paradox since they should have been subject to intense negative selection. The inherent difficulties to study haplolethals have precluded their study so far. Here, we analyzed the case of one of the five haplolethal regions of *Drosophila*, the one associated to the Troponin I encoding gene *wupA*, by measuring the transcriptional effects of mutations and chromosomal rearrangements affecting this gene. The data show that this haplolethality results from the combined insufficiency of a large number of Troponin I isoforms, which are functionally specialized, show interference and require the integrity of the native chromatin structure for their quantitatively regulated expression. These features unveil novel aspects of gene expression and, possibly, on evolutionary gene splitting.

297C Investigating the sex-specific function of *Stonewall* in *Drosophila* female germline stem cells

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The DNA of metazoan nuclei is compartmentalized into distinct domains of euchromatin and heterochromatin to facilitate genome organization and regulate gene expression. Previous studies have shown that the densely packed heterochromatin is enriched at the nuclear periphery in diverse cell types. Heterochromatin localization to the nuclear periphery is thought to function in regulating cell-type-specific gene expression and may also provide mechanical stability to the nucleus. Interactions between multiple DNA-binding and nuclear envelope (NE)-associated proteins are thought to tether heterochromatin to the nuclear periphery. Importantly, mutations in heterochromatin tethering proteins are frequently associated with a class of diseases known as laminopathies, which present with a large variety of clinical symptoms. However, the mechanistic basis for the tissue-specific defects in laminopathies is poorly understood.
In this study, we focused on the *Drosophila* DNA-binding protein, Stonewall, which is predicted to interact with heterochromatin and NE proteins. Stonewall has been previously characterized for its role in female-specific germline stem cell

maintenance although the mechanisms are poorly understood. Our preliminary data suggest that germline-specific knockdown of Stonewall results in the complete ablation of ovaries while testes remain unaffected. Additionally, Stonewall knockdown in XXY females and XO males did not alter the ablated ovary/normal testis phenotype, suggesting that the Y chromosome is not responsible for the sex-specific Stonewall function. Rather, inducible knockdown of Stonewall revealed a NE defect marked by the loss of nuclear lamina specifically in female germ cells. We are currently investigating how Stonewall functions specifically in female GSCs to maintain the integrity of the nuclear lamina.

298A Association of RanGAP to Nuclear Pore Complex Component, RanBP2/Nup358, is Required for Development in *Drosophila*.

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The Ran-GTP/Ran-GDP gradient is critical for nuclear-cytoplasmic transport, nuclear envelope (NE) assembly and mitotic chromosome segregation. This gradient is established through the asymmetric localization of Ran's GTP exchange factor (called RCC1 in vertebrates), which is chromatin-bound, and GTPase activating protein (RanGAP), which is cytosolic. RanGAPs from different multicellular organisms have evolved convergently to be tethered to the cytoplasmic side of NE. However, the functional consequences of its localization remain unknown.
We used cultured mammalian cells and *Drosophila* to investigate the functional consequences of RanGAP localization in metazoans. In mammalian tissue culture cells, we used CRISPR/Cas9 gene editing to mutate human RanGAP (hsRanGAP) in DLD-1 cells, preventing its SUMOylation and NPC targeting. Surprisingly, this change had no impact on cell viability and caused no obvious perturbations of nuclear transport or mitosis. The mechanism of *Drosophila* RanGAP (dmRanGAP) targeting to NE had not been reported. We found that while dmRanGAP associates to the NPC through binding to dmRanBP2, this targeting was different from mammals to the extent that it occured via direct association of dmRanGAP and to dmRanBP2 and it was independent of SUMOylation. We identified the domains in both proteins that mediate their binding and used CRISPR/Cas9 gene editing to generate dmRanBP2 mutants (dmRanBP2^{short}) that abolish dmRanGAP anchorage to NPCs through a 23 amino acid deletion. Homozygous dmRanBP2^{short} mutants exhibited no apparent growth defects as larvae. However, the development of flies was arrested at the early pupal stage without head eversion. This developmental arrest was rescued by a direct fusion of dmRanGAP to dmRanBP2^{short}, indicating that recruitment of dmRanGAP to dmRanBP2 per se was necessary for the pupal ecdysis sequence during development.
Collectively, our results indicate that while the localization of dmRanGAP to the NE is widely conserved in multicellular organisms, the targeting mechanisms are not. Further, we find a previously unreported requirement for this localization in critical tissue developmental processes, and we are currently working to understand the precise molecular role of dmRanBP2-dmRanGAP interaction during metamorphosis.

299B Dissecting the regulation of the *vestigial* gene to explore a potential dual evolutionary origin of insect wings

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The advent of powered flight via the acquisition of wings is often cited as a major contributor to the unmatched diversity and evolutionary success of the insects. However, the origin of the insect wing has remained a hotly debated mystery in biology for over two centuries. Two contrasting hypotheses propose that the insect wing either originated from the dorsal body wall (tergum) or ancestral proximal leg segments which fused to form the lateral body wall (pleuron). Through loss-of-function and expression analyses of an important wing gene, *vestigial* (*vg*), our lab has previously obtained data supporting a third hypothesis: the dual-origin hypothesis. This hypothesis states that both tergal and pleural tissues have contributed to the evolution of the insect wing. However, it is currently unknown to what degree each of these two tissues contribute to wing formation. We will attempt to elucidate both tergal and pleural contributions to wings by leveraging and developing molecular tools in the emerging model beetle, *Tribolium castaneum*. First, our immuno-histochemical analysis in the embryo will more precisely define the position and tissue identity of separate tergal and pleural Vg+ cells in future winged segments. Second, via CRISPR-Cas9 genome editing, we will visualize total Vg expression throughout development, *in vivo*, to clarify tergal and pleural contributions to wing formation at all developmental stages. Third, we will investigate the activity of *vg* regulatory elements (enhancers) via reporter assay. We reason that the presence of separate *vg* enhancers, active in the wings and either tergal *or* pleural body wall, may further indicate a dual-origin. Finally, we will develop additional molecular tools to enhance and streamline gene expression analyses in *Tribolium* and other insects. Obtaining these multifaceted perspectives on *vg*-expression will significantly further our understanding of how tergal and pleural tissues contributed to the evolution of insect wings.

300C Prohibitin connects mitochondrial function to the delta-notch signaling pathway during drosophila oogenesis

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Development in all organisms requires the coordination of cellular metabolism with the proper regulation of signaling pathways. For example, changes in mitochondria metabolism have been associated with cell specification and differentiation in many developmental systems. Similarly, defects in mitochondrial function are associated with many cancers caused by defects in highly conserved developmental signaling pathways. However, despite this association, very little is known about how mitochondrial pathways regulate developmental signaling. Our lab utilizes *Drosophila*, mammalian cells, and mice to examine the conserved metabolic mechanisms that drive development and disease progression.
To understand the mitochondria's role in cellular differentiation, we conducted a genetic screen of nuclear-encoded mitochondrial proteins. Using this approach, we discovered that a specific subset of mitochondrial genes, when disrupted, cause defects in cellular differentiation in the ovary and intestinal epithelia. We focused our studies on the gene prohibitin. We found that inhibition of this gene in germ cells caused a block in follicle cell differentiation, a process known to be regulated by the Notch signaling pathway. We found that prohibitin inhibition causes accumulation/aggregation of the Delta ligand in the cytosol of nurse cells and defective notch activation in follicle cells. Interestingly, we did not observe the accumulation of Notch Extra Cellular Domain (Notch ECD), which is co-degraded with Delta during notch activation, indicating that prohibitin inhibition causes defects in Delta trafficking. Loss of prohibitin in the mitochondrial causes: depolarization of the mitochondrial membrane potential, destabilization of the electron transport chain, and defects in mitochondrial oxygen consumption. Using mitochondrial proteomics, we found that prohibitin loss leads to the turnover of VDAC/porin and accumulation of myosin, indicating remodeling of the mitochondrial outer membrane and changes in the mitochondria's association with the actin cytoskeleton. Overall, our work identifies a novel role for dynamic changes in mitochondrial metabolism as a critical regulator of Delta trafficking and Notch activation during development. Moreover, this work provides a foundation for future studies of how changes in mitochondria metabolism promote cancer progression by regulating Notch signaling.

301A FGF- and Hh-mediated interactions between developing epithelium and muscle precursors revealed by single-cell analysis

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In both vertebrates and invertebrates, generating a functional appendage requires interactions between ectoderm-derived epithelia and mesoderm-derived cells. The wing-imaginal disc of *Drosophila*, the larval precursor of the adult wing blade and thorax, resembles a flattened sac composed of two epithelial monolayers, the disc proper and the peripodial epithelium. In addition, the mesoderm-derived adult muscle precursors (AMPs) reside beneath the notum region of the disc proper and give rise to all adult flight muscles. Previous studies have indicated the importance of epithelial signaling in AMP development, but the signals that influence the identities of AMP subpopulations and those that restrict the localization of AMPs to notum of the disc proper are not fully understood. To investigate epithelium-AMP interactions, we used single-cell transcriptomics to generate a cell atlas of the *Drosophila* wing disc at two time points during larval development. Using these data, we investigated gene expression using a multi-layered model of the wing disc and catalogued ligand-receptor pairs that could mediate signaling between epithelial cells and AMPs. We found that localized expression of the genes that encode two FGF-family ligands, *thisbe* (*ths*) and *pyramus* (*pyr*), provides a niche-like function for AMPs and that this signaling impacts both the location and number of AMPs. Firstly, FGF signaling is critical for maintaining proper numbers of AMPs beneath the notum of the disc proper. Inhibiting FGF signaling caused a decrease in the AMPs and the activation of FGF signaling by the epithelial overexpression of either Ths or Pyr ligand triggered AMP proliferation. Secondly, the ectopic expression of either ligand was sufficient to induce the AMPs to migrate and associate with other regions of the wing disc. The coupling of the AMP numbers to the size of the notum via FGF-signaling suggests a mechanism for organ scaling between the ectoderm and mesoderm.
In addition, we searched for AMP-epithelial interactions that might define different AMP subtypes and identified a unique population that are specified through spatially-restricted *hedgehog* (*hh*) signaling. Hh ligand produced from the posterior compartment of the epithelium specifically activates downstream Hh-signaling in the adjacent AMPs, inducing the expression of both *Neurotactin* and *Midline*. The disruption of Hh-signaling within this subpopulation of AMPs caused severe defects in adult flight muscles, and thus indicates that Hh-signaling within the AMPs is critical for proper adult muscle development. More generally, our annotated atlas provides a global view of potential cell-cell interactions between subpopulations of epithelial and myogenic cells.

302B Regulation of *trn* during the development and evolution of *Drosophila* male genitalia

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Male genital structures diverge rapidly in many animal groups, and in some cases are the only features that can distinguish species that have recently diverged. However, we still lack knowledge about the underlying genetic changes. We recently discovered that the gene *tartan* (*trn*), which encodes a Leucine-rich repeat transmembrane protein, contributes to the difference in surstylus (clasper) size between *Drosophila mauritiana* and *D. simulans*. We also found that *trn* is expressed more expansively and for longer in the developing claspers of *D. mauritiana* consistent with the larger claspers displaying more bristles in this species. Moreover, there are only three non-synonymous differences in the *trn* coding sequence that are not fixed between these two species. These findings imply that changes in the regulation of *trn* must be responsible for the difference in clasper size between the two species. To identify these changes, we first aim to find and characterise the cis-regulatory sequences of *trn*. To do this we carried out chromatin profiling using ATAC-seq in combination with surveying a total of 42 kb of non-coding DNA upstream and downstream of *trn* for enhancer activity using reporter constructs. We have identified one enhancer that is able to drive expression in the developing genitalia in a pattern consistent with *trn* expression. Interestingly, this enhancer region appears to be genital specific and the chromatin is closed in other contexts. The discovery of this enhancer combined with analyses of the expression and function of other genes also allowed us to predict several transcription factors, including Hairy, Grunge and Mirror, that may regulate *trn* and perhaps also contribute to the divergence in clasper size.

303C Evolutionary mechanisms adapting neural circuit structure and function to mosquito visual ecology

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How does something as complex as the brain evolve, and how does its interconnected nature constrain changes that can be made? For example, the Aedes mosquito retina has been entirely rearranged compared to Drosophila. Most flies have retinas that are patterned in a stochastic, probabilistic way which ensures that different color detectors cover the entire visual field. In contrast, Aedes have sacrificed stochastic patterning and instead produce a deterministically specified ventral stripe of unknown function. We investigate how this patterning change occurred, what the stripe might be for, and how the circuits of the brain were shaped in response. We are using CRISPR/Cas9 to test candidate gene function directly in Aedes and making mosquito-like retinas in Drosophila to evaluate the effect on downstream neural circuits in the brain.

We first made an antibody to the transcription factor Spineless (Ss) in Aedes. Ss has been shown to control the stochastic, probabilistic specification of photoreceptor types in flies and butterflies and we find that it is also expressed in the regionalized ventral stripe R7s in developing Aedes retinas. This suggests a change from stochastic to regionalized Ss expression patterns the ventral stripe. We have performed ATACseq on Aedes pupal retinas to identify candidate novel enhancers that give Ss the necessary spatial inputs to specify the stripe. We are cloning candidate regulatory elements into reporter constructs in Drosophila with the goal of driving Ss expression in Drosophila using Aedes enhancers to assess conservation of the genetic program. Concurrently, we are using CRISPR in Aedes to make G0 mosaic Ss knockouts as well as enhancer deletion lines to understand the role of Ss and its regulatory inputs in retinal patterning in this species. This ongoing project is beginning to uncover new insights into how complex, highly conserved developmental genetic networks change and are able to produce diversity in the evolution of animal form.

304A *fs(1)K741* is a female sterile allele of the gene *Sxl* and disrupts *Sxl* splicing

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Several X-linked female sterile screens have been completed and have given rise to an abundant amount of mutants. However, many of these mutants have yet to be identified and assigned to a specific gene and are thus a potentially rich resource for further elucidating the genetic control of oogenesis. One of these EMS-induced alleles is *fs(1)K741*. Our goal was to determine the location of the mutation and explore the role of *fs(1)K741* in oogenesis. We meiotically mapped *fs(1)K741* between the region of crossveinless (*cv*) and singed (*sn*) on the X chromosome. Deficiency mapping and duplication rescue across the *cv* and *sn* region was then completed to further determine the location of the mutation. We found two deficiencies, *Df(1)Sxl-bt* and *Df(1)Sxl*^{fp7B0}, lethal when transheterozygous with *fs(1)K741* and one duplication, *Dp(1;3)DC490*, that rescued female sterility. The gene *Sxl* is the only gene that lies within the overlapping regions of these deficiencies and

duplications. Therefore, we believe *fs(1)K741* is an allele of *Sxl*. We tested a known *Sxl* allele, *Sxl*^{f1}, that was also lethal when transheterozygous with *fs(1)K741*. It is known that *Sxl* is important in sex determination and acts via a sex-specific splicing mechanism. RT-PCR of *fs(1)K741* ovaries showed that both male and female-specific *Sxl* splicing products are present, while RT-PCR on *fs(1)K741* carcasses showed only female-specific *Sxl* splicing. This indicates that aberrant splicing is restricted to the ovary. We have shown that adding additional *Sxl* under the control of otu, a germline specific promoter, rescues female sterility in homozygous *fs(1)K741* flies. Therefore, it is likely that the *fs(1)K741* mutation is disrupting *Sxl* specifically in the germline. Whole genome sequencing of *fs(1)K741* females showed a single point transition mutation (C>T) in the male-specific exon 3 of *Sxl*. We used CRISPR-Cas9 and two different gRNA's directed to the *fs(1)K741* point mutation to recreate the *fs(1)K741* mutation in wild-type females to confirm it to be the cause of sterility. We recovered four unique, sterile alleles that failed to complement *fs(1)K741*. We also used the same method in an attempt to revert the *fs(1)K741* mutation back to the wild-type condition and were able to recover six unique revertants with positive *Sxl* activity and thus restored fertility. We are currently investigating whether the *fs(1)K741* mutation directly disrupts the female *Sxl* splicing mechanism or whether the mutation disrupts the positive autoregulation of the female splice form through a transcriptional mechanism.

305B The Tsh transcription factor and the transcriptional co-regulator CtBP interact in *Drosophila melanogaster* eye development

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Cell fate determination and proliferation need to be coordinated during development of multicellular organisms, and this requires tissue-specific gene transcription. Transcriptional co-regulators mediate between sequence-specific transcription factors and the transcriptional machinery to promote tissue-specific gene transcription. Current evidence suggests that the sequence-specific transcription factor, Teashirt (Tsh), and the transcriptional co-regulator, C-Terminal Binding Protein (CtBP), have roles in coordinating cell fate determination and proliferation during eye development in the fruit fly, *Drosophila melanogaster*. Whether these proteins interact physically during eye development has yet to be determined. We have used genetic and molecular tools to address this question. Over-expressing *tsh* in proliferating eye precursors results in loss of eye tissue, and loss-of-function mutations in *CtBP* suppress the effects of over-expressing *tsh*, suggesting that *tsh* and *CtBP* function in the same process during eye development. Furthermore, *in vitro* Glutathione-S-Transferase (GST)-pulldowns detect direct physical interactions between Tsh and CtBP, and co-immunoprecipitations from lysates of proliferating eye precursors confirm the interaction *in vivo*. These results suggest that Tsh and CtBP interact physically during eye development and that their interaction is important for proper eye development. We have used the genomic editing tool, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), to generate transgenic flies with an insertion of green fluorescent protein (GFP) into the *tsh* gene. Future experiments using this GFP-tagged *tsh* and mass spectrometry will help to identify any proteins complexed with Tsh/CtBP and provide further insight into to how this complex regulates proliferation during the development of the eye.

306C The drosophila PAX6 Eyeless and Twin of Eyeless regulate *decapentaplegic* at the posterior margin of the eye disc for proper eye formation.

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The transcription factor Pax6 is required for eye development in all seeing animals. Mutations in *pax6* lead to serious congenital eye defects such as Aniridia in humans and small eyes in mouse and zebrafish. There are two Pax6 homologs in *Drosophila*, Eyeless (Ey) and Twin of Eyeless (Toy). Together they sit atop the retinal determination network (RDN), which regulates growth and specification of the fly eye imaginal disc. Our *goal* is to characterize the mechanisms by which Ey and Toy specify the fly eye. Here we focus on the initiation of the morphogenetic furrow (mf), a wave of pattern formation through which cells in the eye imaginal disc differentiate into photoreceptors and pigment cells. We hypothesize that Ey is required at the posterior margin of the eye imaginal disc where it regulates *decapentaplegic* (*dpp*) either directly or indirectly. This is based on previously published data where it was shown that Ey is required in the peripodial epithelium, not in the disc proper, to regulate *dpp* expression at the posterior margin of the eye disc. Dpp is required for initiation of the mf. In the absence of Ey, Toy rescues *dpp* expression, albeit weakly, resulting in discs with fewer photoreceptors and flies with smaller eyes than wild type. We postulate that loss of Ey/Toy Toy at the posterior margin of the eye disc result in a downregulation of downstream targets (*dpp, eyes absent, sine oculis, etc.*) which in turn causes defects in or complete failure of the mf to initiate.

307A Two types of cells composing a campaniform sensillum express the patterning gene *wingless* in pupal wings of *Drosophila guttifera*.

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Color pattern formation of insects has been used as a subject for understanding the relationship between gene expression and morphological evolution. Many patterning genes co-opted in the course of evolution have been identified. In order to examine the evolutionary process of color patterns in detail, it is necessary to elucidate the mechanism that spatiotemporally controls the expression of patterning genes.
An adult fly of *Drosophila guttifera* has the species-specific wing spot pattern. The spots occur at specific positions such as around campaniform sensilla on wing veins. The formation is induced by the species-specific expression of the patterning gene *wingless* during the pupal stage (Werner et al. 2010). The expression begins at mid-pupa and induces the subsequent expression of pigmentation genes such as *yellow*. On the other hand, a campaniform sensillum, which is a mechanoreceptor involved in flight control, consists of four differentiated cells: a socket cell, a cap cell, a sheath cell, and a neuron. The expression of the patterning gene *wingless* is synchronized with the differentiation stage of the four cells. Therefore, it is speculated that the gene expression involved in the formation of campaniform sensilla induces the spatiotemporal expression of the patterning gene *wingless*.
Here, as the first step to elucidate the relationship between the differentiation of cells composing campaniform sensilla and the spot formation, we identified cells expressing *wingless*. The identification was performed by the simultaneous detection of *wingless* transcripts by *in situ* hybridization and specific marker protein or cell membrane of campaniform sensilla by immunohistological staining in pupal wings. As a result, we found that *wingless* was expressed in socket and cap cells, which suggests the specific gene network in these cells induces *wingless* expression.

308B Microtubule- and Rab11-dependent apical trafficking of the Fog ligand and apical/junctional proteins regulates apical constriction during tissue invagination

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The Drosophila embryonic salivary gland (SG) forms a three-dimensional tube by invagination by budding. During SG invagination, Folded gastrulation (Fog)-dependent Rho-associated kinase (Rok) promotes contractile apical myosin formation to drive apical constriction. Microtubules (MTs) are crucial for this process and are required for formation and maintenance of apicomedial myosin. However, the underlying mechanism that coordinates actomyosin and MTs networks still remains elusive. Here, we show that MT-dependent intracellular trafficking regulates apical constriction. Key components involved in protein trafficking, such as Rab11 and Nuclear fallout, are apically enriched near the SG invagination pit in a MT-dependent manner. Disruption of the MT networks or intracellular trafficking impairs apicomedial myosin formation and apical constriction. Importantly, MTs and Rab11 regulate apical enrichment of the Fog ligand, the apical protein Crumbs (Crb) and the junctional protein E-Cadherin (E-Cad). Targeted knockdown of *crb* and *E-Cad* in the SG disrupts apical myosin networks and results in apical constriction defects. Our data suggests a role of MT- and Rab11-dependent intracellular trafficking in regulating actomyosin networks and cell junctions, to coordinate cell behaviors during tubular organ formation.

309C *In Vivo* Validation of Candidate Congenital Heart Disease Genes in Drosophila Identifies a Novel Role for the E3 Ubiquitin Ligase *Hyperplastic Discs (Hyd)*

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Developmental defects of the heart are the most common birth defect. Though many genes have been implicated in heart development, many cases of congenital heart disease (CHD) remain without a genetic diagnosis. We sought to identify novel candidate CHD genes from whole exome sequencing data generated from a cohort of patients with CHD by Pediatric Cardiac Genetics Consortium (PCGC) (PMID: 28991257). We identified candidate genes in which there were novel de novo pathogenic variants meeting the following criteria: (1) Drosophila ortholog exists, (2) no studies have investigated the role of this candidate in heart development, and (3) expression in the Drosophila heart during embryonic/larval development based on BDGP in situ database. Candidate genes included *zDHHC8* (a palmitoyltransferase), *Single Stranded DNA Binding Protein 1 (SSBP1*), and *Ubiquitin Protein Ligase E3 Component N-Recognin 5 (UBR5)*. We utilized the 4x*Hand*-Gal4 line developed by Zhe et. al. to knockdown (KD) the candidate Drosophila orthologs by UAS-RNA interference. In each knockdown, we calculated the developmental lethality of the KD by observing the number of flies that eclosed. We also assessed the structure of the adult heart by immunostaining. We found that palmitoylation was not required for survival to adult stages but led to mild dilation of the adult heart. *SSBP1* (*Ssdp* ortholog in Drosophila) similarly did not result in developmental lethality. However, *UBR5* (*Hyd* in Drosophila) KD resulted in a developmental lethality rate of 59% indicating that *Hyd* is required for heart development in Drosophila (χ^2 = 33.96, p<0.0001). The *Hyd* KD flies develop large wing blisters possibly related to defects of the wing heart.

Immunostaining of the *Hyd* KD hearts revealed structural defects of the myofibers of the heart. Our *in vivo* survey of candidate CHD genes identifies *Hyd* and the ubiquitin-proteasome system as a critical molecular component of heart development.

310A Sexually dimorphic gonad development and sex-biased expression depends on karyotype (XX or XY), *tra* (presence or absence) and their interaction

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Drosophila gonads are highly sexually dimorphic with somatic and germ line components. The sex of somatic and germ line components must match for correct sex-specific differentiation of germ cells and functional gamete production. Somatic cells require a sex-specific karyotype (XX and XY) and the germ cells require sex-specific karyotype and *transformer* (*tra*) (non-autonomous signals) from the somatic component in regulating dimorphic gonad phenotypic development that is associated with sex-biased transcript expression. The *tra* locus encodes a splicing factor that regulates female somatic sexual identity. In order to understand the relationship between karyotype and *tra*, we examined the morphology and transcription profiles of late third instar larval (L3) gonads that either null for Tra in ovaries and are ectopically express Tra in testis and compared them to wildtype gonads.
In the absence of *tra* in XX females, the germ cells lost contact with their somatic counterpart hence germline stem cells were depleted from the niche in L3 larval gonad. That suggest a defect in germline stem cell self-renewal and explains the ultimate result in the absence of germ cells in the sex transformed adult gonad. In ectopic *tra* expression in XY males, the germ cells also lost contact with the surrounding somatic cells in the larval gonads which evolved into a germline tumor phenotype in adults. These observations highlight the importance of sexually matched somatic and germ line components for cell-cell contact and communication. At the transcript isoform level, wildtype sex-biased expression depends on both karyotypic and *tra* signal in a combinatorial, additive, or synergistic manner. For example, in testis the XY karyotype and *tra* absence is necessary for the characteristic primary spermatocyte expression program. Certain groups of transcripts are strictly expressed depending only on karyotype or *tra* status. This study highlights the importance of how sex-biased transcript expression is regulated by both karyotype and *tra* that drive sexually dimorphic gonad development.

311B Characterizing the role of *Myosuppressin receptor 2* in the growth of *Drosophila melanogaster*.

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In *Drosophila melanogaster*, developmental transitions and final body size are controlled by the release and production of the steroid hormone ecdysone from the prothoracic gland (PG) in response to various environmental and developmental cues. These cues are communicated to the PG via neuropeptide and peptide hormone signalling pathways. While one well known neuropeptide is prothoracicotropic hormone (PTTH), multiple other neurons innervate the PG[1, 2]. This suggests that more neuropeptides and neuropeptide receptors that regulate ecdysone production remain to be discovered and characterized. Our lab previously conducted an RNAi screen to identify novel neuropeptide receptors that generate growth and developmental timing defects when knocked down in the Drosophila PG[3]. One of the hits from this screen was *Myosuppressin receptor 2 (MsR2).* We have confirmed that there is a delay in time to pupariation when *MsR2* is knocked down in the PG using two independent RNAi lines driven by *phm-Gal4*. Body size was also measured using pupal volume and *phmGAL4*>UAS; *MsR2*-RNAi animals were found to have significantly larger body sizes compared to controls. The *MsR2* developmental delay phenotype was further validated by conducting developmental timing assays for the available *MsR2* mutant, MsR2 MB05984 . Homozygous *MsR2* mutant flies were significantly delayed in their pupariation compared to heterozygous control flies. Additionally, we have shown that feeding ecdysone to *phm-GAL4*>UAS; *MsR2*-RNAi animals restored their development back to normal. qPCR studies are being performed to analyse whether the expression of genes in the ecdysone biosynthesis pathway is altered when *MsR2* is knocked down in the PG. We are also planning to investigate where Ms is being produced, and which environmental cues activate Ms/MsR2 in the PG to further characterise the novel role of MsR2 in growth.

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312C Investigating the role of Notch signalling in the development of the ventral mesoderm in *Drosophila melanogaster*

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Notch signaling is a critical regulator of multiple developmental processes through its ability to control gene expression, and thereby influence cell fate specification and cell proliferation through direct cell-cell communication. Notch signaling is activated through binding of the transmembrane ligand, Delta, to the Notch extracellular domain (NECD) of the transmembrane Notch receptor. Ligand engagement and trans-endocytosis into the signal-sending cell drives removal of the labile NECD, and concomitant cleavage of the Notch intracellular domain (NICD). NICD translocates to the nucleus, where it regulates target gene expression through its interaction with Suppressor of Hairless Su(H). Although Notch signaling has been shown to play a role in regulating *single minded (Sim*) expression in the mesectoderm, Notch activity and function in the directly-adjacent cells of the ventral mesoderm remain unknown. Considering that Delta endocytosis and NECD trans-endocytosis are required for Notch signal activation, and are restricted to the mesectoderm and ventral mesoderm in the early embryo, we hypothesize that Notch signaling is involved in ventral mesoderm development.
To test our hypothesis, we have used a combination of, RNA *in situ* hybridization, qPCR, and Optogenetics. Through *in silico* analysis, we identified 13 potential Notch target genes based on the following three criteria: 1) expressed in the mesoderm, 2) contain a Su(H) binding site, and 3) are expressed differentially during mesoderm formation. These include; *WntD*, *Asph*, *Heartless*, *Traf4*, *Tinman*, *Twist*, *String*, *Stumps*, *Mef2*, *Mes2*, *mir-1*, *Neurotactin*, and *NetrinA.* In order to compare the level of gene expression between loss-of-function mutants in the Notch pathway and wildtype embryos, we have employed two orthogonal techniques; RNA *in situ* hybridization, and quantitative real time PCR. Consistent with our hypothesis, expression of these mesoderm-specific genes requires Notch signaling. These results have prompted us to ask whether Notch signaling is sufficient to drive expression of these mesodermal target genes. In order to address this question, we have developed a set of novel Optogenetic tools to ectopically activate Notch signaling in a precise spatio-temporal manner. We are currently validating these tools in the early embryo, and we anticipate that they will help us to further understand the underlying mechanisms that regulate both Notch signaling, and its role in ventral mesoderm development.

313A Spermatogenesis in *Drosophila pseudoobscura*, a sperm heteromorphic species

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The fruit fly *Drosophila pseudoobscura* is a sperm heteromorphic species, producing three distinct mature sperm morphs. Eusperm are the long (~350µm), fertilising morph. There are two parasperm morphs, which are not capable of fertilisation. Parasperm 1 are short (~50µm), and protect eusperm from spermicides in the female reproductive tract. Parasperm 2 are medium sized (~100µm), also protect eusperm from spermicides, and may have additional functions in sperm competition. While the functions of different sperm morphs have been studied, little is known about their development.
The aim of our project was to investigate the early stages of spermatogenesis in *D. pseudoobscura* and identify molecular mechanisms involved in the differential development of the three sperm morphs. Each spermatocyte cyst generates just one sperm morph, and we hypothesised that the transcriptional activity in these cells is critical for their later differentiation potential. We used RNAseq of manually isolated single cysts and cluster analysis to identify transcriptional signatures in the pre-meiotic spermatocyte cysts. This revealed differential patterns of gene expression between primary spermatocyte classes. We confirmed these patterns by *in situ* hybridisation of *D. pseudoobscura* testes, and identified several transcriptional regulators as candidate genes for further investigation.
We have developed integrated nos-*cas9* lines of *D. pseudoobscura* using the piggyBac transposon system. We are using these to tag and knock out genes identified as differentially expressed between spermatocyte cysts, with the aim of revealing the molecular mechanisms by which differential sperm development occurs in *D. pseudoobcura*.

314B Cofactor-dependent and -independent functions of Hox reveal two distinct evolutionary lineages of insect wing tissues

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The acquisition of novel structures is critical in facilitating organismal adaptation. Insect wings are a classic example of such a novelty whose origin remains a widely debated mystery. Most extant insects possess two pairs of wings (on the second and third thoracic segments, T2 and T3), while T1 and the abdominal segments are wingless. Using the *Tribolium* beetle, we have shown that there are two types of tissues (pleural and tergal) in the wingless T1 segment that are serially homologous to the wing. The suppression of these wing serial homologs (WSHs) from forming wing tissue is achieved by the action of the Hox protein in this segment. Reducing Hox input in T1 allows the WSHs to merge and form an ectopic wing. These observations led us to propose that insect wings have evolved from a merger of two previously distinct tissues that are maintained in a more ancestral state, namely that insect wings have a dual evolutionary origin. Although this hypothesis could potentially unify the

two currently competing wing origin hypotheses (i.e. tergal vs. pleural wing origin debate), it remains unclear how the two WSHs individually contributed to the evolution of the insect wing.
We reasoned that serial homology among WSHs can be further subdivided based on distinct modes of Hox action, allowing us to dissect the contribution of each WSH. We see an example of this distinction in the differentiation of the T3 wing in *Drosophila* (the haltere). The Hox gene acting in this segment (*Ubx*) controls the identity of the entire haltere, while the proximal region requires the action of the Hox cofactor complex, Homothorax/Extradenticle (Hth/Exd). This suggests that there are tissues which are Hox cofactor-dependent or -independent within the wing-related tissues including the WSHs. Here we investigated the Hox cofactor-dependence of the WSHs in *Tribolium* via RNAi for the cofactor genes. In T1 where the Hox gene *Scr* suppresses wing identity, we found that the pleural WSH is cofactor-dependent, while the tergal WSH is cofactor-independent in maintaining its ancestral state. In T3, the entire wing requires Ubx input, yet the proximal region also requires input from Hth/Exd. These results suggest that the pleural tissues contribute to the formation of proximal wing structures, while the more distal portion of the wing is formed from the tergal contribution. Our analysis will contribute in revealing the complex interactions that orchestrated the evolution of the insect wing.

315C Chitinase 10 controls chitin organisation in the *Drosophila* wing

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The *Drosophila* body cuticle is a stratified extracellular matrix produced by the underlying epidermis. The innermost layer of the cuticle, the so-called procuticle contains the polysaccharide chitin that adopts a crystalline organization. For a stereotypic assembly of chitin, we hypothesize that the length of chitin fibers released at the apical plasma membrane of the epidermal cells has to be controlled. Presumably, chitinases that degrade chitin may be involved in the process of chitin trimming during its assembly. In the present work, we have studied the role of chitinase 10 (Cht10) in the formation of the wing cuticle that is the simplest type of cuticle in the adult fly. We found that reduction of *cht10* expression by RNA interference (RNAi) results in increased chitin levels and disorganization of the procuticle. We incorporate these findings in the model of the molecular mechanisms of chitin production and organization.

316A Adherens junctions, transcription factor Mitf and Protein Phosphatase 2A function within the peripodial epithelium of the eye imaginal disc to regulate Yki and prevent retinal displacement

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Sensory organ development relies on a complex series of events, including tissue-level biomechanical and morphogenetic processes that are governed by a limited repertoire of signaling pathways. The *Drosophila* eye is a prime system in which to study these events, and the genetic regulation thereof. The precursor to the adult eye, the bilayer eye imaginal disc, is a flattened epithelial vesicle with apically apposed cell layers. One layer will form the retina of the adult eye, whereas the apposed peripodial epithelium (PE) functions primarily to promote retinal development during larval stages and in disc eversion during metamorphosis. The transition from retinal field to PE occurs along the fold or margin of this flattened vesicle. Here we report a novel phenotype, retinal displacement (RD), in which the PE/retina boundary is shifted at the time of neurogenesis in the eye disc. In RD, a portion of the neural retina becomes mislocalized over the posterior fold of the disc epithelium, shifting onto the PE side of the disc. Ultimately, RD flies exhibit misshapen eyes with abnormal curvature that are incompatible with compound eye vision. We have identified Yki as a critical factor that acts in PE cells to prevent RD, and we document the activity of known and novel regulators of Yki activity in this tissue. First, we demonstrate the critical role of adherens junctions (AJ), through Jub, in segregating Wts to promote Yki activity. Second, we uncover a requirement for the Mitf transcription factor to prevent RD, acting upstream of Wts but independently of AJs. Finally, we find that protein phosphatase 2A (PP2A) complexes with the B' regulatory subunit Wdb function upstream of Hpo and Wts to activate Yki. Unlike the reported role of Cka/B'''-containing STRIPAK-PP2A in down-regulating Hpo, PP2A-Wdb regulation occurs at or upstream of Sav, thus impinging on the antagonistic relationship between Sav and Rassf. Together with PE fate in the early larval disc (Zhang 2011; Neal/Zhou 2020) and photoreceptor subtype specification in the pupal eye (Jukam 2011, 2013; Xie 2019), RD marks the third aspect of *Drosophila* eye development in which the Hpo-Yki signaling axis has been implicated. Further study of RD will reveal potential distinctions in gene networks operating in these other processes, as well as how multiple convergent inputs (AJ, Mitf, PP2A) are integrated by the Hpo-Wts-Yki signaling axis to maintain proper disc morphology during eye development.

317B *Suboptimal intermediates underlie evolution of the Bicoid homeodomain*

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Changes in regulatory networks generate materials for evolution to create phenotypic diversity. For transcription networks, multiple studies have shown that alterations in binding sites of cis-regulatory elements correlate well with the gain or loss of specific features of the body plan. Less is known about alterations in the amino acid sequences of the transcription factors (TFs) that bind these elements. Here we study the evolution of Bicoid (Bcd), a homeodomain (HD) protein that is critical for anterior embryo patterning in *Drosophila*. The ancestor of Bcd (AncBcd) emerged after a duplication of a Zerknullt (Zen)-like ancestral protein (AncZB) in a suborder of flies. AncBcd diverged from AncZB, gaining novel transcriptional and translational activities. We focus on the evolution of the HD of AncBcd, which binds DNA and RNA, and is comprised of four subdomains: an N-terminal arm (NT) and three helices; H1, H2, and Recognition Helix (RH). Using chimeras of subdomains and gene rescue assays in *Drosophila*, we show that robust patterning activity of the Bcd HD (high frequency rescue to adulthood) is achieved only when amino acid substitutions in three separate subdomains (NT, H1, and RH) are combined. Other combinations of subdomains also yield full rescue, but with lower penetrance, suggesting alternative suboptimal activities. Our results suggest a multi-step pathway for the evolution of the Bcd HD that involved intermediate HD sequences with suboptimal activities, which constrained and enabled further evolutionary changes. They also demonstrate critical epistatic forces that contribute to the robust function of a DNA-binding domain.

318C Regulation of Glial Septate Junction proteins by microRNA-184

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Glial cells are crucial for providing structural and nutritional support to neurons, participating in immune responses and maintenance of the blood-brain/nerve-barrier (BBB). Permeability barriers are formed by septate junctions (SJ) in *Drosophila* to restrict diffusion of molecules/fluids/pathogens across tissues. SJs are comprised of a core complex of many proteins including: NeurexinIV (NrxIV), Contactin, Neuroglian, the Na/K-ATPase, Macroglobulin complement-related (Mcr) and the claudin-like proteins kune-kune (k-k) and sinuous (sin). Barriers are also formed at the convergence of three SJ with a complex of proteins Anakonda, M6 and Gliotactin (gli), which form the tricellular junction (TCJ). A specific class of glia, the subperineurial glia (SPG), form auto-SJs with themselves and each other to create the BBB around the brain lobes, the ventral nerve cord and around each peripheral nerve. Loss of any of the core SJ or TCJ proteins compromises the BBB leading to paralysis and lethality. While SJ and TCJs in other tissues such as epithelia have been extensively studied, less is known about the distribution of the SJ proteins in the CNS and PNS and its regulation in the nervous system. microRNAs, in particular microRNA-184 (miR-184) is known to target SJ proteins in epithelia, however the role of miR-184 targeting SJ in nervous system is unknown. We re-confirmed localization of NrxIV, which has already been well-characterized in the glia SJ. We established presence of k-k, Mcr, sin in glial SJ, and M6 and Gli in glial TCJ of CNS and PNS. We found that overexpression of miR-184 in the subperineurial glia leads to loss of these SJ and TCJ proteins and the complete degradation of SJ morphology. We are currently testing the integrity of BBB using dye penetration assays and testing the effects on larval locomotion. Loss of function miR-184 mutants will be assessed for effects on SJ and TCJ protein levels and distribution as well as BBB integrity. SJ mRNA levels in miR-184 overexpression and loss of function mutants will be quantified using qPCR. qPCR results will indicate if miR-184 directly targets all SJ mRNAs listed above, or if targets the mRNA coding for one of the SJ proteins which in turn, affects localization of other SJ proteins. Thus, we would also be able to conclude which and whether some SJ proteins are directly controlling expression/localization of other SJ proteins. Our study will reveal the key SJ proteins in SPG, their post-transcriptional regulation by miR-184 and their importance for BBB integrity. Degradation of permeability barriers in glial cells can result in a compromised BBB—a hallmark of many diseases. Thus, our findings will provide cues to such diseases and are applicable across species.

319A Ventral tissue fate in *Drosophila* leg is controlled in part by three distinct actions of the selector gene *midline*

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The patterning of Drosophila limbs is controlled in part by the regional expression of selector genes, which are transcription factors that mediate binary patterning choices. The T-box transcription factor gene *midline (mid)* (fly Tbx20) acts as a selector gene, causing cells to adopt a ventral pattern instead of dorsal. The ventral signal Wg (fly Wnt) specifies ventral fate by activating *mid* in all ventral cells. Dorsal fate is controlled by Dpp (fly BMP). Our work shows that *mid* specifies ventral fate via at least two pathways. In the first pathway, *mid* inhibits dorsalization by blocking Dpp signaling. Ectopic dorsal fate induced by tkv^{QD}, an activated Dpp receptor, is inhibited by simultaneous expression of *mid*. As well, *mid* mutant cells have increased levels of phosphorylated Mothers-Against-Decapentaplegic (pMad), a readout of the level of Dpp signaling. Expression of *mid* reduces the levels of pMad accumulation. These results are consistent for the Dpp-target gene reporter

dad-lacZ which displays increased expression in *mid* mutant cells and decreased expression when ectopic *mid* is present. Taken together, these results imply that the role of mid in dorsal inhibition is downstream of the Dpp receptor. We suspect that mid is interacting with genes involved in Mad phosphorylation, activation, or nuclear transportation and our research is currently investigating these possibilities. In the second pathway, *mid* directly promotes ventral fate. Genetic mosaics that lack mid and are blocked for Dpp signaling are not rescued to ventral fate in all but one ventral structure. Thus, mid also specifies ventral fate independent of Dpp signaling. Specification of ventral fate by *mid* requires a known repressing domain (eh1) and putative activating domains (TD1/2). Mid is a direct transcriptional repressor of several genes expressed in the ventral domain and a *mid* mutant in the eh1 domain is compromised in ventral fate specification in gain-of-function assays and rescue experiments. Although we have not identified genes activated by Mid, mutants in the *mid* TD1/2 domains are also compromised in ventral fate specification. We propose that mid specifies ventral fate though (1) inhibition of Dpp signaling and (2) coordinating the regulation of genes in the ventral leg.

320B Regulation of EGFR signaling outcome by localized JAK/STAT pathway activity in the posterior domain of the follicular epithelium

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The generation of the many diverse cell types that arise during development is achieved by a surprisingly small number of signaling pathways, implying that the same pathway can generate multiple outcomes depending on the signaling context. As a model, we study the follicular epithelium of the Drosophila ovary, where localized activation of the epidermal growth factor receptor (EGFR) leads to one of two different outcomes depending on the timing and location of signaling. Early in oogenesis, posteriorly localized EGFR activity induces expression of the transcription factors Midline (Mid) and H15. Later, EGFR activity translocates to the dorsal anterior, where it instead induces the transcription factor Mirror (Mirr). EGFR output thus depends on the signaling context, ultimately allowing this one localized signal to define both the AP and DV axes.
The choice between these two alternative EGFR signaling outcomes is mediated by additional positional information provided by opposing gradients of JAK/STAT and BMP activity, each of which promotes EGFR-mediated activation of one target while independently repressing the other. At the posterior, JAK/STAT signaling promotes *mid* and represses *mirr*. At the anterior, BMP signaling promotes *mirr* and represses *mid*. In addition, mutual repression between Mid and Mirr presumably stabilizes these outcomes. We have proposed that this regulatory network generates a bistable switch ensuring proper patterning of this tissue, but how these signals are integrated and interpreted by the cells remains unknown.
Here we address this question by focusing on the opposing regulation of mirr and mid in posterior follicle cells, which in early stages experience both EGFR and JAK/STAT signaling. By characterizing a transgenic reporter bearing a regulatory region recapitulating the early expression of *mirr*, we found a cluster of STAT92E binding sites required for posterior repression, suggesting that STAT92E represses *mirr* through direct interaction with this region. Using this same approach with *mid*, we were unable to find STAT92E binding sites required for expression, suggesting that the positive impact of JAK/STAT signaling on *mid* expression might be indirect. Indeed, we found that the JAK/STAT signaling regulates the EGFR effector *pnt* in posterior follicle cells, and that expression of *pnt* rescues loss of Mid observed in cells unable to respond to JAK/STAT signaling. Together, these results suggest that STAT92E represses *mirr* though direct binding to *mirr* regulatory sequences, but positively regulates the EGFR-mediated activation of *mid* by increasing the levels of the EGFR effector Pnt.

321C A novel transmembrane protein stabilizes damaged photoreceptors and preserves vision

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Varying diets that lack essential nutrients are a challenge for the nervous system. For instance, an insufficient supply of vitamin A causes a lack of light-sensing pigments that initially results in night blindness and, when chronic, causes photoreceptor death. Like human photoreceptors, vitamin A-deprived *Drosophila* photoreceptors are severely damaged and lack functional light-sensing Rhodopsins, but do not die. We hypothesized that the fly eye upregulates molecules that stabilize damaged photoreceptors and preserve vision. To identify such molecules and to elucidate how vitamin A deprivation affects the eye on the molecular, structural, and functional level, we used transcriptomics, proteomics, electrophysiology, and behavioral analysis. We identified the novel transmembrane protein Mps (Major photoreceptor stabilizer) that is dramatically upregulated in damaged photoreceptors. We found that Mps localizes to the membranes of the damaged light-sensing compartments, stabilizes them, and thereby preserves visual function. We also analyzed the intracellular mechanisms that trigger Mps upregulation and found that it is induced by a variety of Rhodopsin processing defects, including a fly model of retinitis pigmentosa. We propose that these findings have the potential to inform novel approaches to preserve photoreceptors and

visual function in human retinopathies.

322A (E)close but no cigar: how the histone modifier KDM5 is required to reach adulthood

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Across a wide variety of life cycles, steroid hormones function as critical signaling molecules that coordinate the cellular processes required for development. In *Drosophila*, the prothoracic gland integrates nutritional status with developmental signals to regulate growth and maturation through the secretion of the steroid hormone ecdysone. While the nutritional signals and cellular pathways that regulate prothoracic gland function are well studied, the transcriptional regulators that orchestrate the activity of this tissue are not yet understood. Here we show that lysine demethylase 5 (KDM5, aka Lid), a histone modifier involved in human cancers and developmental disorders, is essential for prothoracic gland function. Interestingly, animals lacking canonical KDM5 demethylase activity are viable, but *kdm5* null mutants exhibit delayed development and a failure to eclose. Although KDM5 is ubiquitously expressed throughout development, we have demonstrated that restoring KDM5 expression specifically within the prothoracic gland of *kdm5* null mutant animals is sufficient to rescue both the larval developmental delay and pupal lethality. Our studies show that KDM5 functions by promoting the endoreplication of prothoracic gland cells, a process that increases ploidy and is rate-limiting for the expression of ecdysone biosynthetic genes. Molecularly, we show that KDM5 regulates the expression of the receptor tyrosine kinase *torso*, which then promotes polyploidization and growth through activation of the MAPK signaling pathway. Taken together, our studies provide key insights into the biological processes regulated by KDM5 and expand our understanding of the transcriptional regulators that coordinate animal development. We are currently carrying out cellular signaling analyses and genome-wide experiments to define the mechanisms of KDM5-mediated transcriptional regulation in prothoracic gland cells.

323B Knockdown of Mad expression during *Drosophila* wing development results in cell death and pouch duplication

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The *Drosophila melanogaster* wing offers a well-established genetic system to study the effects that molecular and genetic alterations have on developmental processes. These studies can help gain insight on how genetic pathways impact cellular growth and tissue differentiation. As a result, the *Drosophila* wing aids in studying diseases related to genetic changes cellular growth and development, such as cancer. Here, we use the *Drosophila* wing to study the developmental consequences of downregulating Mad (Mothers Against Decapentaplegic), the downstream effector of the Dpp signaling pathway. Knocking down Mad expression levels in the wing results in an increase in cell death as well as pouch duplication, indicating that Mad has a dual role in cell survival and fate determination during development. Further we find that *Engrailed* driving the knockdown of Mad results in pupal lethality of the organism. Additional work investigating the connection between the Dpp and Fat-Hippo signaling pathways shows that while previous studies have shown that Mad has the ability to interact with Yki in overgrown wings, our data suggest that Mad and Yki have more independent roles in standard wing development. This highlights the importance of not inferring normal developmental function from overexpression and overgrowth environments.

324C Negative feedback regulation in *Drosophila* dorsal-ventral patterning

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Development of an organism is dependent upon proper regulation of gene expression. Initiation of gene expression often relies on long-range signals referred to as morphogens; these morphogens form concentration gradients that aid in specific activation of genes responsible for proper body patterning. In *Drosophila*, one such morphogen is Dorsal (Dl), a transcription factor that helps with patterning of the dorsal-ventral (DV) axis in the early embryo. The impact of Dl is further refined by gene regulatory loops that help to control the dynamics of the Dl gradient. One regulatory loop of interest is the negative feedback loop with Cactus (Cact). Cact is initially bound to Dl, sequestering it to the cytoplasm, but Toll signaling on the ventral side of the cell degrades Cact and allows Dl to enter the nucleus. There, Dl can activate target genes, one of which is Cact, suggesting that Dl may regulate its own inhibition.
Our work currently focuses on establishing a system through which Cact can be examined in live embryos. Protein expression and use during development is very rapid; the turnover of Cact happens too quickly for standard live imaging techniques, like fluorescent protein fusions. Fluorescent proteins like GFP do not have enough time to mature and fluoresce before the associated protein is degraded. With the help of the Rao Lab (NCSU CBE), we plan to use several methods (LlamaTags, FRET,

and BRET) through which to detect Cact in live embryos, expand upon our knowledge of its distribution, and examine the effects of a negative feedback loop on the dynamics of the Dl gradient.

325A The detachment of the blastoderm-vitelline envelope interaction and blastoderm chirality

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Gastrulation is a complex and well-coordinated process that, through a precise combination of tissue rearrangements and cellular migrations, leads to the segregation of the germ layers in the developing embryo. The principal issue of gastrulation is movement: both single cells and tissues change dramatically their relative positions over time, being strongly influenced in their movement by the interaction with their surroundings. Of particular interest to us is the role played by the vitelline envelope, the innermost layer of the protective eggshell that surrounds the blastoderm during insect development. Indeed, it has been recently described by our group that integrin-mediated attachment of the blastoderm to the vitelline envelope is required for the proper gastrulation of both *Drosophila melanogaster* and *Tribolium castaneum*. The disruption of the attachment alters dramatically the gastrulation morphogenesis in both organisms and in *Drosophila* the defect is easily scoreable as a twisted gastrulation phenotype. Exploiting this, we searched for additional molecular players involved in the attachment phenomenon besides integrins. Surprisingly, when quantifying the twisted gastrulation phenotype, we detected a bias in the handedness of the twist. This led us to hypothesize that the blastoderm-vitelline envelope attachment could have the role of keeping the embryo symmetric, preventing the germ band from following the intrinsic chirality of the tissue during the process of germ band extension. Since the earliest developmental process showing chirality is the formation of the gut, our results suggest that the left/right asymmetry could be established much earlier during gastrulation. Moreover, the deletion screening on mutants showing the twisted gastrulation phenotype provided a number of interesting candidate genes, which will allow us to look at the left/right asymmetry establishment in *Drosophila* embryo from a novel and surprising point of view.

326B Biosensor mediated detection of physiological cell competition

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In the wild, organisms are exposed to myriad environmental forces ranging from extreme temperatures, oxygen stress and ultraviolet radiation to nutrient deprivation and crowding. Considering these pressures, protection of tissue and organ robustness during growth becomes a crucial issue. *How do somatic tissues respond and adapt to exposure to stress? How does selective pressure influence tissue growth?* Cell competition has evolved as a surveillance mechanism that upholds tissue vigor by selecting for the healthiest cells and eradicating viable but less fit counterparts. Mechanisms underlying cell competition thus sense relative cellular fitness. *Drosophila* larvae feed, forage and grow to their maximum potential over three instars, and serve as an excellent genetically tractable model for study of cell competition. In growing imaginal discs, cell-cell signaling leads some cells to be recognized as relatively less fit (the “losers”), and they are instructed to undergo apoptosis. We speculate that cell competition buffers disc growth against environmental pressures. Here we take advantage of a signaling module, composed of factors co-opted from the immune system, that directs the outcome of cell competition. Activation of this module leads to Dredd/Caspase-8-mediated cleavage and nuclear localization of the active form of the NF-kB factor Relish, expression of the Hid pro-apoptotic factor, and death of the loser cells. We are constructing transgenic sensors to serve as genetic tools to detect competing loser cells. These sensors are designed to sense upstream events (initiation of the signaling cascade) or downstream events (effector activation) during competition, and will allow us to monitor tissues in real time while larvae are subjected to specific environmental stresses. Our goal is to generate tools that will yield mechanistic insights into the effects of ecological stress on tissue growth and development.

327C The walk through the notum: studying the order of macrochaetae pattern growth by the analysis of morphological mirror-like duplications of the adult Drosophila notum caused by the Pentathorax mutation

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Pentathorax (Pth) is a dominant mutation on 2nd with incomplete penetrance and expressivity. It results in a series of phenotypes of the wing, wing hinge, and the notum. The degree of expressivity is independent for the left and the right wing discs. This mutation causes occasional cease at a random stage in the growth of adult notum, accompanied by a mirror-like duplication of the structures already grown. In addition, this mutation, causes a similar effect in the structures of the wing hinge and the axillary apparatus, randomly breaking their growth, and forming posteriorly a mirror-like duplication of the already formed structures. This duplication of axillary apparatus prevents the formation of a normal wing blade, which is almost always absent, replaced by duplications of tegula and costa. In rare cases, a very small, twisted, and residual wing

blade may occur. The size, completeness, and morphological development of the original and the duplicate structures are in the reverse proportion. The configuration with most developed notum and scutellum originals and duplicates from both left and right sides makes a visual impression of five thoraxes in such an individual, that gave the name to the mutation. The phenotypes of each half-notum range into continuous series of incrementally, bristle by bristle, growing half-notum originals, allowing tracing the order of macrochaetae origin in the manner similar to DNA sequencing by analysis of the cumulated frequencies of the presence of each bristle on the half-notum originals of all sizes. More frequently the bristle appears on all the half-notum originals analyzed, the earlier it emerges in the ontogenesis. (This type of analysis we named the Walk Through the Notum.) It revealed that the macrochaetae appear in the order (A-P): Ps-Anp-PnP-Adc-Pdc-Asa-Psa, then (P-A): Psc-Asc-Ppa-Apa, split into two vectors, the anterior-posterior (A-P), and the posterior-anterior (P-A) one. These data were confirmed independently by the alternative bristle mapping method, the mosaic clone analysis. Macrochaetae, more related to each other, coincide in the same mosaic spot more frequently. The revealed model of macrochaetae growth is inconsistent with a simple gradient A-P growth, as well as with the model of diffuse isotropic growth from the same center, or random growth. Instead, we propose the organization of the bristle growth on the notum into two loops, embedded one into another.

328A Investigating the Nature of Transdetermination during *Drosophila melanogaster* Development

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During multicellular development, cells are in a pluripotent state though due to various cell signaling pathways and morphogen gradients cells begin to determine. When a cell does not adopt or retain the correct fate it can lead to various developmental anomalies. One way this happens is by transdetermination when a determined cell switches its fate to that of another tissue type without dedifferentiation. This process can be studied in *Drosophila melanogaster* via ectopic eye formation through the misexpression of the master retinal determination regulator *eyeless* (*ey*). For an ectopic eye to develop the cell must abandon its original fate to adopt a retinal fate. My preliminary observations indicate that ectopic eyes form through a biphasic state suggesting that for an ectopic eye to form, ey misexpression must meet certain criteria involving timing, distribution, and magnitude. Leading to my hypothesis that during transdetermination cells enter a biphasic state where depending on the temporal, spatial, and magnitude of a gene regulatory network misexpression, cells can readopt their original fate or proceed to their new fate. Using the UAS-GAL4 misexpression system I have shown that there is not only a critical spatial expression, developmental time, and expression level to which *ey* misexpression must occur for ectopic eye formation but also an uninvestigated requirement for ey expression in the eye antennal imaginal disc. I will further study these requirements for transdetermination with immunofluorescence, quantitative PCR, and single-cell RNAseq. Understanding the mechanism of transdetermination we can gain a better understanding of cell fate determination.

329B Tissue specific responses to EcR are potentiated by differences in chromatin accessibility

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In *Drosophila*, changes in the levels of the steroid hormone ecdysone titer function as a systemic cue to initiate diverse array of responses in target tissues. Genetically, ecdysone acts through it's its receptor, the transcription factor, EcR. Although decades of work have established that ecdysone pulses initiate a wide variety of transcriptional responses, the means by which this diversity is achieved remains incompletely understood. In this work, we investigated whether tissue-specific EcR binding was involved in this process. We focused on the developing salivary gland and wing imaginal disc because they undergo unique metamorphic responses to the pulse of ecdysone that initiates pupal development. The salivary gland produces and secretes glue gene products before ultimately undergoing programmed cell death, while the wing begins the process of metamorphosis into an adult appendage. We assayed gene expression changes in wildtype and EcR-RNAi wings over time and determined that a large fraction of genes were dependent on EcR for their correct temporal progression. Most of these genes were tissue-specific, including many genes identified as part of the core ecdysone response. To determine the role that differences in EcR binding played in directing these changes, we assayed EcR binding and found that many of its binding sites were specific to each tissue. Additionally, we found that tissue-specific binding was associated with differences in chromatin accessibility. We hypothesized that the association between binding and accessibility could either be a direct consequence of EcR binding, or, alternatively, that pre-existing differences in the open chromatin landscape could dictate the sites EcR could access. To test this, we assayed open chromatin in EcR-RNAi wings. We found that loss of EcR had almost no effect on the open chromatin landscape supporting the second model. An additional prediction of the second model is that is that tissues with similar open chromatin profiles should have similar EcR binding profiles. The developing leg has previously been shown to have a nearly identical open chromatin profile to the developing wing. We assayed EcR's binding and found that, as predicted, there were

almost no differences in EcR binding between wing and leg. Collectively, our data support a model in which differences in the accessibility of regulatory elements genome-wide potentiate EcR binding to promote tissue-specific responses to ecdysone.

330C Matrix Metalloproteinase 2 cleaves and destabilizes cell-surface glypican Dally-like protein to attenuate long-range Wg distribution and function

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The extracellular availability of secreted ligands is critical for activating signaling in target cells during development and tissue homeostasis. Cell-surface glypicans are evolutionarily conserved factors that play an important role in extracellular distribution of many secreted ligands including members of the Wnt family. Because glypicans play an important role in modulating extracellular ligand traffic and their activity, cell-surface glypican levels are likely regulated by other factors. In the *Drosophila* germarium, a tissue where oogenesis initiates, the glypican Dally-like protein (Dlp) promotes long-range extracellular Wg distribution from Wg-producing cap cells to Wg-responsive follicle stem cells inducing their proliferation, which is required for egg development. In genetic experiments, Matrix Metalloproteinase 2 (Mmp2) inhibits Dlp's long-range Wg distribution to restrict Wg signaling in follicle stem cells. Thus, Mmp2 acts as a molecular break on Dlp's long-range function. In this study, we investigate the mechanism by which Mmp2 inhibits Dlp's long-range function. In cell culture, Mmp2 cleaves and destabilizes Dlp on the cell surface. The cleaved N-terminal Dlp is shed in the extracellular media, and the C-terminal Dlp is internalized and degraded. Importantly, exogenously provided Wg is detected on the cell-surface of Dlp expressing cells but not on the surface of cells co-expressing Dlp and Mmp2. Further, the lack of cell-surface Wg localization in Dlp and Mmp2 co-expressing cells was not due to Wg internalization by cleaved Dlp. Intriguingly, when Wg is overexpressed in the same cells that express Dlp and Mmp2, cleaved Dlp sequesters more Wg than intact Dlp. Based on these and our previous observations, we propose a model wherein intact Dlp on the cell surface promotes long-range Wg distribution. In contrast, Dlp is destabilized when cleaved by Mmp2. Further, cleaved N-terminal Dlp sequesters more ligand, and the Dlp-Wnt complex is removed from the cell surface, resulting in attenuation of ligand distribution and function. We are currently investigating if Mmp2 cleaves Dlp *in vivo* and the effect of *Mmp2* gain or loss of function on extracellular Wg distribution in the germarium. Overall, this study identifies the molecular basis of protease-mediated inhibition of cell-surface glypican, which modulates ligand distribution and function.

331A Deciphering mechanisms of Egfr signaling during retinal cell fate determination with single-cell omics

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In the developing *Drosophila* eye, Epidermal growth factor receptor (Egfr) signaling is used reiteratively to initiate differentiation of most photoreceptor subtypes, as well as cone and pigment cells, from a common pool of undifferentiated cells. The exact molecular mechanisms by which a single signaling pathway can control the differentiation of many different cell types in the same tissue has not been elucidated. One hypothesis is that Egfr signaling activates distinct sets of downstream targets to induce differentiation of different photoreceptor subtypes. To test this hypothesis, we performed single cell RNA sequencing (scRNA-seq) on wild-type late larval eye discs. Our results show that the known photoreceptor subtypes form distinct transcriptional clusters. To determine if these clusters express distinct sets of Egfr signaling target genes, we identified a list of 1304 differentially expressed genes in photoreceptors R1-7 and intersected these with ~2500 putative direct target genes of Pointed (Pnt), the nuclear effector of Egfr signaling, generated from previously published (Webber et al., Dev., 2018) and our own Pnt ChIP-seq data. Of the 666 potential Pnt targets that appeared in both the scRNA-seq and ChIP-seq data sets, 31 genes have highly specific subtype expression patterns. We validated the expression patterns of 13 of these 31 subtype-specific genes in late larval eye discs using available transcription reporter lines. To assess the chromatin state of these putative Egfr signaling target genes in specific cell types, we performed single cell Assay for Transposase-Accessible Chromatin sequencing (scATAC-seq) on late larval eye discs. We are integrating our scATAC-seq and Pnt ChIP-seq data to identify potential subtype-specific Pnt bound loci. To test if these genes are indeed Egfr signaling targets, we performed scRNA-seq on late larval eye discs expressing a dominant negative form of Egfr with photoreceptor subtype specific drivers. We are also testing the roles of cell type-specific Egfr targets in photoreceptor subtype differentiation using inducible somatic CRISPR knock downs. In summary, our results show Egfr signaling transcriptionally activates different subsets of target genes in distinct photoreceptor subtypes and this is likely one mechanism by which Egfr signaling initiates the differentiation of multiple distinct photoreceptor subtypes in the eye.

332B Defining the role of the Rap1 GTPase function in eye development

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The fruit fly (*Drosophila melanogaster*) eye has long been a powerful model for how cell-cell interactions lead to complex cell fate decisions. Flies have two basic types of photoreceptors: "outer" photoreceptors on the periphery of each facet that sense motion, and "inner" photoreceptors in the facet center that sense color. The Rap1 protein functions as an on-off switch during cell interactions in multiple contexts in animal development. Reducing dominant *Rap1* function in certain cells during eye development results in a loss of inner photoreceptors, suggesting that Rap1 has a role in "inner" photoreceptor cell fate decisions. One important pathway for cell-cell interactions in multiple contexts in animal development involves the Delta signal from one cell activating the Notch receptor in a neighboring cell and triggering a decision about its fate. Although Rap1 and Delta/Notch are common players in cell fate decisions in many contexts in animal development, little is known about how they interact. We observe an increase in expression of a Notch target gene partly responsible for the cell fate decisions triggered by Notch activation, when Rap1 function is reduced in certain cells during eye development. This result suggests that Rap1 inhibits Notch activation. Accordingly, loss of either Delta or of Notch suppresses the loss of inner photoreceptors, while extra Notch enhances it. These observations set the stage for using this powerful model system to understand the mechanisms between Rap1 and Delta/Notch interactions during animal development.

333C Morphometric and spatial distribution analysis uncovers unexpected progenitor patterns in the adult *Drosophila* midgut

Fionna Zhu[1], Michael Murray[1] 1) University of Melbourne.

A primary function of epithelial tissue is to provide a protective barrier to the outside world. Epithelial maintenance and tissue homeostasis are essential for animal health and wellbeing. To maintain homeostasis, an epithelial tissue must respond to cell death and replace damaged or dead cells through turnover. A powerful system to study these processes is the adult *Drosophila* midgut. The adult midgut is a simple monolayered epithelium comprised of four cell types: absorptive enterocytes (EC), secretory enteroendocrine cells (EE), intestinal stem cells (ISC) and enteroblasts (EB).

To date, most studies on midgut regeneration have focused on cell fate decisions and the regulatory pathways that control ISC proliferation. However, a full understanding of regeneration must also take into account the complex morphogenetic behaviours of the cells that are the functional reservoir of regeneration, the EB cells. Here we explore how EBs maintain their spatial distribution and differentiate into polarised ECs through a mesenchymal-epithelial transition (MET).

To tackle the relationship between gene function and the underlying cell mechanics, we established a novel analysis pipeline that quantifies the morphometric and spatial distribution metrics. Using this system, we have uncovered an unexpected bimodal distribution pattern for wildtype EB cells in which cells either have a "dispersed" distribution pattern or are aggregated into large clusters. Such clusters are associated with newly differentiated ECs, suggesting they represent points of local regeneration. With the same pipeline, we also examined the role of Septate Junction proteins in EBs as potential regulators of the EB MET. While RNAi knockdown of *mesh*, *Snakeskin* and *Discs large* had differential effects on EB morphology, all three disrupted the wildtype bimodal distribution pattern and produced more clusters. EBs with *Discs large* knockdown also exhibits premature expression of EC marker Pdm-1, suggesting precocious MET.

We are currently applying our system to analyse EB dynamic behaviour, morphology and spatial distribution, in response to genetically-induced apoptosis and tissue damage using the colitis model chemical, Dextran Sulfate Sodium. Based on the distinct clustering of EBs and their motile behaviour, we present a working model for damage-induced tissue regeneration in the adult midgut.

334A Characterization of oxidative stress resistance in insulin-signaling impaired *Drosophila melanogaster*

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A disequilibrium in redox signaling and control can lead to overloading of the antioxidant machinery, and thus, to the accumulation of damage in the organism. This phenomenon, known as oxidative stress, contributes to the deterioration of the organism in a vast array of diseases, including diabetes. Flies with homozygous null mutations in insulin signaling are lethal, but viable heteroallelic mutants can be viable, exhibiting several phenotypes, like delay in development, smaller adult size, and hyperglycemia and/or dyslipidemia, which allows its use as a model for diabetes. Using *InR (Insulin Receptor)* and *S6k (ribosomal protein S6 kinase)* heteroallelic mutants and controls with the same genetic background, we investigated diabetic flies' resistance to two prooxidant compounds, H_2O_2 and paraquat. We find that *yw; InR*$^{3t5/el9}$ females, despite presenting an overall more severe diabetic phenotype compared to males (higher hyperglycemia and hyperlipidemia), when exposed to 3% H_2O_2, survive as long as control females, whereas males died earlier than their wild-type male counterparts. Exposure to 20

mM paraquat affected both sexes' survival in the same manner as controls. In *yw; S6k*$^{p1713/l-1}$ mutants, females exhibit higher hyperlipidemia compared to males, but mutant males were again more prone to death in 3% H_2O_2, compared to their wild-type controls. There were again no significant differences in survival to 20 mM paraquat. These results show an important variation in the response to different sources of oxidative stress, and a sexual dimorphism with opposing outcomes in terms of diabetic phenotype (stronger dysregulation in females) and oxidative stress resistance (stronger susceptibility in males), which warrants further exploration.

335B A larval model of cachexia recapitulates key hallmarks of the human disease

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Cachexia is a wasting disorder characterized by loss of skeletal muscle, conversion of white fat towards a brown fat appearance (beige/brite cell formation), and other systemic changes in metabolism. It is estimated to be the direct cause of death for approximately 1 million cancer patients per year globally. Yet, much remains unknown about its cellular and physical origins, its onset, and progression. To date no effective treatments have been identified. In *Drosophila* there are well established tumor models in the larval imaginal discs that generate system wide dysfunction which may yield a useful and genetically tractable model to gain insight into this devastating disease process. Here we present data characterizing the effects of imaginal disc tumors generated by co-expressing Ras^{v12} in cells with RNAi against the cell polarity gene *scribble* (ScribRas) on systemic phenotypes including muscle and fat morphology. In the crawling muscles of the larval body wall we observe thinning of the myofibrils over a period of 5-6 days that is coupled with progressive loss of sarcomeric structure. At late stages muscle fiber breaks are commonly observed. We also observe significant remodeling of the fat including morphological changes that appear to mimic beige cell formation which are detectable very early in the tumor progression. In preliminary screening we have identifed 3 candidate genes that can modify this phenotype when knocked down in the tumor. These results support that the system wide defects observed in the ScribRas tumor model recapitulate key features of the human cachexia and indicate this model may serve as a useful platform for discovery.

336C Investigating the roles of human genes in *Drosophila melanogaster*

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Understanding the signaling pathways that conserved genes are involved in is essential for discovering the etiology of diseases. Genetic screens using the model organism, *Drosophila melanogaster*, are a useful way of testing large quantities of human genes and have been crucial in uncovering the signaling pathways of genetic diseases in humans. Our primary data gathered comes from a genetic screen using GMR-Gal4 to drive UAS-(human gene) fruit flies. We examined the flies for changes in ommatidia and eye bristles. So far, we have already screened around 600 UAS-(human gene) flies and validated 49 human genes with altered eye phenotypes in Drosophila. Using biological databases, information was gathered about the genes that produced phenotypic changes in the Drosophila eye from the genetic screen project. Databases such as FlyBase, Ensembl, NCBI, and UniProt are efficient tools for finding information such as *Drosophila melanogaster* orthologues, evidence-based literature, gene products, and functions. Gene enrichment analysis conducted using the database, Gene Ontology, shows that these sets of genes are involved in the biological process of subpallium development and positive regulation of neuron death. Further analysis of cellular component also indicates that these genes are involved in glial cell projection. In addition, the data from the genomic profile database, PRECOG, shows some of these conserved genes are expressed highly in certain types of cancers, especially nervous system cancers, such as brain cancer glioma and neuroblastoma. In summary, the bioinformatic analysis through databases further provides us valuable information about the gene interactions and disease-relevance, which can be used to draw new hypotheses and later tested with further experimentation.

337A A peripheral HD model reveals dual modes of polyglutamine pathogenicity

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Huntington's disease (HD) is caused by expanded CAG repeats in the first exon of the huntingtin (Htt) gene. Huntingtin transcript and protein are expressed almost ubiquitously at different levels across the body and cellular aggregates have been reported in a wide range of tissues outside of the nervous system in HD model organisms suggesting that some of the symptoms of HD may originate in tissues other than the nervous system.
We used three muscle-specific GAL4 drivers (MHC, Mef2, DJ694) to express an N-terminal fragment of human huntingtin with 72 glutamine repeats tagged with eGFP (UAS-Htt72Q). Although all drivers resulted in the formation of cellular aggregates, the driver inducing the highest level of expression (Mef2) displayed a specific arrangement of the aggregates in a striated pattern and resulted in the most dramatic declines in longevity and locomotion. This led to the hypothesis that there is an expression

threshold in muscles that results in an alternate distribution of the aggregates and a drastic increase of the pathogenicity. This hypothesis was tested by elevating expression with MHC and DJ694 by increasing the number of copies of the UAS and GAL4 transgenes or by feeding antiprogestin (RU486). Although increasing expression level equivalent to that of Mef2 in the adult fly aggravated adverse effects, it did not result in the striated distribution. This would suggest there are two modes of toxicity. It was then hypothesized that striation is not occurring due to the level of expression but due to expression during development. The onset of the striated distribution was examined to occur in the late pupal stage. Unlike during the adult stage, the level of expression is correlated with the striated distribution in the late pupal stage.

338B CRISPR-engineered Drosophila knock-in models to study VCP diseases

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Valosin containing protein (VCP) is a hexameric type II AAA ATPase required for several cellular processes, including ER-associated degradation, organelle biogenesis, autophagy and membrane fusion. VCP contains three domains: a regulatory N-terminal domain and two ATPase domains (D1 and D2). Mutations in the N-terminal and D1 domain are associated with several degenerative diseases, including Inclusion body myopathy with Paget's disease of the bone and frontotemporal dementia (IBMPFD). However, patients with VCP mutations vary widely in their pathology and clinical penetrance, making it difficult to devise effective treatment strategies. Having a deeper understanding of how each mutation affects VCP function could allow us to better predict clinical outcomes and design personalized treatment options. Over-expressing VCP patient mutations in Drosophila has been shown to mimic many pathologies observed in human patients. The power of a genetically tractable model organisms coupled with well-established in vivo assays and a relatively short life cycle make them an attractive system to study VCP disease pathogenesis and novel treatment strategies. Using CRISPR, we have generated individual Drosophila knock-in mutants that include 9 hereditary IBMPFD mutations. We validate that these models display many hallmarks of VCP-mediated degeneration, including progressive decline in mobility, memory impairment and defects in lysosomal and mitochondrial function. These VCP disease models will be useful for studying the etiology of individual VCP patient mutations and test potential genetic and/or pharmacological therapies.

339C Characterizing the Molecular Function of the Mutagen Sensitivity Gene, *mus109*

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DNA repair pathways are essential in repairing damage that otherwise could cause genomic instability and cancer. Because mutations in DNA repair genes are linked to numerous human diseases, elucidating the molecular functions of DNA repair pathways will improve our understanding of disease mechanisms. *Drosophila melanogaster* has orthologs to most human DNA repair genes, so we can study mutants with reduced DNA repair function to investigate mechanism. Our work investigates the function of *mus109*, which is thought to be involved in DNA repair because allelic mutants are sensitive to various DNA damaging reagents. There are three available *mus109* mutant alleles: the lethal mutant loss of function allele $mus109^{ls}$, and the hypomorphic alleles $mus109^{D1}$ and $mus109^{D2}$. Our collaborators at Winthrop University used complementation crosses of these mutant alleles to map the location of *mus109* on the X chromosome and confirmed mutant sensitivity to the DNA alkylating reagent methyl methansulfonate (MMS). We performed a protein sequence alignment comparing *mus109* mutant alleles and found that mutations in each allele result in a loss of nuclease and/or helicase domains. To elucidate *mus109* involvement in various DNA repair pathways, we tested larval sensitivity of different allele combinations to DNA damaging reagents. We have preliminary data suggesting that $mus109^{1S}/mus109^{D1}$ and $mus109^{1S}/mus109^{D2}$ larvae are sensitive to bleomycin, which causes double-strand breaks, but the hypomorph $mus109^{D1}/mus109^{D2}$ larvae are not sensitive to bleomycin nor the single-strand break-inducer, camptothecin. These data indicate that *mus109* is involved in DNA repair pathways that resolve double-strand breaks and that *mus109* alleles have variable sensitivities to different mutagens. We plan to continue to assess *mus109* mutant larval sensitivity to reagents such as the replication fork staller, hydroxyurea, to further characterize the function of the gene in DNA repair.

340A Development of a *Drosophila* model of LGMD1F and drug screening

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The limb-girdle muscular dystrophy type 1F (LGMD1F) is an autosomal dominant disease characterized by progressive muscle weakness and atrophy of the proximal limb and axial muscles. LGMD1F is an ultra-rare disease, as only 60-70 patients worldwide have been diagnosed. The molecular cause leading to LGMD1F is a single nucleotide deletion in the *TNPO3* gene (c.2771delA) that results in the extension of the open reading frame by 15 additional amino acids. TNPO3 is a β-importin responsible for the nuclear import of serine/arginine-rich proteins involved in RNA splicing. TNPO3 has also been identified as essential for HIV-1 infection, and loss of TNPO3 function is protective against HIV-1. However, the role of TNPO3 in skeletal muscle and the effect of LGMD1F mutation in the *TNPO3* gene is unknown. There is currently no animal model in which to test candidate therapeutics and to investigate molecular pathways contributing to the disease. Here we report the first animal model of LGMD1F in *Drosophila melanogaster*. We have generated two transgenic lines that express wild type (*TNPO3wt*) and mutant human *TNPO3* (*TNPO3mut*), respectively, in a background silenced for the endogenous gene. *TNPO3mut* expression directed to the muscles of *Drosophila* impairs locomotive capacity (flight and climbing ability), decreases the half-life, and causes atrophy in the indirect flight muscles and abdominal ones. Moreover, the targeted expression of *TNPO3mut* to *Drosophila* motor neurons also reproduces the previous phenotypes and, besides, causes semi-lethality. Whereas *TNPO3wt* expression both in muscle and in *Drosophila* motor neurons does not produce these phenotypes. Therefore, our data suggest that the 15 additional amino acids present in TNPO3mut are critical for pathogenesis and are sufficient to reproduce the disease in *Drosophila*. Our next goal is to use this semi-lethality phenotype to carry out a high-throughput drug screening with the *Drosophila* developed model. This screening will give us not only the first candidate drugs for treatment against LGMD1F but also clues about how TNPO3mut causes muscular dystrophy.

341B Neurofibromin regulates metabolic rate via neuronal mechanisms

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Neurofibromatosis type 1 (NF1), is an autosomal dominant genetic disorder that results in tumor formation, altered cellular function, short stature, bone abnormalities, and increased rates of cognitive and developmental disorders in humans. NF1 is caused by mutations in the gene encoding neurofibromin (Nf1), a large protein that functions as a negative regulator of Ras signaling and mediates pleiotropic organismal and cellular functions. Emerging evidence suggest that Nf1 may regulate metabolism and energy expenditure, but the mechanisms are not well understood. Here, we show that Nf1 regulates metabolic homeostasis via neuronal mechanisms in *Drosophila*. Loss of Nf1 increases metabolic rate, feeding rate, starvation susceptibility, and alters lipid stores and turnover kinetics. These metabolic effects are independent of locomotion and grooming activity and map to a restricted subset of neurons in the ventral nervous system. The feeding phenotype maps to the same set of neurons as the metabolic effects, suggesting that increased feeding is a homeostatic compensatory effect. Finally, the Nf1 Ras-GAP-related domain is required for normal metabolic function, and that Nf1 regulates metabolic rate via neuronal mechanisms.

342C Identifying novel protein interactors of Abnormal Spindle, a key regulator of proper brain size.

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Autosomal recessive primary microcephaly (MCPH) is a clinical disorder characterized by reduced brain, intellectual disabilities, and reduced life span. The most common cause of human MCPH is homozygous mutations in the *Abnormal Spindle-Like, Microcephaly Associated (ASPM)* gene. *Abnormal Spindle* (*Asp*), the *Drosophila* ortholog of *ASPM,* is a microtubule-associated protein that has been shown to be enriched in mitotic spindle poles and localizes to the minus-end of spindle microtubules, where it functions to maintain proper spindle morphology and centrosome-pole cohesion. Interestingly, depletion of *Asp* in flies also leads to the development of the small brain phenotype, but this is independent of its role at the mitotic spindle, suggesting the involvement of additional cellular roles for Asp that are important for specifying brain size. We recently identified a small N-terminal truncation (Asp Minimal Fragment, or Asp^{MF}) that could rescue the brain size defect in *asp* mutant animals and identified 34 human proteins that could interact with the human ortholog of Asp^{MF} in a high throughput protein microarray. Here, we present an initial validation of these 34 potential interactors in flies. Results thus far show that *vasa intronic gene (vig),* ortholog of the human protein Serpine mRNA-binding protein 1 (SERBP1), is a binding partner of Asp^{MF}. Vig has a known role in RNAI and heterochromatin organization, hinting at potential mechanisms through which Asp might specify proper brain size. Additional experiments to further probe the genetic interaction between *asp* and *vig* in brain growth control and the results of our entire validation will be presented.

343A A CRISPR screen for modifiers of the rare disease DPAGT1-CDG (CDG-Ij)

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Partial loss-of-function mutations in glycosylation pathways underlie a set of rare diseases in humans called Congenital Disorders of Glycosylation (CDGs). Glycosylation is a broad category of sugar modifications on proteins and lipids, with functions ranging from complex post-translational modifications through the endomembrane system (e.g. N-linked glycosylation) to single sugar additions involved in cell signaling (e.g. O-GlcNAc-ylation). CDGs have a range of symptoms, but they commonly cause severe epilepsy, developmental delay, and/or disability. One such CDG is Type Ij which results from loss-of-function of the gene *DPAGT1* – the first step in N-linked glycosylation. Our goal is to better understand the pathways connected to *DPAGT1* loss, as well as glycosylation disorders as a whole, to develop potential treatment options.

We performed a CRISPR knockout screen using the drug tunicamycin (Tun), a known inhibitor of DPAGT1 function and inducer of endoplasmic reticulum (ER) stress, on *Drosophila*-derived S2R+ cells. Because N-linked glycosylation is linked to multiple CDGs, in addition to Type Ij, characterizing the genes that affect this pathway should help us better understand these disorders. Using a pooled format, a whole genome guide RNA library was introduced into S2R+ cells stably expressing constitutive Cas9. Pooled cell populations were grown for 10 generations with either vehicle or Tun. Final cell populations were examined for total guide RNA abundance (correlated with cell survival) to determine candidate genes causing Tun resistance or sensitivity.

Of the candidate genes, one pathway was particularly interesting: loss of major glycosylphosphatidylinositol (GPI) anchor biosynthesis genes (e.g. *PIGA/PIG-A*, *DPM1/CG10166*) was found to cause resistance to Tun. This suggests a novel, uncharacterized genetic interaction between N-linked glycosylation and GPI anchor biosynthesis. Other candidate gene pathways of note include the hexosamine pathway (e.g. *GFPT2/Gfat2*, *GNPDA1/Oscillin*), the mevalonate pathway (e.g. *DPAGT1/CG5287*, *NUS1/Tango14*), ER translocation (e.g. *EMC7/CG8397*, *SEC63/Sec63*), and trafficking (e.g. *RAB6/Rab6*, *RAB40/Rab40*), among others. We will present functional characterization of modifier genes in an *in vivo* *Drosophila* model of ER stress and the development of a new CDG-Ij fly disease model. Better understanding the role of modifier genes and pathways may lead to potential therapies for this rare disease.

344B Modeling age-induced polyploidy in *Drosophila*

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A characteristic of normal aging and age-related retinal disease, including macular dystrophy, is the formation of multinucleated cells within the retina pigment epithelium. Cell multinucleation can arise by endomitosis or cell fusion, resulting in a cell with more than the diploid copy of its chromosomes, hence polyploid. However, it remains unknown whether these polyploid cells function as a beneficial tissue repair strategy or a driver of disease. Like the retina, the adult fruit fly's abdominal epithelium is composed of terminally differentiated cells that when damaged by injury or with age also form multinucleated, polyploid cells. Here we utilize the cellular and genetic tractability of the fruit fly to identify the regulators of age-induced polyploidy. In the fruit fly, multinucleated cells arise by 20 days of age and continue to enlarge, generating giant polyploid cells with more than 30 nuclei in 40 day old flies. Polyploidy is not dependent on the M-phase genes (cdk1 and stg), but does require Rac GTPase. We further confirmed that age-induced polyploidy arises by cell fusion, not endomitosis, using the multicolor labeling technique, dBrainbow. In addition, we have found that knockdown of alpha-catenin, a macular dystrophy linked gene, causes multinucleation of the fruit fly epithelium similar to its effect on the human retina epithelium. Taken together, polyploidy arises within weeks in fruit fly instead of years in mice or decades in human. Using this model, we can now study of how disease linked genes result in polyploid cell growth and its effect on epithelial function with age.

345C Progeroid Barrier-to-Autointegration Factor disrupts tissue homeostasis due to defects in mitosis

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The nuclear lamina is a protein meshwork that lies beneath the nuclear envelope and contributes to transcription, DNA replication and genome integrity. Dysfunction of nuclear lamina components cause human disease, including premature aging or progeroid syndromes. Recently, an atypical progeria syndrome was identified, resulting from mutation of a gene encoding the nuclear lamina protein, Barrier-to-autointegration Factor (BAF). Patients with this Néstor-Guillermo Progeria Syndrome (NGPS) develop hair loss, thin skin, bone loss and stiff joints, but lack cardiovascular or metabolic disease prevalent in other progeroid syndromes. Drosophila BAF is 63% identical in amino acid sequence to human BAF, permitting use of the fly to model

NGPS *in vivo*.To this end, we used CRISPR to generate a progeroid *baf* mutation at the endogenous locus. Whereas complete loss of BAF is lethal, NGPS flies are viable and eclose at the expected frequency, indicating that some functions of BAF are preserved in the progeroid mutant. To identify processes affected by progeroid BAF, we investigated tissue homeostasis of the ovary, a tissue that requires BAF for GSC maintenance. We found that NGPS mutant flies have small ovaries and lay few eggs. Although these defects are not due to GSC death, GSCs display increased levels of DNA damage and death of differentiating germ cells. Indeed, we uncovered that these defects are linked to structural defects in the GSC mitotic spindle that impact chromosome segregation. Additionally, mitotic defects also occur in somatic cells of the ovary. Together, these data support a role for BAF in mitotic progression and suggest that progeroid BAF affects tissue regeneration as a result of genome instability leading to cell death.

346A High-volume functionalization of human *PTEN* variants in *Drosophila*

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With the advent of inexpensive exome and genomic sequencing, identifying disease causing variants has become a routine clinical practice. However, interpreting the functional consequences of identified variants remains challenging. The Clinical Genome Resource (ClinGen) Sequence Variant Interpretation (SVI) Working Group recently published guidelines for 'well-established' experimental assays for clinical variant interpretation. Here, we report the successful implementation of a scalable *Drosophila in vivo* assay in compliance with these guidelines. We screened ~100 human *PTEN* (*hPTEN*) variants implicated in cancer or autism for suppression of PI3K/AKT signaling dependent cellular proliferation in *Drosophila*, a pathway conserved between flies and humans. The assay correctly assigned the function of known pathogenic and benign variants and exhibited a high correlation with available human cell line functional data. We also showed that *hPTEN* functionally replaces its *Drosophila* ortholog (*dPten*) in developmental growth. Our work provides evidence that well-established assays, designed to directly test disease-relevant protein activity in *Drosophila*, can be used to generate reliable functional data appropriate for variant interpretation.

347B Spen modulates lipid droplet content in adult Drosophila glial cells and protects against paraquat toxicity

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Glial cells are early sensors of neuronal injury and can store lipids in lipid droplets under oxidative stress conditions. Here, we investigated the functions of the RNA-binding protein, SPEN/SHARP, in the context of Parkinson's disease (PD). Using a data-mining approach, we found that *SPEN* is one of many astrocyte-expressed genes that are significantly differentially expressed in the *substantia nigra* of PD patients compared with control subjects. Interestingly, the differentially expressed genes are enriched in lipid metabolism-associated genes. In a *Drosophila* model of PD, we observed that flies carrying a loss-of-function allele of the ortholog *split-ends* (*spen*) or with glial cell-specific, but not neuronal-specific, spen knockdown were more sensitive to paraquat intoxication, indicating a protective role for Spen in glial cells. We also found that Spen is a positive regulator of Notch signaling in adult *Drosophila* glial cells. Moreover, Spen was required to limit abnormal accumulation of lipid droplets in glial cells in a manner independent of its regulation of Notch signaling. Lack of Spen in glia was also associated with increased lipid peroxidation-associated damages. Taken together, our results demonstrate that Spen regulates lipid metabolism and storage in glial cells and may contribute to glial cell-mediated neuroprotection.

348C A *Drosophila* model of PIGA deficiency reveals gliopathic mechanisms of epilepsy and may identify potential therapeutic approaches

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PIGA deficiency is an ultra-rare X-linked epileptic encephalopathy caused by partial loss-of-function mutations in the *Phosphatidylinositol glycan class A (PIGA)* gene. It is characterized by early-onset epilepsy, intellectual disability, developmental delay, and several congenital anomalies. PIGA is the catalytic subunit of a complex involved in the first step of glycosylphosphatidylinositol (GPI) anchor biosynthesis, forming the intermediate molecule GlcNAc-PI. GPI-anchors attach the

C-terminus of a protein to the cell surface. GPI-anchored proteins are important for cell signaling, migration, and immunity. In the nervous system, they mediate neuron-glia interactions and are essential for axon guidance, synaptic plasticity, and myelination. It is unclear how loss of PIGA function contributes to the phenotypes observed in patients. We generated a model of PIGA deficiency in *Drosophila* using the GAL4-UAS system to deliver RNAi knockdown of *Drosophila PIGA* (*PIG-A)* expression in neurons and glia. Knockdown of *PIG-A* in neurons with *ELAV-GAL4* results in reduced lifespan and several neurological defects, but no seizure phenotype. Pan-glial knockdown with *REPO-GAL4* leads to reduced lifespan and a severe seizure phenotype, suggesting that defects in glial cells underlie the epilepsy phenotype in PIGA deficiency. Knockdown of *PIG-A* in subtypes of *Drosophila* glial cells with GAL4 drivers specific to surface glia, astrocyte-like glia, wrapping glia, and ensheathing glia will determine which subpopulations of glial cells contribute to the seizure phenotype, providing possible mechanisms underlying these gliopathic seizures. These distinct phenotypes reveal that different cell types underlie the various neurological symptoms in patients with PIGA deficiency. To investigate mechanisms underlying PIGA deficiency and identify potential treatments, we are performing an unbiased drug screen to identify compounds that rescue lethality phenotypes in homozygous *PIG-A* knockout flies and may rescue the seizure phenotype. We are also generating patient-specific models of PIGA deficiency, where each fly line will carry a different patient's disease allele. Characterization of these patient-specific models will reveal the genotype/phenotype correlation in PIGA deficiency and may also provide a path forward for precision medicine approaches to therapies. Altogether, this research will contribute to the understanding of PIGA deficiency and the development of effective therapies.

349A Altered expression of *foxo, Rbf, Buffy* and *Debcl* in novel Drp1-induced PD model in Drosophila

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Parkinson Disease (PD) can be influenced by mitochondrial dysfunction, increasing cellular stress and cell death. *Drp1* plays a key role in mitochondrial dynamics and function. Mitochondrial dysfunction causes unregulated mitochondrial dynamics (biogenesis to degradation), impaired metabolic functions, oxidative stress, loss of proteostasis and abnormal cell death. We have established that both *Drp1* LOF (Loss of function) and GOF (Gain of function) based models have PD-like phenotypes. Mitochondrial health is regulated by various genes such as *foxo*, *Rbf*, and *Bcl-2* (*Buffy* & *Debcl*) in Drosophila. The Bcl-2 family proteins play crucial roles in intrinsic apoptosis and mitochondrial health. Here, we explore the consequences of the altered expression of *foxo, Rbf, Buffy* and *Debcl* in *Drp1* loss and gain of function backgrounds.
In our experiments, we altered the expression of *foxo*, *Rbf, Buffy* and *Debcl* in selected neurons of Drosophila (via *Ddc-Gal4*) along with *Drp1* overexpression and inhibition. The GOF model of *Drp1* has compromised lifespan and health-span. However, when combined with the pro-survival effects of Bcl-2 family genes (*Buffy*: GOF or *Debcl*: LOF) or *foxo* or *Rbf* inhibition, both median lifespan and climbing ability were rescued over time. When combined with the expression of anti-survival transgenes (*Debcl*: GOF or *Buffy*: LOF), the lifespan and locomotor activity are similar to control or further diminished. The novel *Drp1* LOF model has impaired climbing ability over time but does not affect lifespan. Interestingly, if combined with Buffy (GOF) or Debcl (LOF) or Rbf (GOF), the phenotypes are modified but were not rescued. The pro-survival effect of Bcl-2 (*Buffy*: GOF & *Debcl*: LOF) was not sufficient. The Buffy (LOF), Debcl (GOF), foxo (LOF) and Rbf (LOF) either does not affect or further diminished the longevity and climbing ability.
The role of *Drp1* along with *foxo, Rbf, Buffy, and Debcl* in the control of mitochondrial health and cell death pathway appear to be key to the longevity and health-span of flies. The results of our experiments help us understand the regulatory relationship and phenotypic effect of altering the expression of *foxo, Rbf, Buffy* and *Debcl* in combination with *Drp1* that predominantly controls mitochondrial dynamics. Overall, our experiments allow us to contribute to our understanding of mitochondrial health and enhanced conditions of homeostasis. Funded by School of Graduate Studies Fellowship and by an NSERC Discovery Grant.

350B Modeling muscular dystrophy in Drosophila*:* A study of lamins and interaction partners

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Mutations in the human gene *LMNA* cause a collection of disease known as laminopathies, which includes rare muscular dystrophies, fat disorders, and early onset aging syndromes. The muscular dystrophies are characterized by muscle contractures, weakness, and muscle deterioration with different muscles affected and ages of onset. The *LMNA* gene encodes the A-type lamins, lamin A and C, produced by alternative splicing. Lamins are filamentous proteins that form a meshwork inside the nuclear envelope. This meshwork provides a structural scaffold for the nuclear envelope and plays an important role in regulating gene expression. Lamins have a conserved domain structure with an N-terminal head, central coiled-coiled rod, and C-terminal tail, which contains an immunoglobulin-like fold (Ig-fold). The goal of my project is to understand how single amino acid substitutions in lamins cause diverse disease phenotypes.
The first part of this project investigated how the location of amino acid substitutions within lamins A/C correspond to specific

diseases and how they potentially alter interactions with partner proteins. An *in silico* analysis of disease-related amino acid substitutions in lamins A/C revealed no apparent correlation between the location of the amino acid substitution and disease phenotype. Molecular modeling revealed that specific amino acid residues altered in disease mapped to potential protein partner interaction sites.
To test the predictions of our *in silico* analysis of protein partner interactions, we focused on amino acid residue R249 in the rod domain, which when altered to a Q causes Emery-Dreifuss muscular dystrophy. The equivalent amino acid substitution (R264Q) was modeled into the Drosophila orthologue *Lamin C* and used to generate transgenic flies. Wild-type and mutant *Lamin C* was expressed in larval body wall muscles using the Gal4/UAS system. Immunohistochemistry showed that wild-type Lamin C localized to the nuclear periphery as anticipated. In contrast, R264Q caused severe nuclear lobulation and nuclear pore mislocalization. Taken together, these data suggest R264Q disrupts the lamina network, causing other nuclear envelope proteins to mislocalize. Our future directions include analysis of partner proteins that interact with the R264 to determine if a loss of their interaction contributes to nuclear and muscle defects.

351C Conservation of a GAP independent function of the DLC3/Cv-c RhoGAP proteins required for male gonadogenesis

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HIGHLIGHTS:
- *Drosophila* Cv-c RhoGAP null mutations experiment testis dysgenesis akin to human patients with mutations in the homologous DLC3 RhoGAP protein.
-The DLC3/Cv-c male gonadogenesis function is independent of their Rho GAP function and requires a functional START domain.
-Cv-c is required to maintain the germ cell ensheathment in *Drosophila* testis.
- DLC3 rescues testis dysgenesis in *cv-c* null mutants suggesting a deep functional evolutionary conservation.

The DLC3 RhoGAP human protein has been implicated in a case of 46,XY gonadal dysgenesis where two patients inherited a mutation in the START domain, however, no definitive confirmation has been provided yet linking this mutation with male gonadal dysgenesis.
DLC3 belongs to a subfamily of RhoGAP proteins containing three conserved domains: a SAM, a GAP and a START domain. The three domains are also present in the homologous *Drosophila* Cv-c RhoGAP88C protein and DLC3 can functionally substitute for Cv-c. We have previously analysed Cv-c activity in the ectoderm where the GAP domain is absolutely required for its function. However, Cv-c mesodermal requirement has not been analysed yet.
We show Cv-c is specifically expressed and required in the male gonadal mesoderm. *In vivo* analysis of *cv-c* null mutants deleting the GAP and START domains shows normal testis development up to gonad coalescence at st15. However, after st15 the germ cells become extruded from the testis due to their defective ensheathment by mesodermal interstitial gonadal cells, which express lower levels of E-Cad and Neurotactin in the mutants.
Surprisingly, mutants for the *cv-c*^{7} allele, a point mutation only lacking a functional GAP domain, have normal testis. We also find Rho1 mutations do not normalize *cv-c* null mutations indicating a novel Rho GAP independent function in the gonad.
Expression of Cv-c protein variants lacking a functional GAP domain can rescue testis development but not mutants lacking the START domain. Interestingly, human DLC3 can partially rescue *cv-c* null gonad defects but not the DLC3 START mutant allele present in the human patients.
Our results show a new Rho GAP independent specific function for this protein family that is required for testis development and has a deep evolutionary conservation.

352A The oncoproteins H3 K27M and EZHIP inhibit PRC2 by conserved mechanisms in mammals and *Drosophila melanogaster*

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Central nervous system (CNS) tumors are the leading cause of solid tumor death in children. Among the deadliest and most common pediatric brain tumors are diffuse intrinsic pontine glioma (DIPG) and posterior fossa ependymoma type A (PFA). Most DIPG tumors harbor a lysine-to-methionine mutation at residue 27 on histone H3 (H3 K27M). Nearly all PFA tumors feature elevated expression of the previously uncharacterized protein EZHIP. These tumors arise from different cell types and harbor distinct molecular drivers but share remarkable similarities, including a near-complete loss of histone H3 trimethylation at lysine 27 (H3K27me3), a mark that contributes to transcriptionally silent chromatin. We have previously shown that H3 K27M and EZHIP are potent inhibitors of the H3K27me3 histone methyltransferase Polycomb repressive complex 2 (PRC2) in

mammalian cell culture. Given that H3 K27M and EZHIP both robustly inhibit PRC2, it remains unclear why they are enriched in DIPG and PFA, respectively. We suggest that the combination of mechanistic differences by which these proteins inhibit PRC2 and the specific developmental contexts in which they are expressed give rise to distinct disease states. To better understand how these mutations lead to discrete tumor types, it is imperative to study them within the context of organismal development. Because of the high degree of PRC2 conservation between mammals and *Drosophila,* we are using flies to address these fundamental questions. In mammalian cell culture, both H3 K27M and EZHIP reduce global levels of H3K27me3, but residual H3K27me3 remains at sites of PRC2 recruitment. We have shown that both H3 K27M and EZHIP preferentially inhibit catalytically activated PRC2 and propose that by specifically inhibiting this form of the enzyme, which is formed at sites of PRC2 recruitment, EZHIP and H3 K27M prevent further spread of the histone methyl mark. Because sites of Polycomb recruitment are well defined in *Drosophila,* we tested whether inhibition of PRC2 spreading by H3 K27M and EZHIP was conserved. In *Drosophila* cell culture, as in mammalian cell culture, EZHIP expression causes a global H3K27me3 reduction, but the mark is retained at sites of PRC2 recruitment. Thus, despite the fact that PRC2 is recruited to the genome by different mechanisms in flies and mammals, EZHIP-mediated inhibition of PRC2 is conserved. We have extended our studies beyond cell culture and shown that transgenic expression of either H3 K27M or EZHIP inhibits PRC2 *in vivo* and causes mutant phenotypes in the eye or wing. Together these data demonstrate the conserved mechanisms by which these oncoproteins cause disease and provide the groundwork for ongoing studies that will allow us to identify molecular pathways that modify these mutant phenotypes.

353B A small molecule ion channel screen to suppress gliopathic epilepsies

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Duplication 15q syndrome (Dup15q) is caused by the presence of at least one extra copy of the 15q11.2-q13.1 region. Characteristics of Dup15q include hypotonia, intellectual disability, autism spectrum disorder and, in the majority of isodicentric 15 cases, pharmacoresistant epilepsy. We previously established a fly model that successfully recapitulates the seizure phenotype observed in Dup15q individuals by overexpressing Drosophila Ube3a (Dube3a) in glial cells. To identify new drugs to treat epileptic individuals, our lab developed a medium throughput screening method to repurpose previously FDA or otherwise approved chemical libraries for their ability to suppress seizures. We recently used this model to screen 1,280 FDA or otherwise approved compounds from the Prestwick Chemical Library. Eight compounds were identified that reduce seizure recovery time by at least 50% in both male and female flies. Most of these compounds act through serotonin or dopamine receptors and can modulate Na^+/K^+-ATPase activity in glial cells. Here we evaluate 70 compounds from the Screen-Well Ion Channel Ligand Library for their ability to suppress seizures in our glial cell specific epilepsy model. The primary screen was composed of 24 calcium channel modulators, 23 potassium channel modulators, 10 sodium channel modulators, 7 intracellular calcium modulators, and 6 other miscellaneous drugs. We identified 8 compounds that suppress seizures in *repo>Dube3a* flies by at least 50%. Seventy-five percent of these compounds are potassium modulators and 25% are calcium modulators. ATP-sensitive Inward Rectifying K+ channel (KATP) modulation is a shared commonality among 3 of the 8 compounds. To evaluate the potentially critical role KATP modulation plays in seizure suppression, as well as to investigate the importance of glial-specific neuronal modulation, we are currently testing drug efficacy on flies that simultaneously express Dube3a and an RNAi against the *Drosophila* homologue for KATP (*Irk*) in glial cells. We expect to find that KATP modulators fail to suppress seizures in the absence of sufficient irk channel expressions. These studies will lead directly to new candidate drugs, specific ion channel agonists and antagonists, that may eventually be used clinically to suppress seizures in Dup15q syndrome.

354C Transmission of ethanol tolerance to progeny of repeatedly intoxicated parents in *Drosophila melanogaster*

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There has been a renewed interest in the transmission of acquired traits, particularly those related to the acquisition of tolerance to drugs and environmental toxins. Since tolerance is an accepted pre-requisite and strong predictor of addiction, a firmer understanding of the mechanisms underlying the transmission of tolerance could facilitate novel research avenues of importance in public health. Despite the significant advances made in characterizing the epigenetic and molecular mechanisms that underlie the transmission of acquired traits, it is still unclear what genetic pathways may connect acquired traits that operate primarily in metabolic, physiologic or nervous system levels with the necessary epigenetic modifications that must take place in the germline for transmission to take place. While classic model organisms, such *D. melanogaster* and *C. elegans* are prime candidates to dissect conserved aspects of the genetic and epigenetic underpinnings of acquired trait inheritance, little is known about their capacity to transmit acquired drug tolerance to their progeny.

Numerous previous studies over the last 20 years have demonstrated that fruit flies can develop tolerance to ethanol when repeatedly exposed to the drug. However, to our knowledge, no one has systematically addressed whether the progeny of repeatedly intoxicated flies can inherit increased tolerance to ethanol. Here we show that parental flies that are intoxicated multiple times (once a day, for 10 minutes, over a 2 weeks period) give rise to progeny that is significantly more tolerant to the sedative effects of ethanol. Our results indicate that the parental flies need to be exposed multiple times before tolerance transmission can be observed. We have also observed that there is a residual transmission of tolerance to the F2 and that the ability of female flies to transmit tolerance to their F1 lasts several days after their last intoxicating exposure to ethanol. Overall, our results support the exciting potential of *D. melanogaster* in forward and reverse genetics approaches to investigate the genetic pathways connecting repeated exposures of an adult animal to ethanol and the necessary germline modifications that would underlie the transmission of increased tolerance to their progeny.

355A Genotoxicity of water from the La Estanzuela dam in Mexico in the *Drosophila* wing spot test.

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The “La Estanzuela” dam is a water body located between “Mineral del Chico” and Pachuca municipalities in Hidalgo, Mexico, mainly used to supply them with water. Since 2012 there´ve been reports in national newspapers that make evident the contamination of this site, such as the appearance of dead fish and the presence of silver nitrite. The present work attempts to find out if the dam’s water is genotoxic by performing a SMART assay in the wings of the model organism *Drosophila melanogaster*. To accomplish this, three shallow and deep-water samplings of the dam were taken at different times of the year. The assay was carried out on flies belonging to two crosses, a standard cross (ST) and a high bioactivation cross (HB), exposed to water concentrations of 100 and 50%. Furthermore, a chemical analysis was performed in order to detect the presence of certain potentially genotoxic metals in the water samples.
The result of the SMART assay was positive for the three water samples, both shallow and deep-water at 100% for the ST and HB crosses; and in the case of the HB cross, also for shallow and deep water at 50% in the first sampling. The chemical analysis detected, among other data, aluminum concentrations that exceed the limit established by NOM-127-SSA1-1994. These results indicate that the dam water is genotoxic, and hence, that it´s capable of inducing mutations in *Drosophila melanogaster.*

356B *Drosophila* as a model for defining diets to treat inborn errors of amino acid metabolism

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Inborn errors of amino acid metabolism (IEaaM) encompass a large group of 150 monogenic metabolic disorders that collectively impact 1 in 6,500 live births. Most of these disorders have a neonatal onset and are severely debilitating. Fortunately, IEaaM are unique in that dietary-based interventions can successfully prevent or reduce their symptoms (e.g. phenylketonuria). Despite this, only 35% of IEaaM currently have a treatment available. This is owing to their rarity which precludes the ability to conduct clinical trials. We are addressing this problem by the systematic modelling of IEaaM using *Drosophila*. The fruit fly genome contains 100 conserved single orthologs out of the 150 IEaaM human related genes. Using gene disruption technology (CRIMIC-T2A Gal4), we are generating *Drosophila* knockout strains for these genes to characterize the underlying biochemical defects associated with each disorder and performing dietary screening to identify new treatments. Our pipeline incorporates loss-of-function phenotyping, humanization, characterization of metabolomic profiles and the use of fully customisable diets. The phenotypic characterization will provide great insights into the pathophysiology of these disorders in humans. The existence of a fully customisable *Drosophila* synthetic diet offers great advantage. This diet can be systematically modelled to test its ability to rescue lethality or adult phenotypic defects of the mutant flies. The knowledge and diets developed in this work will be an important step forward in characterizing these disorders and guiding clinical interventions for the more than one million IEaaM patients worldwide.

357C Knock-down of the pre-mRNA splicing factor *SNRNP200* causes headless pupae and rough eyed adults

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Retinitis Pigmentosa (RP) is an inherited retinal disorder that affects 1 in 4000 individuals in the United States. The disorder presents in the first two decades of life with night blindness due to apoptosis of rod photoreceptors. As the disease progresses, there is loss of peripheral vision and total blindness due to death of cone photoreceptors. RP is caused by mutations in many

genes, including *SNRNP200,* which encodes an RNA helicase that is a core component of the spliceosome. Helicase activity is needed for proper splicing of pre-mRNAs into mature mRNAs, which occurs in all cell types. Interestingly, degeneration only affects retinal cells despite *SNRNP200* expression in all cells. The mechanisms by which mutations in *SNRNP200* cause retinal cell death are unknown. To address this issue, we are investigating *SNRNP200* function in the Drosophila retina. The Drosophila orthologue of *SNRNP200* is encoded by the gene *l(3)72Ab*, which we term *dSNRNP200*. The two genes share 74% identity and 87% similarity. RNAi knockdown with the *eyeless-Gal4* driver, which is expressed in early eye development, caused pupal lethality. Larvae lacked eye/antennal discs and dead pupae lacked the entire head. This is likely due to the fact that the head capsule forms from cells within the eye/antennal disc. In contrast, RNAi knockdown *dSNRNP200* using the eye-specific *GMR-Gal4* driver, which expresses later in development than *eyeless-Gal4*, led to increased apoptosis in the larval imaginal eye disc, compared to controls, and a rough eye phenotype in adults. Thus, knock-down of *dSNRNP200* in the early eye imaginal disc caused complete loss of all head structures, indicating a requirement in primordial eye discs and suggests that *SNRNP200* is critical for the splicing of *eyeless* and/or *twin of eyeless*, which also produce headless pupae when mutant. Knock-down of *dSNRNP200* by *GMR-Gal4* caused apoptosis of photoreceptors, similar to the human disease, providing support for the use of Drosophila to model human RP patient mutations.

358A Mistargeting of secretory cargo in retromer-deficient cells

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Intracellular trafficking is a basic and essential cellular function required for delivery of proteins to the appropriate subcellular destination; this process is especially demanding in professional secretory cells, which synthesize and secrete massive quantities of cargo proteins via regulated exocytosis. The *Drosophila* larval salivary glands are professional secretory cells that produce and secrete mucin-like “glue” proteins at the onset of metamorphosis. Using mucin secretion in the larval salivary glands as a model system, we have identified a role for the highly conserved retromer complex in trafficking of secretory granule membrane proteins. We demonstrate that retromer-dependent trafficking via endosomal tubules is induced at the onset of secretory granule biogenesis, and that recycling via endosomal tubules is required for delivery of essential secretory granule membrane proteins to nascent granules. Without retromer function, nascent granules do not contain the proper membrane proteins; as a result, cargo from these defective granules is mistargeted to Rab7-positive endosomes, where it progressively accumulates to generate dramatically enlarged endosomes. Retromer complex dysfunction is strongly associated with neurodegenerative diseases, including Alzheimer’s disease, characterized by accumulation of amyloid β (Aβ). We show that ectopically expressed human amyloid precursor protein (APP) undergoes regulated exocytosis in salivary gland cells and accumulates within enlarged endosomes in retromer-deficient cells. These results highlight recycling of secretory granule membrane proteins as a critical step during secretory granule maturation and provide new insights into our understanding of retromer complex function in secretory cells. These findings also suggest that missorting of secretory cargo, including APP, may contribute to the progressive nature of neurodegenerative disease.

359B *Traip* suppresses chromosome bridges via mitotic DNA repair to control brain size

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Microcephaly is a developmental failure to achieve proper brain size and neuron number, and is thought to reflect loss of proliferation and/or increased cell death. Most microcephaly-linked genes function at the mitotic spindle or in DNA damage repair (DDR), but few are well-studied in neurogenesis, and whether they control brain size via common pathways is unknown. Thus, we are characterizing microcephaly genes in *Drosophila*. Here, we found that the microcephaly gene *Traip/nopo* controls brain size by suppressing DNA bridges during mitosis. *Traip* mutant flies have brain structural defects, fewer neurons, and loss of neuroblasts (NBs). *Traip* mutant NBs have increased DNA damage in interphase, consistent with a DDR function. However, we were surprised to also find polyploid *Traip* mutant NBs, suggesting mitotic failure. Live fluorescence microscopy of *Traip* mutant NBs revealed frequent mitotic DNA bridges, providing a possible explanation for polyploidy via cytokinesis failure. Traip has nuclear localization in interphase, whereas in mitosis it localizes on the spindle and furrow, possibly promoting encounters with DNA bridges. A Traip variant lacking the nuclear localization signal (ΔNLS) is evicted from the nucleus and fails to suppress interphase DNA damage; however, ΔNLS Traip localizes normally in mitosis and rescues *Traip* mutant brain phenotypes, showing that a mitotic *Traip* function is sufficient to suppress microcephaly. A possible link between DNA bridges and mitotic failure is the abscission checkpoint; inhibiting the abscission checkpoint suppresses *Traip* mutant brain phenotypes, suggesting this is a key downstream effector. Together, our work challenges current thinking about the relationships between DDR, mitosis and microcephaly by showing that, rather than merely repairing DNA damage during interphase, the primary function of Traip is to resolve mitotic DNA bridges and thus rescue NBs from mitotic failure. Now, using *Traip* as a model microcephaly gene, we are using whole brain imaging and 3D analysis to screen

for suppressors to uncover more downstream pathways in microcephaly. To date, we have found roles for neuronal stress response, Toll signaling, and caspase-dependent cell death. We are now testing whether these pathways also mediate the phenotypes of other microcephaly mutants, and whether these pathways could be potential therapeutic targets to minimize neuron loss in microcephaly.

360C New alleles of *spastin*: a model for Hereditary Spastic Paraplegia and opportunity for undergraduate education

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Autosomal Dominant-Hereditary Spastic Paraplegia (AD-HSP) is a progressive, neurodegenerative disorder characterized by spasticity and weakness in the lower limbs and disrupted mobility. The disease primarily results in degeneration of the longest axons of the CNS, which originate from the upper motor neurons in the brain. Mutations in many different genes have been shown to result in AD-HSP, but about 40% of cases can be attributed to alterations in the SPASTIN gene (SPG4) leading to loss of protein function. Given that *Drosophila* has an evolutionarily conserved ortholog, Spastin, with 67% amino acid similarity, the protein functions can be studied effectively in this *in vivo* animal model. *Drosophila* lacking *spastin* also demonstrate disrupted locomotion, as well as changes in synaptic bouton morphology and weaker synaptic transmission at the neuromuscular junction. These studies have relied primarily upon a single allele, $spastin^{5.75}$, which deletes the entire gene. Using EMS mutagenesis, we generated four putative novel alleles. Here, we took advantage of genetic screening techniques in *Drosophila,* leveraging the reduced eye phenotype that results from overexpression of endogenous Spastin. We screened for mutant lines that, when overexpressed in the eye, resulted in less eye reduction due to less functional ectopic Spastin. We then characterized the new alleles during a semester-long undergraduate lab course. Our class of 32 undergraduates determined the site of mutation and the effect on animal viability, locomotion, and neuronal morphology. This system provided an excellent way to expose students to an array of assays possible in fruit flies, including molecular biology, sequence analysis, animal husbandry and behavior, fine dissection, immunocytochemistry, and microscopy, all within the context of a novel research project. We found that two new alleles ($spas^{EFO6}$ and $spas^{EFO7}$), which contain missense mutations of conserved residues within the ATPase domain, also display the greatest severity in cellular and behavioral phenotypes, consistent with those observed in $spastin^{5.75}$ mutants.

361A A genetic and physiological model of renal dysfunction for Lowe syndrome in *Drosophila melanogaster*

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Lowes syndrome is a rare X-linked recessive genetic disorder characterized by clinical features of renal tubular dysfunction, mental retardation and cataract. It results from the mutations in the *OCRL1* gene encoding an inositol polyphosphate 5-phosphatase ($PI(4,5)P_2$). *In vitro*, OCRL1 is proposed to dephosphorylate $PI(4,5)P_2$ to generate PI4P and thus maintain the maintain the balance of these two lipids. The OCRL gene product required to regulate the phosphoinositide composition of several cellular membranes thus controlling key sub-cellular processes including membrane transport and cytoskeletal function. However, the molecular and cellular basis of renal dysfunction in Lowe patients is poorly understood and the absence of adequate cellular and physiological models remains a limitation.
In *Drosophila,* nephrocytes show anatomical, molecular and functional similarity to human glomerular podocytes. It is reported that *OCRL* gene is conserved across common metazoan model including *Drosophila melanogaster* whose genome encodes a single *OCRL* ortholog (CG3573)-*dOCRL.* To understand the cellular basis of renal dysfunction in Lowes syndrome patients, we generated a null mutant of *dOCRL* (*$dOCRL^{KO}$)* using CRISPR/Cas9 genome engineering. *$dOCRL^{KO}$* animals were late larval to pupal lethal with reduced body mass, reduced nephrocyte size and delayed growth and development of larvae. *$dOCRL^{KO}$* nephrocytes showed significantly elevated levels of $PI(4,5)P_2$ and reduced levels of PI4P at the plasma membrane. *$dOCRL^{KO}$* nephrocytes showed reduced endocytosis of 10Kda dextran and an impaired functional ability to clear silver nitrate when exposed to elevated levels of this heavy metal. These nephrocytes also showed reduced levels of Rab5 and Rab7 positive endosomes, with upregulated lysotracker staining. To test if these nephrocyte defects were cell-intrinsic or not, we generated a nephrocyte specific knockout of *dOCRL* and observed similar results of reduced endocytosis and silver nitrate clearance. These phenotypes could be rescued by reconstitution of *$dOCRL^{KO}$* with wild type *Drosophila* transgene. Phenotypes could also be rescued by reconstitution with a wild type human *OCRL* gene but not with human transgenes carrying patient

specific mutations. Overall, our data support a role for altered endocytic trafficking in nephrocytes leading to altered renal function. These also offer a model to evaluate the functional impact of patient specific sequence variants discovered during next generation sequencing of patient samples.

362B *Drosophila Dyb* Mutants show hearing and proprioception defects: a model for Meniere's disease

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Meniere's disease (MD) is an inner ear disorder defined by recurrent vertigo attacks, sensorineural hearing loss and tinnitus. Evidence from epidemiology and next generation sequencing suggests that genetic susceptibility involves multiple genes. One of these, Dystobrevin (*DTNA*), appeared as the best candidate, but there is no animal model to study the disease mechanisms. The fly's 'inner ear', called Johnston's organ (JO), is a chordotonal organ localized in the 2nd antennal segment, which mediates the sensation of hearing, gravity and wind. In *Drosophila*, *DTNA* orthologue *Dyb* is predicted to be part of the dystrophin-associated glycoprotein complex and is expressed in the auditory/proprioceptive chordotonal sensory organs of the larvae.
In order to investigate whether *Dyb* causes an MD-like phenotype, we analysed *Dyb* null and *Dyb* RNAi knockdown flies. We evaluated proprioception through locomotory coordination using climbing assays in light and dark. *Dyb* flies showed normal mechanosensory under white light but they exhibited climbing defects when assayed in effective darkness. The climbing defect persists over time. The flies thus present a mild proprioception defect that can be compensated by visual input. We assessed JO auditory function in vivo using Laser Doppler Vibrometry. *Dyb* mutant flies show a decrease in auditory active amplification in both males and females. Preliminary analyses of cell-type specific *Dyb* RNAi knockdowns and of a *Dyb* GFP-enhancer transgenic reporter line, suggest that Dyb function is required in ligament cells or sensory neurons.
Our results suggest that disruption in *Dyb* mutant flies generates an MD-like phenotype with hearing and proprioception defects. This supports a causative role for DTNA in human MD. Additionally, our results show that the flies' sense of balance integrates both proprioceptive and visual information: the proprioceptive deficits that arise from a loss of *Dyb* function can be compensated by visual cues. The findings give a firm basis for future analyses to understand the function and mechanisms of *Dyb* in JO hearing and proprioception.

363C An Oatp transporter-mediated steroid sink promotes tumor-induced cachexia in Drosophila

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Cancer cachexia is a devastating tumor-induced metabolic disorder marked by a progressive wasting of adipose and muscle tissues. This is driven by a combination of reduced food intake, insulin resistance, excess catabolism and inflammation. Cachexia greatly impacts the quality of life of patients, reduces the efficacy of chemotherapy and increases susceptibility to infections. This condition is often poorly diagnosed and very few opportunities for treatment are offered. We developed a tumor model in Drosophila larvae causing a cachexia-like syndrome, and used it to evaluate the role of steroid hormone imbalance in cachectic alterations. Cachectic larvae show reduced levels of the circulating steroid ecdysone. Artificially importing ecdysone in the tumor using the Oatp74D Ec-I transporter aggravates cachexia, while feeding animals with ecdysone rescues cachectic defects. This suggests that a steroid sink induced by the tumor promotes catabolic alterations in healthy tissues. We find that Oatp33Eb , another member of this family of transporters, is specifically induced in tumors promoting cachexia. Blocking Oatp33Eb in cachectic tumors restores circulating ecdysone and reverses cachectic alterations. Oatp transporters are induced in several types of hormone- dependent tumors, suggesting that a similar sink effect could modify hormonal balance in cachectic cancer patients.

364A The histone demethylase KDM5 is required for synaptic structure and function at the Drosophila neuromuscular junction

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Mutations in the genes encoding the KDM5 family of histone demethylases are observed in individuals with intellectual disability (ID). Despite clear evidence linking KDM5 function to neurodevelopmental pathways, how this family of proteins impacts transcriptional programs to mediate synaptic structure and activity remains unclear. Using the *Drosophila* larval neuromuscular junction (NMJ), we show that KDM5 is required presynaptically within motor neurons for proper NMJ morphology and function. The histone demethylase activity of KDM5 is a key contributor to its synaptic functions, as animals lacking catalytic activity show a reduction in the number of type Ib synaptic boutons and a decrease in evoked glutamate release. Additionally, we find that KDM5's neuroregulatory functions requires the C5HC2 zinc finger motif, which is

immediately adjacent to the enzymatic JmjC domain but has no currently known function. This is based on our analyses of a missense variant within the C5HC2 motif of KDM5 that is equivalent to an ID-associated mutation in *KDM5C* that reduces the number of synaptic boutons and increases their size. Interestingly, this ID-associated allele does not appear to dramatically alter histone demethylase activity, suggesting that KDM5 uses canonical and non-canonical mechanisms of gene regulation to properly form and maintain NMJs. Consistent with these data, RNA-seq analyses of the ventral nerve cord (VNC), where the nuclei of motor neurons reside, revealed that mutations in either the JmjC or C5HC2 domain cause distinct transcriptional changes. KDM5 therefore uses demethylase-dependent and independent mechanisms to regulate NMJ structure and activity, highlighting the complex nature by which this chromatin modifier carries out its neuronal gene regulatory programs.

365B Understanding the effect of altering excitability in *Drosophila melanogaster* models of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is the most common form of adult-onset motor neuron disease (MND), affecting 3-5 people per 100,000. This devastating disease leads to death within 2-5 years of diagnosis, with no effective treatments available. While ALS can be caused by mutations in many genes, there are a few common pathologies among all patients. One is the near -universal occurrence of cytoplasmic aggregates of normally nuclear proteins, predominantly TDP-43 (TAR DNA-binding protein 43). Another is altered levels of motor neuron excitability, which begins with pre-symptomatic hyperexcitability. Recently, it has been discovered that these two phenomena are linked. Many *Drosophila* models of ALS exist, and *Drosophila* also makes an excellent model for studying neurological systems, with many tools available to alter excitability and target different neuronal subsets. We are determining the effects of both hyper- and hypo-excitability on adult *Drosophila* ALS disease-like outcomes by measuring motor behaviour, lifespan and neuronal pathology. We have shown that constant low levels of hyperexcitability in wildtype motor neurons leads to reduced climbing performance and lifespan in adults, and these phenotypes are more severe when these neurons are hypoexcited instead. We are further manipulating excitability in several ALS models to determine if hyper- and hypo-excitability improves or worsens their disease phenotypes. This will provide valuable evidence to support if altering excitability is a potential therapeutic intervention for ALS.

366C Endurance exercise ameliorates disease progression in *Drosophila* models of Spinocerebellar Ataxias

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Endurance exercise is a potent intervention with widespread benefits proven to reduce disease incidence and impact across species. While endurance exercise supports neural plasticity, enhanced memory, and reduced neurodegeneration, less is known about the effect of chronic exercise on the progression of movement disorders such as ataxias. Here, we focused on three different types of ataxias, Spinocerebellar Ataxias Type (SCAs) 2, 3 and 6, belonging to the polyglutamine (polyQ) family of neurodegenerative disorders. In *Drosophila* models of SCAs 2, 3 and 6, flies progressively lose motor function and accumulate toxic levels of SCA proteins. Excitingly, we observe dramatic protection of speed and endurance in exercised SCA2 flies and modest protection in exercised SCA6 models, while no benefit is observed in SCA3 flies. Importantly, causative protein levels are reduced in SCA2 flies after chronic exercise, but not in SCA3 models, linking protein levels to exercise-based benefits. Currently, we are focusing on the activation of exercise-mimicking genes in SCA-model flies in order to define the mechanisms by which exercise preserves function in polyQ ataxias. In preliminary experiments, the exercise-inducible protein Sestrin suppresses longitudinal decline in mobility in SCA2 flies, even without exercise. Our study suggests differential responses of ataxia disorders to exercise, highlighting the potential for more extensive application of exercise-based therapies in the prevention of polyQ neurodegeneration. Defining the mechanisms by which exercise suppresses polyQ ataxias will inform disease targets driving individual polyQ disorders and will open the door for more effective treatment.

367A Translational efforts towards a better understanding of frequent sleep deficits in Mendelian neurodevelopmental syndromes – from patients to *Drosophila* and back

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Sleep problems and disorders are common in Mendelian neurodevelopmental (NDD) syndromes and related disorders, such as idiopathic autism. They frequently represent a major burden for the affected individuals and their families and their daily

life management. In many of these syndromes sleep anomalies remain poorly characterized, partly due to the challenge and specialized expertise that is needed to objectively assess sleep in these cohorts.
With a team of fundamental researchers, experts in genetic neurodevelopmental syndromes and specialized somnologists, we have started an interdisciplinary project with translational ambition. We aim to characterize sleep and related deficits in individuals with monogenic NDDs, and to correlate them with their genetic defects. Moreover, we assessed sleep in *Drosophila* models of the syndromes of interest, to gain further insight into their sleep pathophysiologies, and ultimately aim to translate our findings back to the clinic.
To this end, we set out to identify and characterize the nature of sleep complaints of patients for a number of NDD syndromes using parent-reported questionnaires and sleep diaries. In parallel, we took advantage of *Drosophila* to create models for these syndromes and evaluated the similarity of deficits across these species in various parameters, including sleep and cognitive phenotypes. We aim to dissect the spatio- and temporal origin and molecular mechanisms that underlie the identified sleep phenotypes, with the purpose of establishing a preclinical model enabling identification of pharmacological and behavioral strategies to ameliorate them (see abstract M. Coll-Tané *et al*.).
Successful treatment approaches will be translated back to the clinic. This way, we aim to improve recognition of sleep problems and treatment efficiency in these patient groups, thereby alleviating the disease burden on a daily basis and enhancing quality of life for the patients and their families. In addition, we will explore whether sleep restoration has the potential to also mitigate cognitive phenotypes, such as learning and memory defects.
Our work aims to provide fundamental insights into the regulation of sleep, the neuropathology of sleep disturbances in rare genetic syndromes, and to explore new approaches towards treatment.

368B The ER stress transcription factor XBP1s blocks CTG repeats-induced toxicity in a *Drosophila* model of myotonic dystrophy type 1

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Myotonic dystrophy1 (DM1) is a neuromuscular disease caused by abnormal expansion of CTG repeats in the 3'UTR of the *Myotonic Dystrophy Protein Kinase* (*DMPK*) gene. DM1 affects muscles, heart and eyes causing myotonic dystrophy, abnormal heart function and cataracts. However, the precise molecular basis underlying DM1 are not fully understood. To shed light on this issue, we investigated the role of the ER stress transcription factor XBP1s (spliced) in transgenic flies over-expressing 480 CTG repeats. The *XBP1* mRNA gets spliced out in response to ER stress and activates the unfolded protein response (UPR), which upregulates ER chaperones and other protective factors. We found that expression of 480 CTG repeats in the fly eyes leads to necrosis and depigmentation, but this phenotype is dramatically alleviated by XBP1s overexpression. In the muscles, over-expression of CTG repeats causes open wing phenotypes, reduced flight ability, abnormal flight muscle integrity and higher levels of p62 compared to control flies. Strikingly, over-expression of XBP1s decreased p62 levels in DM1 flies and rescues the integrity of flight muscles and flight behavioral deficits. XBP1s also increased the expression of the RNA binding protein *muscleblind* and specific transcripts of its downstream target *Serca* in DM1 flies. Our studies suggest that manipulation of the ER stress transcription factor XBP1s may represent a new therapeutic opportunity to approach DM1 pathogenesis.

369C Identifying novel drugs to treat neurofibromatosis type 1 (NF1) tumors using genetic screens in *Drosophila* cells

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Neurofibromatosis type 1 (NF1) is one of the most common inherited neurological disorders, affecting about 1 in 3,000 people. This autosomal dominant condition is characterized by hyperpigmentation of the skin and neurofibromas, benign tumors derived from Schwann cells. *NF1* encodes neurofibromin, a Ras-specific GTPase activating protein (RasGAP) acting as an inhibitor of Ras activity. While loss of one copy of *NF1* does not cause significant overgrowth of cells, biallelic inactivation of *NF1* due to somatic mutation in Schwann cells within the peripheral nervous system results in neurofibroma development. Loss of *NF1* results in aberrant activity of the Ras/MEK/ERK and PI3K/AKT signaling pathways and uncontrolled cell growth and proliferation. Although neurofibromas on the skin are small, those arising on larger nerves (termed plexiform) often cause morbidity or can be fatal if they impinge on vital structures or become malignant. There is an urgent need for effective therapies for neurofibromas.

To identify effective drugs for the treatment of tumors associated with NF1, we have applied powerful screening methods

using *Drosophila* S2R+ cells. *Drosophila* screening is rapid and cost effective; the pared-down complexity of the fly genome reduces off-target effects in RNAi screens, making interpretation of results rapid and reliable. Firstly, we used CRISPR/Cas9 gene editing to generate *NF1* mutant S2R+ cell lines. We then performed a genome-wide synthetic lethal RNAi screen to identify genes that are specifically essential for the survival of *NF1*-deficient cells. This screen identified 46 candidate genes that have unambiguous orthologues in humans. Genetic tests validated synthetic lethality of several RNAi screen hits in a fly model of NF1. We followed up on one candidate gene that could be targeted using an existing drug (chloroquine (CQ), targeting autophagy). This was further tested *in vivo* using both *NF1*-deficient flies and in human cell lines. Further, *NF1* mutant flies were also found to have significantly increased susceptibility to CQ when compared to wild type controls. Importantly, the selective effects of CQ were found to be conserved in human cells derived from NF1 tumors. Our *Drosophila* synthetic lethal screening provides a high confidence set of candidate therapeutic targets for NF1 tumors which we are evaluating for potential use in the clinic.

370A Modeling laminopathies in Drosophila: Comparative analysis of *LMNA* mutations that cause muscle and adipose disorders

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Jill Viles and Priscilla Lopes-Schliep are women who have experienced contrasting adult lifestyles due to differences in their physique. Jill is confined to a wheelchair, while Priscilla is an Olympic bronze medalist for the 100-meter hurdles. Surprisingly, these women each possess a mutation in the *LMNA* gene. This gene encodes A-type lamins, filamentous proteins that form a meshwork on the inner side of the nuclear envelope. Lamins provide structural support for the nucleus and organize genomic chromatin. Mutations in the *LMNA* gene cause a collection of diseases known as laminopathies, which include lipodystrophy, rare types of muscular dystrophy, and early onset aging. Jill possesses a mutation that results in the amino acid substitution R527P, which causes a rare muscular dystrophy called Emery-Dreifuss muscular dystrophy and a rare lipodystrophy called Dunnigan-type familial partial lipodystrophy. Priscilla possesses a mutation that results in the amino acid substitution R482W. This single amino acid substitution causes Priscilla to have Dunnigan-type familial partial lipodystrophy; her skeletal muscles are not affected. The focus of my research is to understand how mutations in the *LMNA* gene cause these different phenotypes.
To accomplish this goal, transgenic Drosophila models expressing the Drosophila orthologue of human A-type lamins, *Lamin C*, were generated. The R residues altered in Jill and Priscilla are conserved in Drosophila Lamin C. Flies expressing the equivalent of R527P, R482W, and a related disease-causing substitution R482Q were generated. Using the tissue-specific Gal4/UAS system, these mutant versions of *Lamin C* were expressed in larval body wall muscles, which have many similarities to human skeletal muscles. Immunohistochemistry showed that each of the mutant lamins had unique patterns of mislocalization. Lamin C R527P showed cytoplasmic aggregation, whereas R482W and R482Q showed different nuclear morphological defects. Interestingly, all three of the mutant lamins caused semi-lethality, with death at the pupal stage. We infer that this death is caused by the failure of the larval body wall muscles to carry out morphogenesis. Larval motility assays are currently underway to assess muscle function. Collectively, data thus far demonstrate that all three lamin mutations cause muscle defects and that Priscilla's muscles might be spared due to second site suppressor genes in her genome.

371B Mechanisms of skeletal muscle and and cardiac disease caused by mutations in *TMEM43*

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Dominant mutations in the human *TMEM43* gene cause Emery-Dreifuss muscular dystrophy (EDMD)-related myopathy, which is characterized by muscle weakness and wasting and joint contractures. Dominant mutations in *TMEM43* also cause arrhythmogenic right ventricular cardiomyopathy (ARVC), characterized by adipose tissue accumulation and fibrosis, primarily in the right ventricle, leading to arrhythmias and sudden cardiac death. *TMEM43* encodes the integral inner nuclear membrane protein TMEM43, also called LUMA. It is unclear if mutations in *TMEM43* cause similar cellular dysfunction in skeletal muscle and cardiac tissue. To better understand the the function of TMEM43 in muscle, we used the Drosophila Gal4/UAS system to express an RNAi transgene against *CG8111*, the Drosophila orthologue of *TMEM43* (referred to here as *dTMEM43*). In addition, we expressed *dTMEM43* transgenes possessing mutations analogus to those found in humans with muscle defects. The transgenes were expressed in larval body wall muscles, adult indirect flight muscles, and cardiac tissue. Knock-down of *TMEM43* in larval body wall muscles reduced viability, with death occurring at the pupal stage. Cytological analyses revealed that the muscles had nuclei with chromatin protrusions, especially at the myotendinous junctions where physical forces are the greatest. These chromatin protrusions were also observed upon expression of the mutant *dTMEM43* transgenes. Knock-down of *TMEM43* in indirect flight muscles caused wing posturing defects characteristic of loss of indirect flight muscle

function. Knock-down of *TMEM43* in cardiac tissue resulted in defective wing heart function, leading to blistered wings, and premature death in adulthood. Viable adults showed an age-dependent elevated heart rate, a shortened diastolic interval, and an accumulation of adipose tissue around the heart compared to controls, similar to the human disease phenotypes. Taken together, these data demonstrate that many of the human disease phenotypes are recapitulated in Drosophila and that mutant *dTMEM43* causes chromatin protrusions in muscle, which has been associated with transient nuclear rupture, leading to increased levels of DNA damage.
These studies are supported by the Undiagnosed Disease Network.

372C Mechanical competition promotes tumor growth via activation of innate immune signaling

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Tissue homeostasis is achieved by balancing stem cell maintenance, cell proliferation, and differentiation, as well as the purging of damaged cells. Mechanical cell competition eliminates less viable cells when tissues grow during development. However, the underlying mechanisms driving competitive growth when homeostasis fails, for example during tumorigenesis, remain largely unresolved. Here, using a Drosophila intestinal tumor model, we find that tumor cells outcompete nearby enterocytes (ECs) by mechanical forces, thereby activating the immune-responsive Relish/NF-κB pathway to induce EC delamination and apoptosis for tumor progression. This requires a JNK dependent regulation on the expression of a regulatory peptidoglycan recognition protein RGRP-LA. In organisms with impaired PGRP-LA function, the tumor growth is delayed and lifespan extended. These data demonstrate a non-autonomous role of a JNK/PGRP-LA/Relish signaling axis that mediates the death of neighboring cells to facilitate tumor growth. We propose that tumors 'hijack' an inflammatory feedback mechanism to eliminate live enterocytes by mechanical cell competition to support their own growth.

374B Effects of saturation deficit on water balance Mechanism in Subtropical Drosophild-Zaprionus indianus:

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Seasonally varying populations of ectothermic insect taxa from a given locality are expected to cope with simultaneous changes in
temperature and humidity through phenotypic plasticity. Accordingly, we investigated the effect of saturation deficit on resistance to
desiccation in wild-caught flies from four seasons (spring, summer,rainy and autumn) and corresponding flies reared in the laboratory
under season-specific simulated temperature and humidity growth conditions. Flies raised under summer conditions showed approximately three times higher desiccation resistance and increased levels of cuticular lipids compared with flies raised in rainy season conditions. In contrast, intermediate trends were observed for water balance-related traits in flies reared under spring
or autumn conditions but trait values overlapped across these two seasons. Furthermore, a threefold difference in saturation deficit (an
index of evaporative water loss due to a combined thermal and humidity effect) between summer (27.5 mB) and rainy (8.5 mB)
seasons was associated with twofold differences in the rate of water loss. Higher dehydration stress due to a high saturation deficit in
summer is compensated by storage of higher levels of energy metabolite (trehalose) and cuticular lipids, and these traits correlated
positively with desiccation resistance. In Z. indianus, the observed changes in desiccation-related traits due to plastic effects of simulated growth conditions correspond to similar changes exhibited by seasonal wild-caught flies. Our results show that developmental plastic effects under ecologically relevant thermal and humidity conditions can explain seasonal adaptations for water
balance-related traits in Z. indianus and are likely to be associated with its invasive potential.

375C Adapting a Drosophila Microbial Pathogenesis CURE for Freshman

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Course-based Undergraduate Research Experiences (CUREs) are an excellent way to provide the benefits of a research

experience to all students progressing through the curriculum. As a part of an upper-level course required for Cell Biology and Biochemistry majors, I have built a CURE in which students investigate how infection impacts immunity by designing their own experiments, collecting data and presenting their results during a five and a half week unit. In the first two weeks, the students learn *Drosophila* husbandry, microbiological techniques, nucleic acid isolation and quantitative PCR using an instructor designed experiment. In week three, students develop their own hypotheses and design experiments to test them. The students pitch their projects to their classmates, and the class then selects a subset of these experiments to execute during the remaining two and a half weeks of the unit culminating in a team-written research paper.

This fall, our department successfully proposed a complete overhaul of our core courses for Biology majors. Starting in Fall 2021, we will have every student in our major participate in a semester-long CURE during their freshman year. While the topics vary based on instructor expertise and interest, all sections will learn about hypothesis testing, experimental design, data collection and analysis by executing student-designed experiments to test their hypotheses. All sections will also emphasize communication of scientific results and include components of both field and molecular work. As part of this endeavour, students will be introduced to the roles published research articles play in the scientific process and how to use the primary literature to both develop their hypotheses and experiments and interpret their results.

I am currently adapting my shorter unit from the upper-level course into a semester-long freshmen experience as one of the initial instructors in our freshmen CURE. Planned alterations include increased emphasis on microbial ecology and the evolution of pathogenesis along with incorporation of *Drosophila* genetic tools to modulate the immune system. Since this is an introductory course taken before any courses focusing on molecular biology, nucleic acid techniques will be streamlined for ease of use. As this is a semester-long experience, we will dedicate approximately six weeks to learning techniques and developing their hypotheses. The final ten weeks will be dedicated to the student-designed research projects to allow for iterative experimentation. Highly motivated students who are inspired by their experimental work during the CURE will be invited to continue their inquiry within my research group.

376A Flying into research excellence: The *Drosophila* Neural Research Course-Based Undergraduate Research Experience

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Gaining an understanding of science requires experiencing science the way it happens in a research setting. However, most undergraduate students do not have access to traditional mentored research experiences. Undergraduate research experiences are often reserved for very few students because of limitations of costs, resources, and student-to-faculty ratios. Course-based Undergraduate Research Experiences (CUREs) have been proposed as a solution. CUREs allow expansion of research experiences to support additional students through a reduction of costs and time invested, while maintaining many of the positive outcomes of a research experience. Further, CUREs integrate research and discovery experiences directly into the curriculum. CUREs also provide an opportunity for scientists to gather novel data to support their research initiatives. At Drexel, we have created and run a CURE focused on using genetic techniques to understand questions in neural development and disease. This course, called *Drosophila* Neural Research, is a key example of how a faculty member's research can be translated to a classroom environment to support students in gaining skills through an undergraduate research experience. To date, this course has been offered for seven years and has enrolled over 65 students. Work of the students in this CURE has directly led to published research outcomes and the establishment of at least one project taken on by a Ph.D. candidate. Here we present outcomes from this CURE, including undergraduate student outcomes and how they compare with traditional mentored research experiences at our institution. Our analysis reveals that students who participated in this CURE reported similar outcomes to students engaged in traditional mentored research experiences. We will provide suggestions and tips about how faculty can translate their research into a CURE, and the benefits of this approach for faculty and students.

377B Identification of hematopoietic genes in *Drosophila* by undergraduates participating in a course-based research experience

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Undergraduates participating in the UCLA Undergraduate Research Consortium for Functional Genomics (URCFG) have

conducted a functional genomics screen in *Drosophila* using RNA interference (RNAi) in combination with fluorescent reporter proteins to identify genes important for hematopoiesis. This screen identified 137 candidate genes for which loss of function leads to observable changes in the hematopoietic development in wandering larvae. Targeting RNAi to maturing, progenitor, and regulatory cell types identified key subsets that either limit or promote blood cell maturation. Bioinformatic analysis of identified gene sets revealed enrichment in several areas, including RNA processing and vesicular trafficking. Student participation in this course-based undergraduate research experience (CURE) correlated with increased learning gains across several areas, as well as increased STEM retention, thereby providing further evidence that authentic, student-driven research in the form of a CURE represents an impactful and enriching pedagogical approach.

378C Fly-CURE, a multi-institutional undergraduate CURE using *Drosophila*, investigates the relationship between research dosage and student impact on research attitudes

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The Fly-CURE is a multi-institutional Course-based Undergraduate Research Experience (CURE) in genetics. Through this CURE, undergraduate students at higher education institutions across the country (including public, private, community colleges, and minority-serving institutions) spend a semester mapping and characterizing novel *Drosophila melanogaster* mutants isolated from a Flp/FRT genetic screen. Undergraduate researchers' successful mapping of over 10 unique EMS mutants has led to local and national scientific presentations by students, as well as four peer-reviewed publications with a combined total of over 130 student co-authors. This project has provided more than 300 undergraduate researchers with hands-on research experiences within the classroom setting. We have developed and validated assessment tools to understand both the impact of Fly-CURE on students' attitudes toward research and how the impact of the Fly-CURE relates to dosage (number of prior research experiences students had prior to Fly-CURE). These data will help to inform undergraduate programs about the learning gains afforded by course-based and other research experiences. As an NSF-IUSE funded project, we are expanding Fly-CURE to twenty higher education institutions to increase student exposure and access to an authentic research experience.

379A Annotation of D element contig3 and a F element contig59 of *Drosophila ananassae*

Norma Velazquez Ulloa[1], Malcolm Arnott[1], Vy Lam[1], Blake Tellinghusen[1], Elizabeth Kolb[1], Xena Jaffe[1], Jack Allen[1] 1) Lewis and Clark College.

The Genomics Education Partnership (GEP) is a collaboration of over 100 institutions that have developed curriculums to teach bioinformatics and genomics to undergraduates by providing real research opportunities. This project investigates F element expansion in unannotated *Drosophila* species. Students annotate genomes of *Drosophila* species with expanded F elements and genes in the unexpanded D element for comparison. We used the GEP gene annotation workflow, which uses evidence tracks in the GEP UCSC Browser to annotate two contigs of the *Drosophila ananassae* genome: contig3 on the D element and contig59 on the F element. The first step in the process is to identify, then examine the gene structure of the *D. melanogaster* (*D. mel*) ortholog to determine the number of isoforms and CDS usage of that gene in *D. mel*. Next, each isoform is annotated by mapping each CDS sequence from the *D. mel* ortholog onto the contig sequence to find a region of homology. Coordinates for each CDS in *D. ananassae* are refined on the genome browser using evidence from RNAseq experiments, splice site prediction tracks, gene predictor tracks, and examination of the sequence to determine the best supported start codon, stop codon, and splice donor/acceptor sites. The BLASTX alignment to *D. mel.* protein track identified five putative orthologs in contig3 on the D element and six in contig59 on the F element. Of these, 3 orthologs were found in contig3 (Nopp140, P5CDh1 and CG14563) and 1 in contig59 (CaMKII). Only the coding sequence was annotated. Untranslated regions and transcription start sites were not annotated. In *D. ananassae*, Nopp140 isoform PB appears to be missing, with identical coding sequence to PF, but similar to *D. mel* Nopp140-PA had 3 CDSs, compared to 4 in other isoforms. All isoforms are ~60% conserved, with no structural changes over *D. mel*. Exon 2 has poor conservation and complexity across all isoforms. For P5CDh1 all annotated isoforms were highly conserved, except the first exon. For CG14563, the gene was not well conserved, requiring adjustments to BLASTp parameters. For CamKII, conservation with *D. mel* is high, despite minor disagreements between blast predictions and other data. The *D. mel* alignment browser track misses exons 6 and 9 entirely, but all other evidence supports it. Gene annotations generate gene models that expand knowledge of *Drosophila* genomes, which answer research questions on F element expansion. Gene models can also be used to ask new research questions. This work provided research opportunities

during the pandemic for 6 undergraduate students, ranging from sophomores to seniors. Google Classroom was used to deliver curriculum and we had weekly meetings on Zoom to check progress on the annotations. The students worked in small groups and checked each others' work. Completed annotations will be submitted to the GEP for reconcialiation to contribute to the data set on the expanded F element project.

380B Subcellular localization of Tip60 HAT and HDAC2 in the *in vivo Drosophila* brain: implications for Alzheimer's disease

Ellen Armour[1], Julia Perhacs[1], Felicity Khoa[1], Haolin Zhang[1], Felice Elefant[1] 1) Drexel University, Philadelphia, PA.

Neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and Amyotrophic Lateral Sclerosis (ALS) that involve cognitive defects are becoming increasingly common in our aging population. Epigenetic mechanisms, such as histone acetylation, have proven to reinstate cognition and are critical for proper neuronal function, although the exact mechanisms are yet to be elucidated. Previous work in our lab has shown the imbalance of Tip60 histone acetyltransferase (HAT) and histone deacetylase 2 (HDAC2) in a well characterized *Drosophila* AD model results in epigenetic repression of synaptic plasticity genes and functional cognitive defects. Remarkably, Tip60 overexpression restores AD-induced neuropathology, emphasizing Tip60's neuroprotective role. Recent work in our lab has identified Tip60 as having shuttling capabilities between the nucleus and the cytoplasm. Moreover, we have shown that in the human AD hippocampus, Tip60 is largely excluded from the nucleus, suggesting that disruption of Tip60 subcellular localization may contribute to AD. To further elucidate the importance of localization and the HAT/HDAC balance, we examined the subcellular localization of Tip60 and HDAC2 in the *Drosophila* brain. Under normal conditions, Tip60 is localized mainly in the cytoplasm, while HDAC2 is localized mainly in the nucleus. By elucidating Tip60 HAT/HDAC2 shuttling mechanisms, we can better understand the interaction between these two epigenetic modulators in an attempt to develop more specific therapeutic targets for neurodegenerative diseases.

381C

Disentangling siRNA, epigenetic modifications, and X chromosome recognition *Sudeshna Biswas*[1], Victoria Meller[1] 1) Wayne State University.

Flies correct for imbalance of chromosome dosage between the sexes by a mechanism called dosage compensation that increases X-linked gene expression in males. The dosage compensation machinery is made up of proteins and one of two redundant, long non-coding RNAs that assemble into the Male Specific Lethal (MSL) complex. X-localization of the MSL complex is essential for upregulation of X-linked genes, but how the X is identified remains unclear. Small interfering RNAs (siRNA) and satellite repeats enriched on the X contribute to X recognition. H3K9me2 enrichment in and around these repeats may play a role. Previous studies from our lab showed that ectopic expression of siRNA from 1.6883F (at cytological position 3F) partially rescues roX1 roX2 males and restores localization of the MSL complex, but siRNA from other repeats with similar sequence did not. Su(var)3-9 deposits H3K9me2 around repeats and mutations in Su(var)3-9 enhance the male lethality of mutations that reduce X recognition. The links between 1.688X repeats, siRNA, H3K9me2 deposition and X recognition remains unclear. It is possible that only siRNA with 1.6883F sequence is capable of rescuing roX1 roX2 males. Alternatively, details of construction of transgenes expressing dsRNA from different repeats could determine function. To answer this question, I am engineering an siRNA-producing construct with 1.6881Asequence (from cytological position 1A) but with size, phasing and orientation identical to that of the biologically active 1.6883F construct. I will analyze siRNA production from this construct and measure its ability to rescue of roX1 roX2 males. I will take advantage of existing siRNA-producing transgenes that differ in their ability to promote X recognition to determine if H3K9me2 deposition at 1.688X repeats is only enhanced when transgenes that promote X recognition are present. Finally, I will test the role of Su(var)3-9 in X recognition by artificially tethering this protein close to an autosomal reporter for compensation. If H3K9me2 enrichment promotes X recognition by the MSL complex, we predict that a closely linked reporter will be able to capture the MSL complex. These studies test the idea that H3K9me2 enrichment, rather than DNA sequence, is important for recruitment by 1.688X repeats and will serve to disentangle the role of siRNA, Su(var)3-9 and H3K9me2 in X recognition.

382A Investigating the role of Polycomb repression in *Drosophila* eye specification

Haley Brown[1], Justin Kumar[1] 1) Indiana University.

During metazoan development, gene regulatory networks (GRNs) are activated in undifferentiated tissues to induce a specific fate. However, when GRNs are disrupted, the tissue can *transdetermine* – losing the programmed fate to adopt another. Epigenetic factors, such as the Polycomb Group (PcG) proteins, ensure proper spatiotemporal control of GRNs. PcG proteins function as a set of complexes to add a repressive histone mark (H3K27me3) and condense chromatin. In turn, the accessibility

of chromatin – or lack thereof – regulates differential transcription of genes in certain tissues. While the correlation between GRNs and chromatin modifications in development is widely established, the underlying mechanisms linking the two during transdetermination has yet to be discovered. *The overarching goal of this project is to determine how epigenetic modifications affect tissue fate specification.* An excellent model to study the mechanisms underlying fate plasticity is the eye-to-wing transformation of *Drosophila* eye-antennal discs (EADs). Previous work from our lab discovered that the EAD-specific removal of one PcG protein, Polycomb (Pc), transforms the eye imaginal tissue to wing – indicating that the loss of epigenetic repression is sufficient to allow cellular reprogramming.
To investigate the molecular mechanism underlying this transformation, I have performed RNA-seq on wild-type (WT) wing discs (WDs) as well as WT and *Pc* mutant EADs throughout third instar development. This analysis identified 55 candidate genes that could be responsible for promoting reprogramming of the EAD. My preliminary data suggest the most promising of these candidates is *vestigial* (*vg*), as this locus is directly regulated by Pc. Furthermore, overexpression of *vg* in the EAD grants an eye-to-wing transformation, and ectopic *vg* expression is detected in the developing wing pouch of the transformed disc. I will further investigate how repressive and active histone modifications are changing in this system to allow for reprogramming by using a novel epigenome profiling technique, CUT&RUN. I hypothesize that the inability of *Pc* mutants to read H3K27me3 marks allows the epigenome to become malleable, activates wing determination genes, and transforms the eye into a wing. Outcomes of this study will elucidate the mechanistic role epigenetic factors play in tissue fate determination, ultimately providing insight into how mutations in epigenetic proteins result in human developmental disorders.

383B Epigenetic regulation of reproductive arrest in *Drosophila*

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Genes and the environment interact to determine developmental transitions. Extensive and exquisitely coordinated changes in gene expression accompany the initiation, cessation, and pausing of such transitions. To investigate the determinants of coordinated gene expression changes associated with developmental pausing, we exploit an environment-dependent reproductive arrest phenotype called “diapause” in *Drosophila melanogaster*. Female *D. melanogaster* suspend egg development in response to low temperatures and short days that signal the start of winter. When warm temperatures and long days return in the spring, the arrested ovaries re-initiate egg development. These awakened females age and reproduce as if the arrest had never occurred. We discovered that diapause is regulated epigenetically – isogenic females respond differentially to winter conditions, entering diapause at a 40% rate. This differential response allows us to compare individuals in the same conditions, with the same genome, in different developmental states. We hypothesize that chromatin mediates the gene expression program that preserves reproductive potential in diapausing females. Consistent with this possibility, using Western blots we found that histone mark H3K36me3 is elevated and H3K4me3 is depleted in diapausing ovaries. Moreover, germline knockdown of the enzymes that deposit these histone marks (Set2 and Set1, respectively) alter diapause incidence in the expected opposing directions. We are currently using CUT&RUN to define the genome-wide occupancy of these histone marks to identify the genes both differentially expressed and differentially marked, ultimately revealing the chromatin-mediated gene expression program that preserves reproductive potential.

384C Investigating the rate of paramutation in *Drosophila virilis*

Ana Dorador[1], Justin Blumenstiel [1] 1) University of Kansas, Lawrence, KS.

Paramutation is the process by which a silent allele can turn off the expression of a wildtype allele in an epigenetic manner. The silenced state of the wildtype allele can persist through generations even when the original paramutagenic allele is no longer present. The mechanism underlying paramutation in *Drosophila* is poorly understood. However, it appears that maternal transmission of piRNAs derived from the silent locus is critical. In a system of paramutation in *Drosophila virilis,* studies suggest that the silent state of the *center-divider (cdi)* gene may persist for two generations in the absence of the paramutagenic allele. In this study, we seek to estimate the rate of persistence of the silent state across generations. This will be achieved by performing *cdi* qPCR on the ovaries of 25 F1 heterozygous mothers as well as 25 backcross daughters lacking the original silent allele. To estimate rates of paramutation, it is essential to quantify *cdi* expression in backcross progeny lacking the silencing allele. In order to do this, a PCR indel genotyping assay was performed on backcross progeny. If there is substantial persistence of paramutation, we expect that *cdi* will be silenced in all backcross daughters homozygous for the wildtype allele. However, the extent of the silencing is not known. Thus, we may observe decreased expression rather than complete silencing. These findings will serve as a baseline for future potential studies seeking to investigate how paramutation is regulated.

385A The COMPASS-like complex members Ptip and Trr regulate cardiac cell development in the *Drosophila*

***melanogaster* embryo**

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Heart development requires a precise spatiotemporal regulation of gene expression responsible for mesodermal induction, cardiac mesoderm specification, and cardiac cell differentiation. These genes consist of transcription factors associated with regulatory machinery that activate or repress target gene expression. A central mechanism of transcriptional activation and maintenance involves the recognition of epigenetic modifications that alter local chromatin into a favorable environment for transcription. A predominant epigenetic modification associated with open and actively transcribed chromatin is Histone 3, Lysine (K) 4 methylation (H3K4me). H3K4me is primarily deposited by the evolutionarily conserved COMPASS/COMPASS-like complexes in *Drosophila melanogaster*. One of the essential COMPASS-like complexes, the Trithorax-related (Trr) H3K4 methyltransferase complex, is recruited by Ptip to gene loci thereby activating and maintaining proper gene expression. In mammals, Ptip and the Trr-ortholog, KMT2D, are essential for proper mouse heart development and cardiomyocyte electrophysiology. Additionally, global levels of H3K4me, as well as local levels of Trr-mediated H3K4me, have been shown to be reduced in *Drosophila* embryos lacking Ptip or Trr function. Together, these findings implicate Ptip and Trr in an epigenetic regulatory network important for heart development.

We investigated the loss of Ptip and Trr function using developmentally lethal strains in Drosophila melanogaster and examined cardiac cells for abnormal development at embryonic stage 16. Quantitative analysis revealed numerous cardiac cell division defects that indicate disruption to both symmetric and asymmetric cardiac cell divisions in two separate hypomorphic *Ptip* strains predicted to disrupt the protein›s function: the *Ptip[c450]* CRISPr-mediated deletion and *Ptip[MI03338]* MiMIC insertion mutants. Additionally, a developmentally lethal *trr[C2375X]* allele with a predicted premature stop codon located in the C-terminal SET domain showed symmetric, asymmetric, and earlier cell division defects. Overall, our results suggest that Ptip and Trr may cooperatively regulate proper cardiac cell development in the *Drosophila melanogaster* embryonic heart. Our future experiments will investigate the epigenetic and gene regulatory function of the Ptip-Trr complex during embryonic heart development, cardiac cell differentiation, and cardiac cell morphology.

386B CBP maintains Polycomb chromatin occupancy and silencing competency

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Maintenance of appropriate cell states involves a finely tuned balance of gene activation and repression. In *Drosophila*, Polycomb group (PcG) proteins are recruited to Polycomb response elements (PREs) to repress important developmental genes through formation of compact chromatin marked by trimethylation of lysine 27 in histone H3 (H3K27me3). PcGs must remain stably bound at PREs to ensure the maintenance of repression, yet the mechanisms by which chromatin compaction is repelled at the PRE to maintain PcG occupancy are unclear. The coregulator CBP/p300 positively regulates transcription by acetylating H3K27 (H3K27ac) at enhancers and promoting the recruitment and release of RNA polymerase II (Pol II) at active promoters, yet *Drosophila* CBP also occupies repressive PREs devoid of H3K27ac where its function is unclear. Here we uncover a role of CBP in modulating the chromatin occupancy of PcGs. We find that pharmacological and genetic perturbation of CBP result in rapid destabilization of PcGs at PREs, which leads to ectopic derepression of PcG-repressed genes. Perturbing CBP leads to nucleosome compaction and to disruption of R-loops at PREs. Overall, we identify a novel activity of CBP that supports PcG repression by transcription-dependent maintenance of nucleosome depletion at the PRE.

387C Conservation of Modular Polycomb Group Complexes in Drosophila

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Polycomb group (PcG) proteins, first identified as silencing factors of homeotic gene clusters in Drosophila, are critical regulators of developmental genes in all metazoans. PcG proteins have mammalian orthologs that are highly conserved in domains and functional activities as well as their assembly into PRC1 and PRC2 complexes. The study of mammalian PcG proteins is complicated by their number, diversity, and the capacity to form a wider array of PcG complexes than thought to occur in Drosophila. Here, we identify complexes in fly embryos that are orthologous to the mammalian variant PRC1.1 and PRC2.2 complexes. We provide evidence that these complexes have conserved modularity and function, highlighting that Drosophila remains a valuable model system for understanding the critical molecular mechanisms underlying epigenetic regulation of development. Functional analyses, through overexpression and RNAi knockdown of key alternative PcG subunits

such as Jing and Jarid2, complement our continued proteomic and genomic analyses of variant PcG complexes in Drosophila.

388A Uncovering effects of epigenetic silencing of transposable elements on differential gene expression in Drosophila .

Lorissa Saiz[1] 1) University of Houston.

Transposable elements (TEs) are genetic parasites that proliferate and increase in copy number in the germlines of organisms to guarantee transmission to offspring. TEs also play a major role in gene regulation, both by providing or disrupting cis-regulatory sequences, and by establishing a local heterochromatic state through the recruiting small RNA mediated silencing. Epigenetic effects of TEs on host gene expression are of particular interest because they potentially represent a negative side effect of TE silencing. However, it is difficult to isolate the epigenetic effects of TE silencing from the genetic effects of the insertion itself, because in most genotypes both are present.
To isolate the epigenetic effects of TE silencing on gene expression from other genetic effects, we will compare the expression of genes between individuals with and without knockdown of TE silencing machinery. We will focus on knockdown of Aubergine and Argonaute 3, two components of the piRNA pathway that establishes transcriptional silencing. We will take advantage of previously-published RNA-seq data from ovarian knockdown of Aubergine and Argonaute 3, as well as corresponding genome resequencing data revealing the genomic positions of TEs in the same genotypes. The frequency and direction of expression changes among genes adjacent to TE insertion sites will reveal the prevalence and impact of epigenetic effects on host genes. (1434 characters)

389B Consequences of the Loss of Multiple HP1 Proteins in *Drosophila melanogaster*

Sarah Sims[1], Mina Momeni[1], Nicole Riddle[1] 1) University of Alabama at Birmingham.

Chromatin, a complex of DNA, histones, and various chromosomal proteins, occurs in two main forms: euchromatin, which is typically transcriptionally active, and heterochromatin, which is typically transcriptionally silent. Proteins of the Heterochromatin Protein 1 (HP1) family are involved in the maintenance of chromatin states and are conserved across eukaryotes. The genome of *Drosophila melanogaster* contains three somatically expressed HP1 genes: *Su(var)205* encoding HP1a, *HP1b*, and *HP1c*. Loss of each of the three proteins has significant impacts, including the misexpression of hundreds of genes and decreased viability and/or fertility. When mutations in HP1 proteins are studied, the impact on the other HP1 family members typically is not assayed, despite them occurring together in protein complexes. Here, we investigate how HP1 proteins interact by examining double-mutant fly strains lacking pairs of HP1 proteins. Our initial studies focus on the *HP1b/HP1c* double mutant. We find that the viability of these animals is dependent strongly on genetic background, with one background producing a homozygous viable, fertile, and healthy stock despite lacking two HP1 proteins, while another background allows less than 10% of animals to survive. Initial examination of polytene chromosomes in these *HP1b/HP1c* mutants suggests that their morphology is affected, but that HP1a continues to localize mainly to the centromeres and telomeres. These data illustrate that complete loss of HP1B and HP1C is survivable in *D. melanogaster* and demonstrate the importance of genetic background. In addition, our study highlights possible crosstalk between and cooperative functions of HP1 proteins.

390C Testing models of centromere specification using genome editing

Prachi Tandale[1], Barbara Mellone[1] 1) University of Connecticut.

The centromere is a region of specialized chromatin necessary for accurate chromosome segregation during cell division. Centromeric chromatin is marked by the presence of the histone H3 variant CENP-A and previous studies support the model that this specialized chromatin is the major determinant for centromere identity and propagation. In contrast, the function of the highly repetitive centromeric DNA (CenDNA) remains elusive. Investigating the role of CenDNA in multicellular organisms has been challenging due to the lack of full centromere DNA assemblies. We recently reported the sequence and organization of all the centromeres of *D. melanogaster* obtained using ChIP and long-read sequencing. The centromeres have a shared organization, where CENP-A occupies primarily complex DNA islands enriched in retroelements that are flanked by blocks of simple satellite repeats, yet they differ in sequence composition. Only one DNA element, the G2/Jockey-3 non-LTR retroelement, is present at all five centromeres and is also centromere-associated in three other species in the *melanogaster* subgroup. To determine if centromeric islands are essential for centromere identity, we have engineered

two *in vivo* systems to delete the Centromere 3 island, called *Giglio*. In the first system, transgenic flies were constructed such that two unique gRNAs complexed with the Fok1-dCas9 protein would target either side of *Giglio* and induce double stranded DNA breaks that may be repaired by non-homologous end joining resulting in a complete deletion. We are currently testing the efficiency of this system using Oligopaint FISH for *Giglio* combined with immunofluorescence for the kinetochore marker CENP-C in larval neuroblast squashes. In the second system, we are introducing attB/attP recombination cassettes on either side of *Giglio* to induce its excision upon somatic or germline expression of the phiC31 recombinase. If *Giglio* deletion is successful, we will determine the faith of chromosome 3, specifically whether the centromere forms again on the satellites flanking the deletion site or elsewhere along the arms. We will also investigate chromosome 3 segregation accuracy at the cellular level as well as identifying viability and fertility phenotypes.

391A A novel Dbf4-Gcn5 HAT complex is necessary for histone H3 acetylation and viability in *Drosophila*

Eliana Torres-Zelada[1], Vikki Weake[1] 1) Purdue University.

There are two homologs of the Gcn5-binding protein Ada2 in *Drosophila*, Ada2a and Ada2b, which nucleate formation of either the ATAC or SAGA transcription coactivator complexes respectively. Ada2b has two splice isoforms that share the N-terminal domain and differ in their C-terminal. Both Ada2b isoforms are required to fully complement *ada2b* mutations, although expression of the 418aa Ada2b-PA isoform alone can support development and partially restore histone H3 acetylation. Recent studies in our lab, demonstrate that Ada2b-PA forms a complex with Chiffon, the *Drosophila* homolog of Dbf4. This novel HAT complex, CHAT (*Chiffon Histone Acetyltransferase*), which contains Gcn5, Ada3, Sgf29 and Ada2b-PA is necessary for histone H3 acetylation in flies. Dbf4 activates the cell cycle kinase Cdc7 to phosphorylate the Mcm helicase, initiating DNA replication. Chiffon in flies is necessary for gene amplification, a specialized type of DNA replication in ovary follicle cells in flies. However, the HAT activity of CHAT is not essential for gene amplification since our data shows that *ada2b* mutants show normal gene amplification in ovary follicle cells. Although the HAT activity of CHAT is dispensable for DNA replication, this complex is essential for viability in flies. Y2H and recombinant proteins co-immunoprecipitation experiments showed that the Chiffon C-terminal domain nucleates CHAT formation. Notably, this C-terminal domain, but not the N-terminal domain required for Cdc7 activation, is essential for fly viability. These data suggests that CHAT histonce acetylation activity has a critical role in fly development. Our hypothesis is that Chiffon regulates gene expression of a subset of genes required for development by activating transcription. Indeed, our current RNA-seq studies support the idea that Chiffon regulates gene expression in late stage embryos.

392B Epigenetic and transcriptional changes in *Drosophila* mushroom bodies neurons due to restrictive nutrition during development on neuroblast stage.

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Nutrition can affect the brain during early life and development. There are many studies in humans exposed to famine, which revealed how low nutrition in-utero correlates with mental disorders like schizophrenia and other personality pathologies. This long lasting effect may be the result of epigenetic changes in brain cells that set a stable chromatin configuration related with altered channel expression and metabolic misbalance. In this work, we used *Drosophila* as model to investigate how low nutrition (sugar and proteins) during development influences brain cells, specifically the mushroom bodies neurons. The mushroom bodies are associated with olfactory and visual memory, behavior and sleep. Using a nuclear isolation technique, from adults flies grown in restrictive and full nutrition, the nuclei of mushroom bodies neurons were separated to perform mRNA-seq and ATAC-seq libraries, making cross-data from these two profiles. The most relevant genes gave insights about the molecular mechanisms underlying behavioral which are manifested on changes on sleep distribution and movement, with more activity on entire circadian cycle.

393C Somatic pairing loss in interspecies Drosophila hybrids reveals genome features driving pairing

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Homologous chromosome pairing is essential to all eukaryotes. Although we often associate chromosome pairing with meiosis, pairing can also occur in somatic cells. Near-complete somatic pairing is the wild type state in dipterans such as *Drosophila*, but highly reproducible patterns of unpaired regions are observed across the genome in interspecific *Drosophila* hybrids. Hybrids generated from more diverged *Drosophila* species show a greater degree of pairing loss, raising the possibility of a causal relationship between somatic pairing and reproductive incompatibility. Understanding what makes some regions pair while others do not is an avenue toward understanding the molecular components that drive pairing generally.

We crossed *Drosophila melanogaster* and *Drosophila simulans*, then used Hi-C to measure the rate of chromosome pairing with high resolution across the genome. This Hi-C shows dramatic regions of high and low pairing. We also showed that this reduction in chromosome pairing is unique to polytene tissues in hybrid individuals, and demonstrate that there is no signal of reduced chromosome pairing around inversion breakpoints in a pure-species cross, in opposition to existing hypotheses.

While past experiments using light microscopy to measure pairing rates were able to show the existence of non-pairing regions, our high-resolution methods show a rich tapestry of high- and low- pairing regions of varying sizes and intensities. The narrow, clearly resolved peaks are consistent with the "button" model of chromosome pairing, rather than the "zipper" model. We identified local pairing maxima and minima, showing that they are apparently uncorrelated with classical genome features believed to drive chromosome pairing rates, such as sequence similarity, chromatin state, and insulator binding site density. Future work will include a survey of *D. melanogaster* genome features to identify features associated with pairing peaks, as well as experimental dissection of the trait of hybridization-driven pairing changes using knockdowns of the pairing-influencing gene complex Condensin II.

394A Temperature, rainfall and wind variables underlie environmental adaptation in natural populations of *Drosophila melanogaster*

María Bogaerts Márquez[1,2], Sara Guirao-Rico[1,2], Mathieu Gautier[3], Josefa González[1,2] 1) Institute of Evolutionary Biology (CSIC-Universitat Pompeu Fabra); 2) The European Drosophila Population Genomics Consortium (DrosEU); 3) CBGP, INRA, CIRAD, IRD, Montpellier SupAgro, Université de Montpellier, Montpellier, France.

While several studies in a diverse set of species have shed light on the genes underlying adaptation, our knowledge on the selective pressures that explain the observed patterns lags behind. *Drosophila melanogaster* is a valuable organism to study environmental adaptation because this species originated in Southern Africa and has recently expanded worldwide, and also because it has a functionally well-annotated genome. In this work, we aim to decipher which environmental variables are relevant for adaptation of *D. melanogaster* natural populations in Europe and North America. We analyzed 36 whole-genome pool-seq samples of *D. melanogaster* natural populations collected in 20 European and 11 North American locations. We used the BayPass software to identify SNPs and transposable elements showing signature of adaptive differentiation across populations, as well as significant associations with 59 environmental variables related to temperature, rainfall, evaporation, solar radiation, wind, daylight hours, and soil type. We found that besides temperature and rainfall, wind related variables are also relevant for *D. melanogaster* environmental adaptation. Interestingly, 23% to 51% of the genes that showed significant associations with environmental variables were not found overly differentiated across populations. Besides SNPs, we also identified ten reference transposable element insertions associated with environmental variables. Our results showed that genome-environment association analysis can identify adaptive genetic variants that are undetected by population differentiation analysis while also allowing the identification of candidate environmental drivers of adaptation.

395B Evolution of Y-linked genes through expression analysis of *Drosophila*'s spermatogenesis

Carolina de Athayde Mendonca[1,3], Camila Correia Avelino[1], Mara Maria Lisboa Santana Pinheiro[1], Gabriel Nassar Reich Goldstein[1], Antonio Bernardo Carvalho[2], Maria Dulcetti Vibranovski[1] 1) Universidade de Sao Paulo, USP, Sao Paulo, SP, Brazil; 2) Universidade Federal do Rio de Janeiro, UFRJ, Rio de Janeiro, RJ, Brazil; 3) Harvard University, HU, Boston, MA, USA.

The study of *Drosophila melanogaster* Y chromosome has always been slow even in the genomic era mainly because of its heterochromatic nature. Ingenious methods lead to the mapping of six fertility factors and the identification of 12 single-copy genes. These genes were originated from duplications of autosomes. The comparison of the expression profile of a gene that is Y-linked in one species and autosomic in a second species may shed light on the evolution of the expression profiles of Y-linked genes. To test different evolutionary hypotheses, stage-specific (mitosis, meiosis and post-meiosis) spermatogenesis RNAseq were conducted for *D. melanogaster*, *D. willistoni* and *D. mojavensis*. Differential expression analysis revealed that the increase in expression from mitosis to meiosis is commonly found in Y-linked genes and in its autossomic orthologs. This suggests that the increase in meiosis is an ancestral character that was selected among genes duplicated to the Y. On the other hand, the maintenance/increase in post-meiosis seems to be more common among Y-linked genes in relation to their autossomic ancestors. The Y genomic context may favour the expression in later phases of spermatogenesis and some possible mechanisms are the formation of *lampbrush-like loops* and epigenetic marks. Thus, natural selection and genomic context are relevant on determining the expression profiles of Y-linked genes.

396C Using fruit fly chaetotaxy to Infer Mechanisms of Morphological Evolution in Different Insect Clades

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Insect exoskeletons have taxonomically identifiable bristle patterns which perceive environmental cues and have proven to be a valuable model system for studying aspects of evolution including evolutionary innovations, developmental constraints and effects of artificial selection. Here we use leg bristles for studying convergent evolution. We studied the leg chaetotaxy of fruit fly wild type and mutant flies by comparing numbers and locations of mechanosensory and chemosensory bristles .We provide known examples in which *Drosophila melanogaster* mutations can mimic aspects of chaetotaxy in other *Drosophila* species as well as other insect clades . We also document the gain and loss of vertical bristle rows several times in evolution, suggesting that their functions change during evolution and thus the gains and losses can result in convergent evolution. Combining the existence of mutant phenotypes that mimic patterns in different insect clades suggests that there may be a basic "ground plan", which can allow rapid changes during evolution. Here, we expand our model and propose the potential cellular and developmental processes responsible for the cases of convergent evolution.

397A Timing and Pattern of Early Diversification in Drosophilidae (Diptera): A Phylogenomic Approach

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Drosophilid flies are extensively used as model organisms in biological sciences, and many studies rely on phylogenetic hypotheses of the group for conclusions. Notwithstanding, most phylogenetic studies to date focused only on *Drosophila*, and thus the relationships and divergence times of other genera in the family remain unclear. Here, we used a set of 1,292 single-copy ortholog genes to infer a phylogenetic hypothesis and estimate divergence times for 21 species of drosophilids (including representatives of most tribes proposed for both the subfamilies Drosophilinae and Steganinae). We estimated divergence times using a fossil-based calibration and a Bayesian method (MCMCTree) and inferred species trees using both concatenation and multispecies coalescent approaches. In these inferences, we consistently recovered the monophyly of the two subfamilies, despite the high levels of gene tree heterogeneity (*e.g.*, 58.3% of the gene trees recovered Steganinae as paraphyletic). Yet, we recovered as paraphyletic most of the tribes proposed to date. We estimated that the most recent common ancestor of the drosophilids has lived ca. 58 Ma (42-75 Ma) and that the speciation events leading to current genera have occurred in quick succession during the Eocene and the Oligocene (23-56 Ma). This scenario may have favored the retention of ancestral polymorphisms (incomplete lineage sorting), which is probably the primary cause of the high levels of gene tree heterogeneity found in Drosophilidae. This heterogeneity may have biased previous studies that relied on only a few genes to infer the phylogeny of the family, and we overcame this issue by using genomic data. Thus, we propose here a robust dated phylogenetic hypothesis for the drosophilids.

398B Regulatory changes underlying chromatin accessibility divergence within and between *Drosophila* species.

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Chromatin accessibility is an important mechanistic step in eukaryotic transcription, as physical access to DNA allows for *trans*-regulatory factors to bind *cis*-regulatory elements. Previous work has shown extensive chromatin accessibility variation within and between species at loci enriched for regulatory elements, suggesting that genetic changes altering chromatin accessibility may represent a mechanism underlying gene expression differences across genotypes. However, the relationship between chromatin accessibility and gene expression variation is complex and remains unclear. In particular, whereas the contribution of *cis* and *trans* changes both within and between species is well characterized for gene expression, this is not the case for chromatin accessibility. To address this shortcoming, I have collected chromatin accessibility data using ATAC-seq from *Drosophila melanogaster* (North American and African populations), *D. simulans*, and their F1 hybrids to determine the contribution of *cis* and *trans* changes to chromatin accessibility differences at different evolutionary timescales. These data suggest that 1) unlike gene expression, the relative contribution of *cis*-regulatory changes to chromatin accessibility differences does not increase with divergence time and 2) similar to gene expression, the effect size (i.e. chromatin accessibility difference) of *cis*-regulatory changes is greater than that of *trans*-regulatory changes.

399C Evolution of mRNA Localization in Drosophila Oogenesis

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Maternal gene products supplied to the egg during oogenesis drive the earliest events of embryogenesis in all metazoans. The subcellular localization of these maternal mRNAs in oocytes is also a conserved feature in a number of vertebrates and invertebrates, and critical to their development. For instance, the crucial first step in setting up the major body axes is the asymmetric localization of maternal mRNAs in a number of model systems, including Drosophila, Xenopus, and zebrafish.

Additionally, there are examples where specifically localized transcripts essential to organism survival have changed over evolutionary time. Even within Diptera, recent studies have shown that different maternally localized RNAs act as the primary anterior determinant in different species of basal Diptera. However, no systematic study has been done to examine how localization of maternal transcripts can evolve over shorter periods of evolutionary time, or to investigate the mechanisms behind the evolution of localization. Previous experiments in the lab used a large comparative dataset to show that hundreds of maternal transcripts are differentially supplied across the 50 million years of divergence time represented by the genus Drosophila. However, the extent and rate of gains or losses in localization of maternal transcripts in the egg between these species is unknown. Given that we observe a significant number of changes in the pool of maternal transcripts deposited across *Drosophila*, my hypothesis is that considerable evolution of novel maternal mRNA localization will occur on this evolutionary timescale. In order to test this hypothesis, I bisected oocytes, extracted and sequenced the RNAs from the anterior and posterior halves, and analyzed how the transcripts present in each half differ. This experiment has been performed in 5 species spanning the Drosophila genus, with increasing divergence times from *D. melanogaster.* Findings reveal which transcripts evolve changes in localization and the rate of change of mRNA localization across the Drosophila genus. Analysis of the differences between cis regulatory regions will determine what changes are required for these differences. This novel localization may ultimately underlie adaptive changes during embryogenesis necessary for survival of early development under different conditions.

400A Multiple sex chromosome-autosome fusions associated with high satellite DNA content in *Drosophila virilis*

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Robertsonian translocations are one of the most common chromosomal aberrations involved in disease and genome evolution. They occur when an acrocentric chromosome experiences a break near the centromere, and during the repair process, becomes fused to another acrocentric chromosome. The occurrence of chromosome fusions might be completely random, or it could be that natural genetic variation influences their probability. Pericentromeric satellite DNA abundance varies by several megabases among *Drosophila virilis* strains, and we hypothesize its abundance influences the rate of DNA breakage and thus Robertsonian translocation events. We discovered a strain of *D. virilis* that is an outlier with extremely high satellite DNA abundance, and also contains two independent chromosome fusions. We isolated both fusion substrains and used cytogenetics and Mendelian segregation experiments to demonstrate that one fusion is Y-Chr3 and the other is X-Chr4. To quantify DNA breakage levels between strains with different satellite DNA abundances, we performed the comet assay after feeding flies gemcitabine and administering low-dose gamma radiation. We find that DNA breakage levels vary significantly among lines with varying satellite abundances. We also hypothesize that these chromosome fusions have fitness costs with increased rates of non-disjunction. Overall, our work offers genome instability as a potential constraint on satellite DNA abundance. We also believe the model we discovered will be valuable in studying the dynamics of neo-sex chromosomes, neo-centromeres, centromere inactivation, and meiotic drive.

401B Copper Tolerance in Natural Populations of European *Drosophila melanogaster* is Shaped By a Complex Interplay of Regulatory and Environmental Variables

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Industrialisation and increased anthropogenic activity have resulted in a greater release of pollutants such as heavy metals into the environment, of which copper is one of the most abundant. However, due to its highly heterogeneous distribution and its dual nature of as both an essential micronutrient and toxic element, the genetic basis to copper tolerance has the potential to involve a complex interplay of physiological and environmental factors. As long-standing sentinel of environmental toxins, *Drosophila melanogaster* makes for an excellent model for the study of copper tolerance in arthropods.
In this study, we characterised the degree of copper tolerance in *D. melanogaster* across Europe by screening 97 inbred and iso-female lines taken from twelve populations on copper sulphate. We found that copper tolerance is a highly variable phenotype both within and between locations. While these locations covered a wide range of different atmospheric and soil pollution levels, we found that the environmental factor most strongly linked to copper tolerance was the degree of urbanization at the collection sites. Using a combination of genomic and differential expression analysis, we revealed that multiple copper-related genes are regulated by the transcription factors *sir2* and *HNF4*, and are potentially affected by transposable element insertions. We demonstrate that the greatest transcriptomic response to copper toxicity occurs in the midgut; where the preservation of gut acidity is strongly linked to greater copper tolerance. In addition, we show that these changes are predominantly physiological and are not linked to changes in feeding behaviour Overall, our study provides a new perspective in regards to the genetic and environmental factors that shape copper tolerance in *D. melanogaster* across Europe

and identifies new gene and regulatory candidates involved in this complex phenotype.

402C CRISPR mutants of Y chromosome genes in *Drosophila melanogaster*

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Gene-poor, repeat-rich regions of the genome are poorly understood and have been understudied due to technical challenges and the misconception that they are degenerating "junk." Yet multiple lines of evidence indicate these regions may be an important source of variation that could drive adaptation and species divergence, particularly through regulation of fertility. The ~40 Mb Y chromosome of *Drosophila melanogaster* contains only 16 known protein-coding genes and is highly repetitive and entirely heterochromatic. Most of the genes originated from duplication of autosomal genes and have reduced nonsynonymous substitution rates, suggesting functional constraint. We previously devised a genetic strategy for recovering and retaining stocks with sterile Y-linked mutations and combined it with CRISPR to create mutants with deletions that disrupt three Y-linked genes – *PRY*, *FDY*, and *CCY*. In the process we encountered instances of Y chromosome truncation, low CRISPR efficiency, and sterility of mutants despite the presence of an extra wild-type Y chromosome, suggesting that perturbation of the Y chromosome can lead to dominant sterility. We now present a modified approach for creating CRISPR mutants of Y-linked genes that accounts for these special challenges. We also present our progress in generating knockouts of additional Y-linked genes and analyzing their phenotypes.

403A Environmental and genomic interactions determine *Wolbachia* maternal transmission and infection frequencies

Michael Hague[1], Chelsey Caldwell[1], Dylan Shropshire[1], William Connor[1], Brandon Cooper[1] 1) University of Montana.

Maternally transmitted *Wolbachia* infect about half of all insect species, as well as other arthropods. *Wolbachia* rapidly spread within host populations by increasing host fitness and manipulating host reproduction (e.g., cytoplasmic incompatibility). Many *Wolbachia*, including *w*Mel infecting *D. melanogaster*, persist at intermediate frequencies that tend to fluctuate across space and time for reasons that are poorly understood. Here, we integrate genetic, genomic, and cellular analyses to dissect the contributions of imperfect maternal transmission and *Wolbachia* effects on host fitness and reproduction to fluctuating *w*Mel-like *Wolbachia* frequencies in the *D. melanogaster* subgroup of hosts. We find that the fidelity of *Wolbachia* maternal transmission is strongly dependent on temperature. Although transmission is near perfect under standard laboratory conditions, rearing hosts in relatively cold temperatures reduces *Wolbachia* titer in host bodies, resulting in imperfect maternal transmission. Using mathematical models of *Wolbachia* frequency dynamics and equilibria, we infer that temperature effects on maternal transmission can plausibly explain spatial fluctuations in *Wolbachia* frequencies. Notably, our analysis of reciprocally introgressed *Wolbachia* and host genotypes indicates that both the *Wolbachia* and host genomes also contribute to imperfect maternal transmission, revealing that certain *Wolbachia*-host genotype combinations have a higher fidelity of transmission. These results highlight the complex interplay among *Wolbachia*, hosts, and the environment underlying *Wolbachia* maternal transmission and the maintenance of infections in host populations. Identifying how environmental and genetic conditions contribute to fluctuating *Wolbachia* dynamics is key to explaining the global *Wolbachia* pandemic and improving biocontrol efforts that rely on maintaining *w*Mel infections in mosquito populations to prevent human diseases, including dengue and Zika.

404B The evolution of sex-biased gene expression in the *Drosophila* brain.

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Genes with sex-biased expression in *Drosophila* are thought to underlie sexually dimorphic phenotypes and have been shown to possess unique evolutionary properties. However, the forces and constraints governing the evolution of sex-biased genes in the somatic tissues of *Drosophila* are largely unknown. Using population-scale RNA sequencing data we show that sex-biased genes in the *Drosophila* brain are highly enriched on the X Chromosome and that most are biased in a species-specific manner. We show that X-linked male-biased genes, and to a lesser extent female-biased genes, are enriched for signatures of directional selection at the gene expression level. By examining the evolutionary properties of gene flanking regions on the X Chromosome, we find evidence that adaptive cis-regulatory changes are more likely to drive the expression evolution of X-linked male-biased genes than other X-linked genes. Finally, we examine whether constraint due to broad expression across multiple tissues and genetic constraint due to the largely shared male and female genomes could be responsible for the observed patterns of gene expression evolution. We find that expression breadth does not constrain the directional evolution of gene expression in the brain. Additionally, we find that the shared genome between males and females imposes a substantial constraint on the expression evolution of sex-biased genes. Overall, these results significantly advance our understanding of the patterns and forces shaping the evolution of sexual dimorphism in the *Drosophila* brain.

405C Cryptic suppression reveals intragenomic conflict in the *Sex Ratio* system of *Drosophila pseudoobscura*

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In *Drosophila* species, segregation distorting *X* chromosome variants called *Sex Ratio* (*SR*) chromosomes are hypothesized to fuel an evolutionary arms race between *X*-linked distorters, *Y*-linked targets, and autosomal suppressors. However, several well-studied *Sex Ratio* systems exhibit no evidence of suppression despite ancient origins of their *SR* chromosomes. This "ancient gene drive paradox" can be resolved if the present-day absence of *SR* suppression is just a transitory phase in an ongoing evolutionary arms race. Here, we test the intragenomic arms race hypothesis by surveying for historical, now cryptic, suppressors of the ancient, unsuppressed *SR* chromosome in *Drosophila pseudoobscura.* First, we performed segregation assays using rare recombinant *SR* chromosomes which reflect a historical state of *SR*chromosomes in North American populations. Second, we examined genetic variation for suppressors in a recently diverged South American subspecies of *Drosophila pseudoobscura* that lacks endemic *SR* chromosomes. We discovered a partially dominant autosomal suppression system only capable of affecting recombinant *SR* chromosomes in North American populations and a recessive autosomal suppression system of non-recombinant *SR* chromosomes in South American populations. We present a historical scenario where the same intragenomic conflict favors evolution of autosomal suppression leading to the extinction of *SR* chromosomes in South American populations, but in North American led to an escalation of the molecular arms race in the form of a suppressor-of-a-suppressor. The cryptic evolution of *D. pseudoobscura's Sex Ratio* system discovered here indicates an intragenomic evolutionary arms race driving both the continued evolution and extinction of this same enigmatic *SR* chromosome.

406A Honeybee queen pheromone induces a potentially conserved starvation response in *Drosophila melanogaster*

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Eusociality is the social structure where one female (the queen) reproduces, and her workers have their reproduction repressed (e.g. a classical honeybee hive). In the insects, this system has evolved independently at least 20 times. This raises the question of how this group living evolves, and which mechanisms underlie its function. Are the same pathways of repressing reproduction involved each time it arises? Or are new mechanisms evolved each time? This is the question this project has addressed.
To do so, *Drosophila* were exposed to QMP- the pheromone which queen bees use to prevent reproduction in their workers. Theoretically, these *Drosophila* should not have their reproduction affected, as they are not eusocial, are never exposed to QMP in nature, and diverged from honey bees ~350 million years ago. Despite this, they do show a reduction in their fecundity. This research into this response in *Drosophila* has allowed for the identification of mechanisms which we believe to be key to this process, in particular nutrient sensing. This leads to changes in foos consumption behaviour, as well as ovarian checkpoint activity. Overall, the mechanisms which underpin this reproductive constraint in response to QMP are likely ancient, conserved mechanisms for responding reproductively to environmental changes. These processes appear to be co-opted during the evolution of eusociality in honey bees.

407B Novel odorant receptors tuned to mustard oils facilitate evolution of herbivory in Drosophilidae

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Plant toxins are effective defenses because they typically cause aversion to herbivores. These molecules, however, are often co-opted by specialist herbivores as host-finding chemical cues. It is not understood, however, how these shifts in chemosensory behavior evolve. To address this question, we used *Scaptomyza flava,* a herbivorous drosophilid fly that specializes on mustard plants that produce isothiocyanates toxins (ITC). We identified three olfactory receptor (*Or67b*) paralogs experiencing positive selection since its divergence from a microbe-feeding common ancestor (<10 million years ago) as candidate receptor genes that mediate host-seeking behavior. *In vivo* electrophysiological recordings of neurons expressing *S. flava* Or67b proteins -but not homologs from microbe-feeding relatives- responded exclusively to volatile ITCs. Consistent with this observation, we found that *S. flava*, but not its microbe-feeding relative, is attracted to ITCs. Ancestrally aversive chemicals can be co-opted as attractants through gene duplication, contributing to behavioral valence shifts in herbivorous insects.

408C Nuclear transport genes experience high turnover in *Drosophila* but not in other insects

Ayda Mirsalehi[1], Mohammadmehdi Eslamieh[1], Dragomira Markova [1], Esther Betran[1] 1) University of Texas at Arlington.

Although the nuclear transport system is highly conserved and its components are highly expressed in every eukaryotic cell, previous studies have shown recurrent and convergent duplication of some of its genes in different *Drosophila* lineages. Losses of these gene duplicates, fast evolution, and male-biased expression have also been observed. This particular gene evolution pattern is attributed to their involvement in male germline genetic conflicts. Nuclear transport machinery is involved in several known male meiotic drive systems in *Drosophila*, and gene duplication of nuclear transport genes has been an underlying mechanism in almost all of them. Using a comprehensive, detailed phylogenomic study, we examined 52 species of *Drosophila* and non-Drosophila insect genomes for duplication of nuclear transport genes. We find duplications of nuclear transport components that have not been reported before and additional independent duplication events for the genes shown to have undergone duplications in previous studies. Furthermore, while the vast majority of duplications are RNA mediated, we detect a few DNA-mediated duplications that are new because only retrogenes were studied for some genes in the past. We also recovered several additional pseudogenes. Some of them are very young and contribute to the turnover. Although we find a few duplications of nuclear transport genes in Diptera species outside of *Drosophila*, most of them are in *Drosophila* species and not other insects. These findings and the detected fast evolution of the duplicates, compared to their parental counterparts, strengthens the hypothesis that they evolve as suppressors of meiotic drive systems or as another male-specific adaptation that is specific to flies.

409A *Cis*-regulatory variation and sex-biased gene expression in *Drosophila melanogaster*

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Males and females may move towards their respective fitness optima by evolving differences in expression at certain genes. Following this, genes with sex-biased expression may continue to be under sexually antagonistic selection, maintaining gene regulatory variation through the resulting balancing selection. Alternately, they may represent a resolution of past sexual conflict over gene expression. While numerous studies have documented sex differences in expression, less is known about how segregating variation affects expression in males and females. Here, we study allele-specific expression (ASE) to gain an understanding of genome-wide patterns of *cis*-regulatory variation in *Drosophila melanogaster*. We explore three main questions: (1) Are *cis*-regulatory variants found more commonly at sex-biased vs unbiased genes, implying the maintenance of greater regulatory variation in such genes? (2) Do *cis*-regulatory variants affect both sexes similarly, and is the concordance of effects across the sexes reduced for sex-biased genes? (3) Are *cis*-regulatory variants more numerous in strongly (gonads) and weakly (heads) dimorphic tissues, even after for controlling for differences in sex-biased gene expression? Insights from this study could help elucidate the regulatory framework underlying sex-biased gene expression, and by extension, the resolution of sexual conflict.

410B Diet-induced changes in titer support a threshold effect of Wolbachia-associated plastic recombination in *Drosophila melanogaster*

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Plastic recombination in *Drosophila melanogaster* has been associated with a variety of extrinsic and intrinsic factors such as temperature, starvation, and parasite infection. The bacterial endosymbiont *Wolbachia pipientis* has also been associated with plastic recombination in *D. melanogaster*. Wolbachia infection is pervasive in arthropods and this infection induces a variety of phenotypes in its hosts, the strength of which depend on bacterial titer. Here we test the hypothesis that the magnitude of Wolbachia-associated plastic recombination in *D. melanogaster* depends on titer. To manipulate titer, we raised Wolbachia-infected and uninfected flies on diets that have been previously shown to increase or decrease Wolbachia titer relative to controls. We measured recombination in treated and control individuals using a standard backcrossing scheme with two X-linked visible markers. Our results recapitulate previous findings that Wolbachia infection is associated with increased recombination rate across the yellow-vermillion interval of the X chromosome. Our data show no significant effect of diet or diet by Wolbachia interactions on recombination, suggesting that diet-induced changes in Wolbachia titer have no effect on the magnitude of plastic recombination. These findings represent the first step toward investigating the mechanisms behind Wolbachia-associated plastic recombination and demonstrate that the effect may be threshold-based as opposed to dose-dependent.

411C Modeling satellite DNA organization

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Repetitive DNAs comprise large portions of eukaryotic genomes. Satellite DNAs (satDNAs) are abundant tandemly repeated DNA sequences found near centromeres, telomeres, and on sex chromosomes. SatDNAs originate through polymerase

slippage, recombination between repeat elements, or TE-mediated mechanisms. Arrays of satDNA repeats are highly dynamic over short periods of evolutionary time: they vary in copy number and organization through unequal exchange, mutation, and other processes. The expansion of satDNA arrays is thought to decrease organismal fitness but the relative importance of processes shaping satDNA evolution in natural populations is not well understood. Models of unequal crossing over and selection on satDNA arrays mainly focused on copy number number changes. We used an Approximate Bayesian Computation approach to fit one of these models (Stephan 1986) to simulated data and determine the suitability of this model for estimating parameters of recombination (and selection) in populations. Summarizing satDNA arrays by properties of the copy number distribution alone, however, was not informative. Instead, conditioning on the structure and organization of the arrays may be more informative about underlying mutation and recombination processes. We developed a model that tracks structural changes and mutations in satDNA arrays. Our model simulates both sequence and copy number evolution for a population of size n over x generations. We designed a method to determine the site of recombination breakpoint based on satDNA copy numbers using the transition probabilities described in Stephan's 1986 model and the sequence composition of the monomers. We also incorporate random nucleotide mutation and natural selection on copy number. Introducing sequence composition and organization to models of satDNA evolution may prove useful for estimating the impact of recombination and natural selection on satDNA arrays from empirical data.

412A Transcriptome responses to compensatory selection: study of *net* and *rho* suppression

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Regulatory networks are tuned and reorganized during evolution but how easily these networks can be rewired during rapid evolution is poorly understood. Major mutations and environmental insults can expose cryptic genetic variation in populations, and compensatory selection can utilize this variation to restore phenotypes and potentially also fitness. We ask how does selection on cryptic genetic variation lead to the restoration of phenotypes and modify regulatory and developmental circuits? To address this question we studied gene expression in strains of *Drosophila melanogaster*, that had undergone genetic perturbation and subsequent artificial selection leading to compensatory evolution of major phenotypes. Mutations in the genes *net* and *rhomboid*, known to act antagonistically during development to pattern wing veins, were introgressed into a wild type population. Replicate populations were subject to artificial selection for improved wing shape (evolved) and "natural" selection against the harmful effects of the major mutations (control). Full compensation of venation defects was achieved in the evolved flies but none was observed in the controls. We did RNA seq of several distinct populations: Reference (wild-type) population, two introgressed populations (*net* and *rhomboid*), and three evolved and three control populations for each mutation. Transcriptome libraries were prepared from wing-discs of 3rd instar larvae. The *net* mutation impacted expression of more genes than *rhomboid*. Compensation of the wing phenotypes did not seem to be mediated by transcriptional restoration of these two genes, arguing against compensatory evolution of upstream factors in the same pathways. The data suggest the compensation was achieved by restored expression/function of the target genes of *net* and *rhomboid*, or by changes in parallel pathways. Interestingly, expression of several genes was altered by the compensatory selection in both backgrounds. One set of genes showed the same pattern in *net* and *rhomboid* backgrounds, and slightly fewer genes displayed antagonistic patterns in the backgrounds. Analyses of the evolved responses to permutations of genes in overlapping or antagonizing pathways offers unique way to study the evolution and modulation of regulatory networks.

413B Phylogenetic analysis of the Neotropical anthophilic species *Drosophila lutzii*

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The species diversity of Drosophilidae is well documented, and it is known that several species use a wide range of resources as feeding and breeding sites. Despite the efforts of many researchers to extend knowledge to species that use "unconventional" resources, like flowers, there is a lot to be clarified about anthofilic drosophilids, as the species belonging to the *Drosophila lutzii* species group. Until now, only one phylogenetic hypothesis using molecular markers is reported. According to it, *D. lutzii* is placed in the subgenus *Drosophila*. A precise phylogenetic reconstruction of this group will allow further studies of niche specialization and ecological traits. This study aims to improve the hypothesis of the evolutionary relationships of this group. In this way, the genome of *D. lutzii* was sequenced for the first time, and 1,521

single-copy orthologous genes were identified, concatenated, and used to generate a distance tree using the Neighbor-Joining algorithm. Also, phylogenetic trees were reconstructed using Bayesian Inference and Maximum Likelihood, based on five mitochondrial genes (*COI*, *COII*, *CytB*, *12S*, *16S*) and six nuclear genes (*Amyrel, Adh, Amd, Ddc* and *GPDH*), 70 species were analyzed, covering mostly the subgenus indicated by the previous analysis. The distance-based tree, reconstructed with 1,521 single-copy orthologous genes, recovered the phylogenetic relationships described previously. This analysis placed *D. lutzii* within the *Drosophila* subgenus, more specifically, as sister group of *D. innubila* (*quinaria* group). The reconstruction using 11 molecular markers, with emphasis on the *Drosophila* subgenus, positioned *D. lutzii* as sister group of *D. bandeiratorum* (*tripunctata* group, subgroup III), closely related to *D. pallidipennis* (*pallidipennis* group), within the "*tripunctata* lineage", described previously. The results indicate that the *D. lutzii* group has strong evolutionary relationships with species of the *Drosophila* subgenus, especially lineages from the *Drosophila tripunctata* group.

414C Evolving a novel trait through co-option of the *shavenbaby* gene regulatory network

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In order to understand the mechanisms that led to the diversity of forms found across the tree of life, it is vital to determine how new traits are generated. Network co-option is a process thought to facilitate novelty through the deployment of a set of interacting genes into a new developmental context. Most models of co-option suggest the existence of an upstream factor whose ectopic expression is sufficient to initiate the activation of an entire network in a new tissue. However, these transcription factors have seldom been identified, and their sufficiency to induce a trait has rarely been established. **Here, we identify and validate a well characterized genetic network that was redeployed to generate a novel morphological feature.**

Among *Drosophila* species, a wide variation of genital morphologies exist, including the phallus of *Drosophila eugracilis*, which is covered with over a hundred spike shaped structures that have been implicated in wounding females during copulation. The homologous tissue in *Drosophila melanogaster* lacks these spikes, providing a convenient outgroup for comparisons of development and genetic manipulations. We have found that the spikes in *Drosophila eugracilis* are produced by single cell extensions, similar to the small hairs (trichomes) that adorn the body in *Drosophila*. The transcription factor *shavenbaby* activates a well-studied genetic network responsible for trichome formation. We find that *shavenbaby* and its downstream targets are expressed in spike-forming tissues, suggesting the co-option of this network underlies this dramatic phenotype. We have also discovered that ectopic expression of *shavenbaby* in the phallus of *Drosophila melanogaster* induces phallic spikes. These results indicate that co-option of the *shavenbaby* network is sufficient for the gain of this dramatic novel trait.

415A Recurrently domesticated PIF-transposases

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Transposable elements (TEs) are genetic units that move and amplify within a host genome. In recent years, an increasing number of studies have shown that TE encoded proteins can also be co-opted by their host for its own benefit through a process called molecular domestication. Thus, TE proteins are an important contributor to the emergence of novel host proteins. Despite the relative abundance of RNA TEs in eukaryotic genomes, DNA TE proteins and most notably their transposase, responsible for the excision and movement of the elements within the host genome, are generally considered to be more likely to be co-opted by the host than any other TE-derived protein. We have been studying four domesticated transposases from the *PIF/Harbinger* DNA family of TEs in *Drosophila melanogaster*, named *Drosophila PIF Like Genes* (*DPLGs*). All four *DPLGs* in *D. melanogaster* are highly diverged, under purifying selection, and most likely arose through independent domestication events. Using protein localization studies and null mutant lines for *DPLG1-4*, we show that *DPLGs* are potentially domesticated as regulatory proteins and a subset of these genes is also involved in neuronal and gonadal functions, and affect the viability, fertility and survival of *D. melanogaster*. Moreover, given the functional relatedness of these independently domesticated PIF transposase, we propose a model in which the domestication of a transposase might promote the domestication of related transposases from same TE family. To provide further support to this stepping stone model, we have investigated other Dipteran species genomes for additional evidence of recurrent transposase domestication events.

416B *Drosophila suzukii* Avoid Commensal Acetic Acid Bacteria in Oviposition Choice

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Oviposition site selection is a critical factor in determining the survival rate of the offspring in insect species. A nutritionally suitable resource may be heavily utilized by other insects and the offspring may suffer from intense competition. Therefore, to avoid competition, the strategy of developing new oviposition sites has evolved frequently in insects.
The majority of *Drosophila* species including *D. melanogaster* and *D. biarmipes* lay eggs on ripe and fermented fruits. Whereas, females of *D. suzukii* pierce the skin of a ripening fruit and lay eggs into it by using their serrated ovipositors. The behavioral shift to deposit eggs into ripening fruits must have been accompanied by changes not only in the ovipositor morphology but also in the sensory systems to evaluate the oviposition substrate. The evolution of firmness preference for the oviposition site has been investigated previously and it has been shown that *D. suzukii* are able to lay eggs on hard agarose gels, which are not used by other species. The chemosensory changes to sense and respond to attractants from ripening fruits have been well characterized. However, the repellent substances of fermenting fruits have not been well understood. We hypothesize that *D. suzukii* avoid microbes on fermenting fruits that have been deposited by conspecific and heterospecific individuals.
We investigated the effects of commensal microbes on oviposition site preferences in *D. suzukii*, *D. biarmipes* and *D. melanogaster*. In our assay, *D. suzukii* exhibited a strong avoidance of microbes transferred from other flies. This response was distinct from the other two species suggesting that the behavior has evolved in the lineage leading to *D. suzukii* after the split from *D. biarmipes*. Furthermore, we tested the combinatorial effect of the hardness and the presence or absence of microbes on the oviposition site selection. The mechanical stimuli provided by substrate hardness superseded the influence of microbial chemical signals. This property was conserved among the three species despite differential preference towards hardness and microbial stimuli.

417C Experimental evolution for longevity differentiation in *Drosophila melanogaster*

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According to the evolutionary theory of aging, the forces of natural selection start to decline after the first age of reproduction, and continue to steadily decline until the last age of reproduction, where these forces stabilize at zero or become weaker than random genetic drift. Past studies have used *Drosophila melanogaster* populations to show that gradually postponing the first age of reproduction, postpones the age at which the forces of natural selection begin to drop, and results in delayed aging and increased longevity. However, efforts to use short-lived and long-lived populations of *D. melanogaster* to study aging genes have faced difficulties due to limited replication and small population sizes. In this study, we aim to harness the full potential of experimental evolution and resequencing by completing a ten-fold replicated selection experiment, evolving ten *D. melanogaster* populations for extreme longevity. Long-lived populations were created by progressively postponing the first age of reproduction from 14 days to 70 days. Comparisons of selected populations to controls which were maintained on 14-day discrete generation cycles revealed a response to selection, including increased development time. Additional phenotypic and genomic differentiation between long-lived and short-lived populations will lead to identification of candidate genes affecting aging. This experimental evolution study at an unprecedented scale will enrich our understanding of specific loci that affect longevity.

418A Epigenetic conflict on a degenerating Y chromosome increases mutational burden in Drosophila males

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Large portions of eukaryotic genomes consist of transposable elements (TEs) that replicate at the expense of host fitness. During early embryonic development in Drosophila, the establishment of transcription-repressing heterochromatin at the maternal-zygotic transiton safeguards the genome from deleterious TE mobilization. However, prior to full establshment of heterochromatin after cellularization, TEs may exploit the ramp-up of zygotic expression and incomplete suppression for proliferation. Repeat-rich Y chromosomes, in particular, can act as reservoirs for TEs that takes advantage of this window of compromised defense. Here, we contrast the dynamics of early TE activation in two Drosophila species, *D. psueoobscura* and *D. miranda*, with vastly different Y chromosomes of disparate sizes and ages. In both species, zygotic TE expression is elevated in male embryos relative to females, mostly due to expression of Y-linked TEs. Interestingly, male-biased TE expression diminishes across development in *D. pseudoobscura*, but remains elevated in *D. miranda*, the species with the younger and larger Y chromosome. Using ChIP-seq against the heterochromatin mark H3K9me3 in sexed single embryos, we find that the formation of heterochromatin is compromised on the Y of *D. miranda*. This is because the younger Y still contains thousands of actively transcribed genes with open chromatin interspersed among high density of repeats. The close proximity of genes and repeats create an epigenetic conflict between activating and suppresive chromatin environment that TEs exploit for expression. We further find that, surpisingly, the elevated TE expression in males is associated with more *de novo* insertions compared to females. These results lend support to the idea that the 'toxic' Y chromosome can create a mutational burden in males especially when genome-wide defense mechanisms are compromised, and suggest a previously unappreciated

epigenetic conflict on evolving Y chromosomes between transcription of essential genes and silencing of selfish DNA.

419B Monarch flies on milkweeds: genetic basis of toxin resistance in an ecological context

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Herbivorous insects have played an important role in understanding how ecology drives fundamental evolutionary processes. Recently, the genetic basis of resistance to dietary toxins was elucidated in the milkweed butterflies (Danaini). Milkweed butterflies evolved to become specialists of toxic cardenolide-producing milkweeds, gaining increasing levels of resistance through a set of amino acid substitutions in the α-subunit of the Na/K ATPase, the physiological target of cardenolides. Additionally, some species in the lineage sequester toxins from their host plants, retaining them through development and using them to deter predators. We generated six gain of function knock-in D. melanogaster lines carrying mutations which mimic those found in the milkweed butterfly lineage, as well as "path-not-taken" mutations which are not found in the lineage, but also provide some cardenolide resistance. Our previous work established that these gain of function mutations progressively increased *in vivo* resistance to the widely studied hydrophilic cardenolide ouabain, which is not found in milkweeds. Here, we turned our attention to how these mutations affect performance of flies in a more ecologically relevant context. We addressed the hypothesis that the progression of substitutions in the milkweed butterfly lineage's adaptive walk is important for performance on a diversity of milkweed host plants that vary in cardenolide concentration and composition. First, we compared the survival and development of six knock-in fly lines on media containing seven different milkweeds which vary 14-fold in cardenolide content. We show *in vivo* that the milkweed butterfly lineage's adaptive walk conferred increasing resistance to high toxicity milkweeds, and provide additional insight into the selective pressures underlying the evolution of toxin resistance in medium and low cardenolide plants.

420C Friend or Foe: Modeling the genetic interaction between the reproductive parasite Wolbachia and a host germline stem cell gene

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Relationships between hosts and symbionts consist of a broad range of interactions that can result in rapid evolution of the involved genes. One widespread symbiont is the intracellular bacteria *Wolbachia. Wolbachia* lives in symbiosis with many arthropods, including *Drosophila melanogaster,* where it resides in the reproductive tract. Our lab has previously identified a genetic interaction between *Wolbachia* and the rapidly evolving *bag-of-marbles* (*bam*) gene, which acts as the key switch gene to promote differentiation of germline stem cells in female *D. melanogaster*. We hypothesize that *Wolbachia* could be contributing to the adaptive evolution of *bam* and are exploring two models from which this could occur. We call the first the "Conflict Model" as it is based on a potential arms race conflict between *bam* and *Wolbachia;* such conflict has been predicted to explain the evolution of numerous host-parasite interactions. Here, there is positive selection at *bam* to "escape" manipulation by *Wolbachia.* We call the second model the "Buffering Model." It is specific to the observed genetic interaction between *bam* and *Wolbachia* in which the reduced fertility of a partial loss of function *bam* mutant is rescued by *Wolbachia*. In this model, *Wolbachia* infection allows for a period of relaxed constraint at *bam* as it buffers the effect of slightly deleterious mutations that arise, and the positive selection is seen after *Wolbachia* is lost, when *bam* is selected to return to its optimal function.
We have implemented each model with the forward simulator SLiM and on the resulting simulated sequences, we have performed McDonald-Kreitman tests to identify parameter spaces that result in positive selection and performed preliminary analyses on sequence composition using amino acid metrics. We have found that the Conflict Model consistently gives signatures of positive selection across different parameter values of selection coefficients and *Wolbachia* infection time periods. In contrast, we see that while the Buffering Model can give signatures of positive selection, it is far less likely under our current setup. Additionally, we see that the amino acid sequence composition of sequences simulated under our two models are biochemically distinct. We are continuing to further refine the parameter spaces of interest and explore other methods of analysis. Next, we plan to relate our findings here to the empirical population-level data we have on *bam* across *Drosophila* species.

421A Unravelling the transcriptomic and physiological basis of desiccation tolerance in natural European *Drosophila melanogaster* populations

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Climate change is altering precipitation and water availability thus exposing species and ecosystems to increasing harnessing conditions such as desiccation. Insects are especially threatened by these challenging dry environments, because of their small size and thus large surface area to volume ratio. *Drosophila melanogaster* is a great model to study the response of populations to rapidly changing conditions, because it originated within southern Africa and has spread all around the world recently. Desiccation stress response is a complex and extensively studied trait, however, the natural variation in tolerance, the underlying transcriptomic and physiological mechanisms, and the causative genomic variants are still not well-understood. Here, we subjected to desiccation stress 74 natural European *D. melanogaster* strains belonging to five different climate zones. We found that the strains from cold semi-arid climates are more tolerant compared to the ones from hot summer Mediterranean climate zones. Moreover, the variance in the tolerance of the strains correlated with the interaction of altitude and evaporation. We found that the tolerant strains had a lower level of initial water content and lose less water during desiccation stress. We showed that the reduction in the water loss is possibly due to the decreased respiration rate in desiccation stress conditions, and to the cuticular hydrocarbon composition identified in the tolerant strains. Moreover, we found that the genes related to response to stimulus and environmental sensing are up-regulated only in the tolerant strains. Furthermore, we identified several desiccation candidate genes that can be targeted by tRNA derived fragments (tRF), known to be important in post-transcriptional gene regulation in several stress responses. Overall, our study describes for the first time the physiological and transcriptomic changes underlying the desiccation tolerance of natural European *D. melanogaster* strains, and puts tRFs in the scope of desiccation related studies as possible regulators of desiccation tolerance.

422B Functional validation of an Alzheimer's Disease susceptibility gene network

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Genome-wide association studies have implicated approximately 30 Alzheimer's disease (AD) susceptibility loci, including the candidate genes *PTK2B, CD2AP,* and *CASS4* which have been similarly implicated in cell adhesion and integrin mediated signaling. Genetic analysis of *CD2AP* and *PTK2B* highlight roles in synaptic function and axonal trafficking, respectively. We are testing the hypothesis that these, and perhaps other AD risk genes, comprise a susceptibility network, functioning coordinately to maintain brain health in aging. Using *Drosophila* models, we have studied conserved homologs of *PTK2B, CD2AP,* and *CASS4* (*Fak56*, *cindr* and *p130CAS*, respectively), examining for genetic interactions with Tau-mediated neurotoxicity. We have also assessed for neurodegenerative phenotypes independent of Tau, including both single and multiple combinations of mutant alleles. Immunoprecipitation-mass spectrometry (IP-MS) analysis was also performed to define a protein-protein interaction network in fly brains. In order to identify other network members, we also considered candidate key drivers from AD-associated gene coexpression networks derived from the Accelerating Medicines Partnership-AD Consortium analyses of human brain transcriptome and proteome profiles; 43 targets were screened for genetic interactions with Tau-induced retinal toxicity. Knockdown of *Fak56*, *cindr* and *p130CAS* similarly enhance human Tau neurotoxicity. In the absence of Tau, mutant analysis reveals little or no evidence of age-dependent neurodegenerative changes, based on either retinal neurophysiology or brain morphology. However, Fak56 mutants exhibits an age-dependent survival phenotype and under conditions of activity-induced neuronal stress, *Fak56* mutants reveal impaired neurophysiologic responses with aging. A similar phenotype is observed in *Fak56/cindr/p130CAS* triple heterozygotes, and genetic interactions are recapitulated in survival assays, supporting our susceptibility network hypothesis. Moreover, co-immunoprecipation experiments confirm physical interactions between Cindr and p130CAS within the adult fly brain. IP-MS identifies several other proteins as potential shared interaction partners for Fak56 and Cindr, including Ubiquilin. In our Tau modifier screen of conserved, candidate key drivers from AD-associated coexpression networks, we further identified fly orthologs of *Plectin* and *Moesin*, which are similarly implicated in cell adhesion. We highlight an nascent AD susceptibility gene network with potential roles in synaptic adhesion and neuronal vulnerability to Tau pathology. Results from IP-MS and a targeted genetic modifier screen identify additional promising network members for further investigation.

423C Bioinformatic and cell-based CRISPR tools for functional genomics in mosquitos

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Mosquito-transmitted diseases are among the deadliest human diseases and an ever-growing global health threat. Although CRISPR and other genetic technologies are well established for *Drosophila*, comparable tools have not yet been developed for CRISPR-based studies in mosquito species. Here, we present our work towards the development of a platform comprised of bioinformatic tools and CRISPR-ready reagents that make it possible to generate CRISPR knockouts and perform pooled CRISPR screens in mosquito cell lines. First, to facilitate CRISPR engineering in mosquitos, we developed an online bioinformatics resource for retrieval of pre-computed single guide RNA designs for mosquito genomes. The resource also supports ortholog mapping from *Drosophila* to mosquito species. Second, we experimentally identified optimal U6 promoters for expression of sgRNAs in cell lines derived from four major disease vectors: *Anopheles coluzzii*, *Culex quinquefasciatus, Aedes aegypti* and *Aedes albopictus*. Third, we engineered an *Anopheles* cell line following an approach developed for *Drosophila* cells, and we performed the first proof-of-concept pooled-format CRISPR screen in a mosquito cell line, using a drug resistance assay. Finally, we are further extending this work by generating CRISPR screen-ready mosquito cells for multiple mosquito species. The bioinformatics and cell-based resources described represent a powerful and widely-applicable approach for reverse and forward genetics in mosquitos and establish a paradigm for similar studies in other arthropod species.

424A Up, down, and out: new developments in loss- and gain-of-function CRISPR screens in fly cells

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High-throughput screening in cultured cells provides a valuable complementary approach to *in vivo* studies. In a pooled-format genetic screen in cultured cells, each cell receives one reagent at random from a complex library of reagents. We previously developed a delivery method for pooled DNA elements compatible with *Drosophila* cells, and used this approach to conduct the first genome-wide CRISPR knockout screens, identifying essential genes and new components of the ecdysone response pathway. In new work, we first introduce new screening-compatible strategies: fly cell-based CRISPR activation (CRISPRa) using the Synergistic Activation Mediator approach for systematic gene overexpression, and Cas13-based targeting of RNA for systematic gene knockdown. Next, we performed **genome-wide CRISPRa screens** and identified several genes that confer resistance to rapamycin when overexpressed using several different sgRNAs. Validation experiments showed that the pooled screen was able to accurately predict guide RNAs that permitted growth in the presence of rapamycin and elevated target gene expression. We have also found that Cas13 can knock down reporters and cause visible phenotypes in fly cells. Further, we can titrate Cas13 levels to reveal dose-dependent gene knockdown phenotypes. Finally, we report the **first genome-scale pooled screen for cell-essential genes using Cas13** in any system. Altogether, we report significant progress towards establishing genome-wide pooled CRISPR screening with reagents for overexpression and knockdown, providing approaches that complement our previously established pooled CRISPR knockout screening system.

425B Soma-Germline communication and the role of cortical polarity in signalling regulation during in Drosophila spermatogenesis.

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Stem cells are essential for the development, maintenance and repair of tissues in multicellular organisms. Communication between stem cells and their differentiating daughter cells with the surrounding tissue is vital to balance stem cells maintenance vs. differentiation. In the *Drosophila* testis, germline stem cells divide asymmetrically giving rise to daughter germ cells, which progressively differentiate to form mature sperm cells. During this process somatic cyst cells enclose the germline as a pair, forming a cyst-like structure that is the functional unit of differentiation. However, several aspects of communication between germline and somatic cyst cells are not fully known. We have previously shown that cortical polarity has the ability to impact signal transduction in cyst cells, which in turn affects the survival of the enclosed germ cells. Knockdown of the cortical and scaffolding components *dlg, scrib, lgl* or clathrin-mediated endocytosis (CME) in cyst cells results in germ cell death and elevated levels of MAPK and the membrane phospholipid PtdIns(4,5)P2 (PIP2), similar to increased signal transduction via the EGFR. Lowering EGFR signaling levels can rescue the observed defects, restore germ cell survival and the integrity of the testis-barrier. Interestingly, membrane phospholipid PIP2 contributes to EGFR/Ras-mediated activation of the MAPK/dpERK. Comparative analysis using septate junction knockdowns revealed that germ cell death and maintenance of the permeability barrier seem to be two differentially regulated events. These results provide a better understanding of how cell polarity and endocytosis fine-tune signal transduction to sculpt developing tissues and provide insights for the identification of new approaches in infertility and regenerative medicine.

426C Broad is sex and cell type specifically required in gonads

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The *Broad* (*Br*) locus encodes a family of BTB domain DNA-binding proteins with any of four different C2H2 zinc fingers. Br is best known as a modulator of the ecdysone-response. We examined *Br* expression in third instar larvae when ecdysone levels are high.
In our RNA-seq experiments, we detected 11 out of 15 Br transcripts in gonads encoding 4 different protein isoforms of Br (Z1-Z4), on the basis of distinct Zinc-fingers at C-terminus. Single Cell RNA sequencing (scSeq) and immunostaining with antibody show that, Br, was expressed only in somatic cells of testis and ovary. In the testis, Br expression was enriched in cyst cells that enclose the spermatogonia, which are mitotic germline cells near the apex, but not the cyst cells surrounding spermatocytes. Br was also expressed in terminal epithelium, which ultimately attaches to the reproductive tract derived from the genital disc, and the pigment cells that ensheath the testis. In ovary, we found Br expression in all somatic cells (Sheath cells, Terminal Filament, Cap cells, Intermingled cells, follicle cell and Swarm cells). To determine the function of Br in these cell types we knocked down of all of its isoforms (by targeting common BTB domain) in the enclosing the germ cells (male cyst cells and female intermingled cells) using a *traffic jam* driver. This led to female-specific sterility due to germline defects including loss of germ cells. Br expression was lost from the spermatogonia cyst cells, but did not result in an overt phenotype. However, knockdown of Br using a *doublesex* driver resulted in sterility in both sexes. Testes failed to elongate and attach to the reproductive tract. This indicates that Br is required in terminal epithelium to mediate attachment to the reproductive tract. In summary, we show that Br is required in both sexes, but in fundamentally different somatic cell types. This highlights the context-dependency of gene expression in sexual development.

427A A meiotic switch in lysosome acidity supports spermatocyte development in young flies but collapses with age

Tyler Butsch[1], Olga Dubuisson[1], Alyssa Johnson[1], Adam Bohnert[1] 1) Louisiana State University.

Animal fertility requires the production of functional gametes. Understanding fundamental mechanisms that direct animal gametogenesis, and how they go awry with age, may reveal new entry points to combat infertility. Recently, we found that lysosomes activate during oocyte meiotic maturation in *C. elegans*, suggesting that a developmental switch in lysosome activity promotes female germ-cell health in young animals. Whether lysosomes are similarly regulated during sperm development is unknown. Using *Drosophila melanogaster*, we report that lysosomes are specifically activated as spermatocytes enter meiosis, and not before. In developing spermatocytes, active lysosomes support cell-membrane integrity and prevent germ-cell multinucleation, by turning over membrane-associated E-cadherin to limit its accumulation at ectopic sites. Importantly, we find that this function naturally declines with age as lysosomes lose acidity. In old testes, diminished lysosome acidity immediately precedes E-cadherin build-up and germ-cell multinucleation, and thus may contribute to age-related reproductive decline in males. These findings demonstrate that lysosome activity is tightly linked to meiotic progression in both male and female germ cells and hint that lysosomes may be key determinants of male reproductive aging.

428B Characterization of *CG4511* as a Novel Regulator of Spermatogenesis

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In the malaria parasite, *Plasmodium berghei*, Phosducin-Like Protein 3 (PhLP-3) has been found to contain redox activity and it has been hypothesized to function as a co-chaperone in the folding and regulating of cytoskeletal proteins. CG4511 is the *Drosophila* homolog of the *Plasmodium berghei* PhLP-3. In order to explore the role of PhLP-3 and its homologues in the regulation of the cytoskeleton, we are examining the role of CG4511 in *Drosophila* spermatogenesis. *Drosophila* spermatogenesis is an excellent model for studying regulation of the cytoskeleton given the requirement of actin and microtubules during multiple stages of sperm production, and a level of organization in the testis that allows one to view the stages of spermatogenesis simultaneously. Following meiosis, the microtubule-based axonemes elongate, while and actin-rich individualization complex (IC) aids in spermatid individualization and cytoplasm removal. Males homozygous for a P element insertion in the *CG4511* were found to be sterile. Quantitative PCR analysis of the RNA expression levels for *CG4511* in males homozygous for this insertion reveals a significant reduction compared to wild-type. In order to determine what may be happening in spermatogenesis to cause this sterility we next examined sperm morphology in the distal testis and the seminal vesicle. At this stage, wild-type sperm individualize and nuclei exhibit a needle-like appearance. However, in *CG4511* homozygous mutants individualizing sperm were not observed and no needle-like nuclei were observed. In addition, seminal vesicles appeared smaller in size, consistent with decreased sperm production.
Given the predicted function of PhLP-3 as a regulator of cytoskeletal protein folding, we performed phalloidin staining to visualize the actin-based structures in the testes. In the male mutants there were no actin cones, which are found in wild-type flies. These actin cones are important structures that are important in spermatid individualization. Using differential

contrast microscopy we have seen that there is a lack of sperm bundles in these male mutants, another indication that spermatogenesis is not proceeding normally. Experiments are being conducted to determine when spermatogenesis arrests. Further examination is needed to determine if the microtubules are affected due to the mutation.

429C Triglyceride lipase *brummer* is required for spermatogenesis in *Drosophila melanogaster*

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Lipid metabolism is essential for spermatogenesis in many animals, including flies; mutations that disrupt fatty acid, phospholipid, and neutral lipid metabolism are all known to disrupt male fertility. While the importance of fatty acids and phospholipids in regulating various stages of sperm development have been described, few studies have examined the cellular mechanisms by which changes to neutral lipid metabolism disrupt fertility. Here, we show that loss of triglyceride lipase *brummer* (*bmm*) reproduces fertility defects observed in mice with null mutations in *bmm* homolog *adipose triglyceride lipase* (*ATGL*). These fertility defects were observed upon loss of *bmm* either in the whole-body or autonomously in the germline, and were caused by impaired progression of spermatogenesis both at the transition from spermatogonial cells to spermatocytes and at meiosis. Our analysis revealed that loss of *bmm* caused an abnormal accumulation of lipid droplets in the testis, and a reduction in many membrane species in the whole-body. Given that blocking triglyceride synthesis rescued the abnormal lipid droplet accumulation and testis defects in *bmm* mutants, our data suggests that *bmm* normally prevents abnormal triglyceride accumulation during spermatogenesis to promote differentiation.

430A Investigating the Roles of *asteroid* and *Star* during Oocyte Selection and Oogenesis in *Drosophila*

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The intricate process by which gametes are formed from the germline stem cells is a fundamental question in biology. In *Drosophila*, oogenesis begins by asymmetric division of the germline stem cells, ultimately producing a cyst of 16 cells surrounded by a layer of somatic cells. One of these 16 germline cells is selected as the oocyte, the future egg, while the remaining become supporting cells. A genetic screen in *Drosophila* identified mutations in several evolutionarily conserved genes that result in a failure of oocyte fate determination, leading to loss of mature eggs and fertility. Strikingly, when the germline cells are mutant for *Star* or *asteroid*, the resulting cysts contain no oocyte. Further characterization of *asteroid* mutants revealed a persistence of double-stranded DNA breaks during meiosis. The protein encoded by *asteroid* and its human ortholog (*ASTE1*) both contain XPG domains, suggesting they act as nucleases, possibly during DNA repair. Interestingly, *ASTE1* is mutated in a subset of patients with colorectal cancers, although its molecular function is yet unknown. To address the necessity of *asteroid*'s predicted nuclease-encoding domain during *Drosophila* oogenesis, we recently made a CRISPR-generated allele lacking the XPG domain. Surprisingly, loss of this domain does not result in severe defects during oogenesis in *Drosophila*. We are now investigating the relationship between *Star* and *asteroid* by generating individual gene knockouts using CRISPR/Cas9 gene editing and genomic rescue. Further insight into the roles of *asteroid* and *Star* during oogenesis will shed much needed light on the molecular mechanisms controlling oocyte fate determination.

431B Specific effects of chronic thermal stress on *Drosophila melanogaster* oogenesis

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All organisms exhibit phenotypic plasticity, adjusting their physiology and re-allocating resources according to ever changing environmental conditions. For example, various animal populations have been altering their behaviors in response to climate change. Oogenesis is highly sensitive to a variety of environmental and physiological factors; however, it remains largely unknown how exposure of *Drosophila melanogaster* adult females to chronic thermal stress affects oogenesis. To directly address this question, we incubated newly-eclosed *y w* adult flies (raised at room temperature, 23°C) at 18°C (cold), 25°C (optimal), or 29°C (hot) for 20 days at constant humidity (>70%), and found that the rate of egg production was reduced both at 18°C and 29°C relative to that at 25°C. We next determined what steps of oogenesis were affected at 18°C and 29°C and found that different cellular mechanisms account for decreases in egg production. While 29°C had no impact on germline stem cell (GSC) numbers, we found that chronic exposure of females to 18°C improved GSC maintenance over time. By contrast, chronic heat stress (29°C) negatively impacted early germline cyst survival, follicle growth, and vitellogenesis, while exposure to 18°C did not affect those processes. Fertility can be affected not only by the number of eggs produced, but also by the ability of laid eggs to support embryo development. To assess how well embryos develop from eggs produced at different temperatures, we measured the hatching rates of eggs laid by females chronically exposed to low or high temperatures relative to 25°C. Interestingly, we found that eggs produced by females exposed to 18°C had improved hatching rates relative

to those of 25°C females, whereas the hatching rates of eggs produced by 29°C females were drastically reduced over time, significantly decreasing fertility. Notably, the reduction in hatching rates of eggs produced at 29°C resulted from both maternal and paternal effects. Our findings provide a foundation for future research on the mechanisms underlying temperature sensing and downstream effectors regulating specific steps of oogenesis. This broad question is widely relevant not only to cold-blooded organisms, which have limited ability to regulate their body temperature, but also to warm-blooded organisms, which are subject to hypothermia, heatstroke, and fever.

432C Spargel/dPGC-1 controls mitochondria-driven embryonic development in *Drosophila*

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Drosophila Spargel/dPGC-1 is the ancestral form of mammalian PGC-1. Murine PGC-1 is a robust activator of mitochondrial biogenesis and is primarily expressed in the liver and skeletal muscles. Spargel/dPGC-1 though is predominantly expressed in the Drosophila ovary and is essential for nutrient-mediated ovarian growth. Germline-specific knockdown of *srl* arrests late-stage egg chamber development, resulting in arrested oogenesis and female sterility. Similarly, *srl* hypomorphic mutants (srl^{1}/srl^{1}) have a marked decrease in growth rate, body size, life span, and fecundity. We generated a CRISPR-mediated *srl* deletion or amorphic mutant, which displays homozygous lethality, indicating a zygotic requirement for Spargel. To bypass the lethality, trans-heterozygous mutant mothers were generated (srl^{del}/srl^{1}) to investigate the effects of low dosage of Spargel on oogenesis. To determine the maternal requirement of Spargel we performed a classical reciprocal cross of srl^{del}/srl^{1} females with wild type (+/+) males and vice versa. Only trans-heterozygous (srl^{del}/srl^{1}) females produced deformed embryos, most of which remained unhatched, indicating a maternal requirement of Spargel for embryogenesis. Furthermore, srl^{del}/srl^{1} females produce ventralized embryos with phenotypes including single dorsal appendage (DA), 2 short DAs or 2 DAs close together as compared to the control. In approximately 42% of srl^{del}/srl^{1} post-vitellogenic oocytes, Gurken is mislocalized with cytoskeletal localization defects. We also found gaps in distribution of the syncytial nuclei (nuclear fallout) in a majority of the embryos from srl^{del}/srl^{1} mothers, which, also show cytoskeletal defects in pseudocleavage furrow formation. The metaphase furrow formation is an essential energy-dependent step. The localized mitochondrial distribution of ATP supplies this energy demand to support the early syncytial divisions. Cytoskeletal transport maintains the localization of mitochondria in early embryos. Lack of Spargel causes mitochondrial dysregulation, which explains why Spargel is essentially required both maternally and zygotically.

433A Natural tolerance to transposition is associated with increased expression of DNA repair machinery

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Transposable elements (TE) are mobile genetic parasites whose unregulated activity in germline causes DNA damage, thereby disrupting gametogenesis. Hosts respond to this fitness cost either by evolving resistance, where transposition is regulated, or by evolving tolerance, where the germline is robust to the damaging effects of TE proliferation. Host resistance to TEs through small RNAs, particularly piRNAs is the focus of extensive research. However, little is known about host factors that could confer tolerance to TE activity. We sought to uncover natural variation in tolerance to P-element DNA transposons, whose unrestricted activity causes severe DNA damage and germline loss in D. melanogaster.

To uncover genetic factors that determine tolerance, we took advantage of recombinant inbred lines (RILs) that do not establish piRNA silencing of P-elements. We performed quantitative trait locus (QTL) mapping of variation in P-element induced germline loss and identified a genomic region associated with tolerance. We then examined ovarian gene expression differences between tolerant and sensitive genotypes, in order to isolate candidate genes and pathways that produce tolerance. We discovered two members of a chromatin remodeling complex involved in double-strand break (DSB) repair, TIP60, which are located within QTL and upregulated in tolerant ovaries. Additionally, tolerant ovaries exhibit increased chorion gene expression: a potential indicator of more efficient DSB repair. In contrast, sensitive ovaries exhibit increased expression of histones, which may inhibit DNA repair by competing with repair machinery. Our findings suggest that DNA repair pathways are an important source of natural variation in tolerance to transposable elements.

434B Analysis of Me31B's Role in Regulating *Drosophila* Germline RNAs

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Me31B is a protein component of *Drosophila* germ granules and is believed to play an important role in germline development by interacting with germline RNAs such as *osk*. To understand the role of Me31B on the RNAs in oogenesis, we measured the amount of representative germline RNAs, *osk, bcd*, and *nos*, in loss-of-function *me31B* heterozygous strains as well as

strains trans-heterozygous with strong alleles of germline genes *vas, tud, aub, tral*, and *cup*. We found that losing a copy of *me31B* does not cause significant change in *bcd* mRNA levels but a significant increase of *nos* mRNA levels. We compared this with Tral knockdown strains (Tral complexes with Me31B in germ granule RNPs) in which *bcd*, *osk* and *nos* mRNA levels showed contrasting changes. In addition, we discuss our ongoing experiments to profile Me31B-associated mRNAs and small RNAs during oogenesis via RNA sequencing and the strategic plan to generate novel *me31B* alleles by using CRISPR gene editing.

435C Characterizing CG14545 in *Drosophila* oogenesis

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CG14545 is a previously uncharacterized gene that exhibits highly enriched expression in the *Drosophila* female germline. Using CRISPR-Cas9 we knocked out *CG14545.* Homozygous null males are viable and fertile. While mutant females are also viable, they exhibit a sterile phenotype, marked by the formation of both agametic ovarioles and germ cell tumors. The germ cell tumors contain single cells and multi-cellular cysts but do not enter meiosis. Loss of *CG14545* results in an expansion of Nanos expression, resembling *snf* and *mei-P26* mutant tumors. *CG14545* encodes for a 16 kDa protein with no defined protein domains. HA-tagged endogenous CG14545 and a HA tagged CG14545 cDNA transgene both localize to the oocyte starting in 8 cell cysts. However, introducing the N-terminal HA tag within the endogenous *CG14545* gene results in a weak hypomorphic phenotype. Homozygous or hemizygous HA-CG14545 females exhibit a dumpless phenotype, indicating a defect in the transport of material from the nurse cells to the oocyte during stage 11. However, these mutants display normal ring canals and actin bundles, suggesting the dumpless phenotype may arise because of a defect in actin-myosin-based contraction. Strikingly, the expression levels of HA::CG14545 in homozygotes ovaries are more than two fold higher than in heterozygotes, suggesting that CG14545 may repress its own expression. Based on these and other results, we will test if CG14545 represses translation of its own mRNA or whether inclusion of the HA tag changes the stability of the protein. We are also assaying whether CG14545 genetically and physically interacts with Sxl, Bam, Bgcn, and Mei-P26 to repress *nanos* translation.

436A Investigating the role of eRpL22 ribosome-mediated regulation of Mitochondrial Assembly Regulatory Factor (Marf) expression in mitochondrial morphogenesis in the male germline in Drosophila melanogaster

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Recent studies support the hypothesis that heterogeneity in ribosomal protein (Rp) content within ribosomes alters translation specificity, leading to the "specialized ribosome" hypothesis. Our laboratory has focused on expression and differential function of the essential, eukaryotic-specific Rp paralogues, eRpL22 and eRpL22-like, in the male germline of *Drosophila melanogaster.* While eRpL22 expression is ubiquitous, eRpL22-like is specifically expressed in the germline, giving rise to ribosome heterogeneity in the germline. Paralogue depletion studies demonstrate that eRpL22 and eRpL22-like are not completely functionally equivalent since eRpL22 knockdown with eRpL22-like overexpression restores viability, but results in significant phenotypes including decreased longevity, an excess of immature spermatocytes, and diminished fertility. The mechanism contributing to accumulation of immature spermatocytes and infertility is unknown. Using RNAseq, we have recently shown differential enrichment of subsets of mRNAs involved in sperm maturation on eRpL22 or eRpL22-like polysomes. Therefore, we hypothesize that diminished eRpL22 expression in rescued flies depletes the germline of eRpL22-specific ribosomes and essential proteins required for sperm maturation; and eRpL22-like ribosomes cannot replace this function. Among mRNAs preferentially enriched on eRpL22 polysomes is the essential transmembrane GTPase, Marf, which is required for mitochondrial fusion. Marf loss leads to mitochondrial fragmentation. Differential Marf expression within the germline can be used to track potential mitochondrial defects within elongating spermatocytes in rescued flies. In a comparative study using wildtype flies and flies depleted of eRpL22 but rescued with eRpL22-like or eRpL22, we are assessing Marf expression in the testis by Western blot and immunohistochemistry. Reduced levels of Marf protein in flies rescued with eRpL22-like, but not with eRpL22, would provide corroborating evidence that eRpL22 ribosomes play a specific role in mediating Marf translation. With Marf depletion, we expect accompanying defects in mitochondrial morphology in eRpL22-like rescued flies, but not in flies rescued with eRpL22. Collectively, these outcomes would support the hypothesis that eRpL22 and eRpL22-like ribosomes play specialized roles in sperm maturation through differential translation of proteins essential for fertility.
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437B Ring canal formation in the Drosophila testis occurs via reorganization of germline midbodies

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During male and female gametogenesis in various invertebrate and vertebrate species, cytokinesis is incomplete and occurs in the absence of abscission to yield cells connected by a shared cytoplasmic intercellular bridge. These bridges, called ring canals (RCs) in Drosophila, are membrane-attached cytoskeletal structures that stabilize the cytoplasmic opening between cells and are comprised of several contractile ring (CR) components suggesting that RCs are derived from the CR; however, the mechanism that transforms a CR into a RC is not known.

Our time lapse imaging of the CR and RC component, Pavarotti (Pav/MKLP1/Kif23), a subunit of the centralspindlin complex and major component of RCs, in the Drosophila male germline revealed novel insights into RC biogenesis. Pav, at cleavage furrows and central spindle microtubules, condenses to a large midbody-like focus upon constriction of the CR that resolves into an open ring over the course of one hour. RC formation is preceded by a period of maturation during which the dense Pav focus undergoes a 5-fold reduction in fluorescence intensity. Imaging of Pav::Dendra2 suggests that the reduction in signal is due to Pav degradation in the midbody prior to RC formation. Interestingly, purification of putative Pav interactors in the RC identifies many proteins implicated in ubiquitin homeostasis, including several deubiquitylases and ubiquitin ligases.

We find that known somatic midbody ring and/or RC components, namely Septin, Anillin, and Sticky/Citron Kinase, localize in a ring around the Pav-labeled focus, similar to the localization of these midbody ring proteins during complete cytokinesis. However, in contrast to complete cytokinesis, the midbody does not recruit the abscission machinery components Tsg101/ESCRT-I, ESCRT-III, or Spastin, and RC formation occurs in the absence of microtubule severing or depolymerization. However, the upstream ESCRT-associated protein ALIX::GFP is enriched at midbodies and nascent RCs, but not mature RCs. These data suggest a possible role for additional proteins/protein modifications at the germline midbody in the inhibition of abscission required for RC formation.

Taken together, these data inform a model wherein midbodies fail to initiate abscission and instead reorganize into open RCs. We are testing this model using a combination of electron microscopy, genetics, and proteomics approaches to identify potential targets for future investigation.

438C Domain specific deletions of Spargel/dPGC-1 highlight its importance on growth, fertility, and mitochondrial function during oogenesis

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PGC-1 is a transcriptional co-activator that plays a key role in the regulation of mitochondrial energy metabolism in mammals. A PGC-1 homolog designated as Spargel/dPGC-1 in Drosophila is predominantly expressed in the ovaries. Ovary-specific knockdown of *srl* induces complete sterility indicating that Spargel plays an essential role in oogenesis and ovarian growth. Spargel shares many structural features with PGC-1 proteins including the Serine-Arginine rich repeats (RS), RNA recognition motif (RRM) and a nuclear localization signal (NLS). Gain of function studies of PGC-1 paralogs have established the role of PGC-1 on mitochondrial biogenesis. In contrast, domain specific deletions of PGC-1 failed to influence mitochondrial function in mice. To further establish the role of Spargel in oogenesis, we generated two *srl* mutant lines using CRISPR/CAS9 system: (1) An amorphic *srl* mutant with 3336 bp deletion beginning from Exon 2 (srl^{del}), and (2) RRM domain specific deletion (ΔRRM). Homozygous srl^{del} mutants fail to proceed beyond embryogenesis, suggesting a postzygotic requirement of Spargel. Trans-heterozygotic combination of srl^{del} and a hypomorphic allele of *spargel* (srl^{1}) helped us bringing down the Spargel level to a minimum without the adverse effect of *srlRNAi*. Lower number of mature egg chambers were observed in homozygous *srlΔRRM* and *srlΔRRM/* srl^{del} females as compared to controls. On the other hand, Spargel gain of function through overexpression induce the rarely observed phenotype of faster development of egg chambers with comparatively more post-vitellogenic stages with a significantly increased egg laying rate compared to controls. In contrast drop in egg laying capacity was noticed in srl^{1}/srl^{del} females. Domain specific *srlΔRRM* mutants also suffers from a decrease in egg laying capacity with mutant females laying cup-shaped eggs indicating dorsoventral polarity defects. Consequently, transgenic mutant lines overexpressing truncated Spargel protein in the ovary without the RRM and RS domains (Srl ΔRRM and Srl ΔRS) showed a dominant negative effect as the ovarioles failed to proceed beyond pre-vitellogenic stages. Together, this collection of *spargel* mutants are helping us to address (1) the role of Spargel on ovarian growth and development, and (2) to establish how this ancestral PGC-1 still retains its influence on mitochondrial function.

439A Examining Size Scaling Relationships in the Developing *Drosophila* Egg Chamber

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Proper regulation of the size of intracellular structures is essential for the normal function of cells, and a scaling relationship is

often seen between cell size and the size of intracellular structures and organelles. For instance, larger cells often have larger structures or more organelles than smaller cells. Although these scaling relationships have been observed, little is known about how they are established and maintained during tissue growth. We are using the developing fruit fly (drosophila) egg chamber as a model system to study size scaling relationships throughout development. We have chosen to initially focus on the size scaling of two intracellular structures: ring canals, the germline intercellular bridges which connect the nurse cells and oocyte, and the nurse cell nuclei. We have found that both nuclear size and ring canal size scale with egg chamber size across four Drosophila species (D. melanogaster, D. pseudoobscura, D. santomea, and D. yakuba). Additionally, similar analysis on a collection of 10 artificially selected D. melanogaster lines that produce either big eggs or small eggs suggests that this size scaling relationship observed across Drosophila species is also maintained when looking within a single species. Future studies will follow up on these data and begin to more deeply explore intracellcular size scaling relationships within other model organisms.

440B A Role for Notch Signaling in Drosophila Spermatogenesis

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The Notch signaling pathway has diverse functions in male and female gonad development. In *Drosophila* male embryos, Notch specifies hub cell fate in somatic gonadal precursor cells (SGPs), which are important for maintaining germline stem cells and somatic cyst stem cells throughout the lifetime of adult flies. However, a role for Notch signaling in adult males has only started to emerge recently. Others have demonstrated that Notch signaling appears to be necessary for the survival of the germline stem cell lineage. We have found that reducing Notch function with a temperature sensitive allele in adults results in the absence of hub-like structures in males. In addition, overexpression of an activated Notch receptor in the somatic cells causes defects in spermatogenesis. Overexpression of activated Notch in somatic cells results in the presence of two or more hub-like structures in 57% males and testes with abnormal morphology. We also found that overexpression of activated Notch in somatic cells results in the continued expression of the transcription factor *traffic jam* (*tj*), an early somatic cyst cell marker, at later stages when *tj* expression is usually turned off. This indicates that the somatic cyst cells maintain an early somatic cyst cell identity. Consistent with a failure of somatic cyst cells to mature properly, we observe a failure of sperm bundles to form properly, indicating further defects in spermatogenesis. Currently, we are exploring when germline development arrests, and the mechanism by which Notch functions to promote spermatogenesis. Others have identified cofactor proteins shown to cooperate with Notch in gonad development. Our lab studies Ribbon, a transcription factor that is part of the BTB protein family, containing domains that allow it to interact in multiple ways with other proteins. Ribbon is important in gonad development and continues to be expressed in adult gonads. We predict that it likely plays a role in spermatogenesis as well by working cooperatively with the Notch pathway. Genetic interaction experiments will help us explore this possibility further.

441C Potential Role of *CG5050* in *Drosophila melanogaster* Sperm Transfer and/or Storage

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Mitochondria are crucial organelles in the eukaryotic cell that harness energy for cellular metabolism. Mitochondria are dynamic and undergo a series of shape changes throughout the life of the cell. In *Drosophila melanogaster* spermatogenesis, mitochondria undergo these dramatic changes, and disruption in genes that govern these changes can lead to male sterilization. *CG5050* is a gene highly expressed in the testes, closely related to the *CG5043* gene. When *CG5043* is mutated, abnormal mitochondrial shaping is observed. CRISPR/Cas9 mutagenesis produced *CG5050* mutant strains, some of which had frameshift mutations and early stop codons. Those strains with early stop codons were infertile, despite producing normal amounts of motile sperm. This suggests that the mitochondria are still undergoing necessary shape changes and points toward a different role of *CG5050* in fertilization. In dissections of females mated to *CG5050* mutant males, the presence and motility of sperm in female sperm storage organs was tested. In those strains that are fertile, sperm is present in the female sperm storage organs and moves in characteristic patterns. However, in infertile strains, sperm is either absent from the sperm storage organs, or the motility of the sperm in these organs is altered. Changes in *CG5050* therefore seem to affect fertility after sperm leave the male reproductive tract. The differential presence and motility of sperm in the female reproductive tract that varies with fertility indicates *CG5050* may play a role in sperm transfer and/or storage.

442A Disrupted satellite transcripts in the selfish *Segregation Distorter* system of *Drosophila melanogaster*

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Meiotic drivers are selfish genetic elements that bias their transmission during gametogenesis, breaking Mendel's law of segregation. One of the well-known examples of meiotic drive is *Segregation Distorter* (*SD*)—an autosomal, male-specific

driver—found in nearly all populations of *Drosophila melanogaster*. Male flies heterozygous for an *SD* chromosome and a wild type chromosome transmit the *SD* chromosome to nearly all of their progeny (>95%) by causing a defect in sperms bearing wild type chromosome during spermatogenesis. Two key components have been identified in the *SD* system: the driver, *Segregation distorter* (*Sd*), and its target, *Responder* (*Rsp*). *Sd* encodes a truncated duplication of the gene *RanGAP*, and *Rsp* corresponds to a block of tandem 120-bp satellite DNA repeats in the pericentric heterochromatin. *SD* targets *Rsp*-bearing sperm for destruction, however, the role of *Rsp* in this process and how is it targeted by *SD* remain unknown. Here we used a combination of genomic and cytological approaches to detect the localization and expression level of *Rsp* satDNAs in testes with or without *SD*. We found that not only *Rsp*, but also other satDNAs (*e.g. 359-bp* satellite), show disrupted expression patterns in *SD* system. In testes with *SD,* the satellite transcript signals are more aggregated than in flies without *SD*, suggesting possible localization defects and/or phase separation of satellite-associated RNA-binding proteins. In addition to altered localization, we also found that satDNAs show reduced expression levels, and generate fewer piRNAs in the presence of *SD*. The expression level of other types of repeats like TEs and genomic piRNA clusters remain unchanged, suggesting that the disrupted expression is exclusive to satellites. Taken together, our results suggest that *Rsp* satellite regulation is disrupted in *SD* testes, not only providing insights into our understanding of the role of *Rsp* satDNA in meiotic drive, but also shedding light on vulnerabilities of gametogenesis during reproduction.

443B Soft repression: Subtle transcriptional regulation with global impact

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Transcriptional repressors are often thought of as mediators of decisive on/off activity that underlies cell-type, or condition-specific, gene expression. However, our recent assessments of global regulators Retinoblastoma (Rb) and SIN3 led to the realization that another important, but often overlooked repression activity involves measured but critical modulation of expression that is considerably less than two-fold. This so-called “soft repression” involves promoter-proximal regulation by Rb and SIN3 that may interfere with only a subset of incoming signals that drive transcriptional initiation. Soft repression has been overlooked because of challenge of teasing out pleiotropic, yet subtle influences, but the types of target genes involve many integral components for central metabolic processes. We will discuss current approaches to identify and characterize soft repression in the context of development and disease, and focus on new technologies that can uncover this new layer of regulation underlying metazoan gene regulation.

444C Investigating the sex-specific, tissue-specific, and segment-specific regulation of the *branchless* gene in *Drosophila*

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One of the interesting challenges in developmental biology is to understand how different cues are integrated to determine where, when, and under what conditions a particular gene is expressed. A striking example of such a gene is *branchless* (*bnl*), which is expressed in a sex-, segment-, and tissue-specific manner in the *Drosophila* genital disc. Analysis of the regulation of the heretofore unidentified *bnl* gene enhancer would therefore provide insight into how these sex-, segment, and tissue-specific cues are integrated. We hypothesize that the integration of sex-, segment-, and tissue-specific manner cues are achieved by transcription factors (TFs) specific for each of these cues binding to and regulating one or more *bnl* gene enhancers. Using ChIP-seq and Dam ID binding data, we have mapped clusters of binding sites of Doublesex (the TF providing sex-specific cues), abdominal-B, and caudal (two TFs providing segment-specific cues) in the neighborhood of the *bnl* gene to identify several putative *bnl* gene enhancer(s). These putative enhancers will be assessed using enhancer-reporter transgenic constructs to identify the true *bnl* enhancer(s): only the latter will recapitulate the sex-, segment-, and tissue-specific expression patterns of the endogenous *bnl* gene. We will then functionally analyze the roles of each of these TFs on the validated *bnl* enhancer-reporter construct(s) by examining the effects of mutating the TF binding sites (*cis*-assays) or removing the TF in loss-of-function clones (*trans*-assays). Collectively, our results will shed light on the interactions and integration between different regulatory inputs and further our understanding of *Drosophila* sexual differentiation.

445A *Daltonien* is a novel mutation that affects color photoreceptor fates

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Color blindness in humans is usually attributed to mutations in *opsin* genes that encode red- or green-sensitive pigments. Analogous to the expression of different *opsins* in different subtypes of human cones, the color-sensing photoreceptors in *Drosophila* are divided into different subtypes; for instance, one photoreceptor subtype expresses the blue-sensitive

pigment Rh5 while the other one expresses the green-sensitive pigment Rh6. The conserved Hippo Pathway, well-known for its role in growth control and tumor suppression, plays a noncanonical role in the specification of these two photoreceptor subtypes. When the Hippo pathway is active, it gives rise to Rh6-photoreceptors (Hippo 'on') and when the pathway is off, it gives rise to Rh5-photoreceptors (Hippo 'off').
The *daltonien* mutant, originally discovered in the Desplan lab at New York University, shows a dramatic increase of Rh6-photoreceptors (Hippo 'on') and a loss of Rh5-photoreceptors. The ratio of Rh5 to Rh6 changes from the approximate 30% (Rh5) to 70%(Rh6) for wildtype to approximately 5% (Rh5) to 95%(Rh6) for the mutant. This suggests that an unknown mutation affects a negative regulator of the Hippo Pathway. Our goal is to identify the gene that is disrupted in the *daltonien* mutant and to determine if its mammalian ortholog also plays a similar role in color vision. We hypothesize that the gene that is mutated in *daltonien* promotes the expression of Rh5 by repressing the Hippo pathway. We performed recombination mapping and complementation test with defined deletions, which narrowed the mutation down to a 56kb region on the X chromosome. Moreover, epistasis analysis revealed that *daltonien* acts downstream of the Babo type I receptor (which mediates the signal that induces Rh5 fate) and upstream of the PH domain containing protein Melted, which is a negative regulator of Warts, the nexus of the Hippo pathway.
Taken together, the *daltonien* mutation affects an unknown gene that is required for the proper specification of two subtypes of color-sensing photoreceptors. Since color blindness in humans has a genetic basis, it will be important to determine if the ortholog of the mutated gene plays a similar role in human color vision and photoreceptor differentiation.

446B The influence of chromosomal environment on X-linked gene expression in *Drosophila melanogaster*

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Heteromorphic sex chromosomes in flies are associated with their uneven presence in males and females of *Drosophila*. This in turn determined the development of regulatory mechanisms specific to the sex chromosomes. In the somatic tissues of *D. melanogaster* males, X-linked genes are upregulated approximately twofold through a process mediated by the dosage compensation complex (DCC), thus restoring the balance in gene expression between males and females and between the X chromosome and the autosomes. Previous studies of endogenous X-linked gene expression suggested that the proximity to the nearest DCC binding site may be one of the major factors determining the pattern of sex-biased gene expression in somatic tissues. To test this hypothesis while avoiding the confounding effects of gene- or tissue-specific regulatory factors, we examined the expression of a reporter gene inserted at many random locations on the X chromosome. We used a *lacZ* reporter gene (encoding b-galactosidase) from *Escherichia coli* and a minimal human cytomegalovirus promoter, both of which are foreign to the *Drosophila* genome. We found a negative correlation between a gene's male-to-female expression ratio and its distance to the nearest DCC binding site in somatic tissues. In gonads, where dosage compensation is assumed to be absent, no significant correlation was found. Our results demonstrate that the positional effect associated with the proximity to a DCC binding site contributes to sex-biased gene expression and, for our reporter genes, has a greater influence than local regulatory factors associated with nearby endogenous genes. Average levels of sex-biased expression did not differ between head and brain, but there was a greater variation in reporter gene expression among regions of the X chromosome in the brain. This may explain the observed excess of endogenous sex-biased genes located on the X chromosome in this tissue.

447C Function of a transcriptional Mediator complex subunit in cell proliferation, competition and apoptosis

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The correct development of complex organism requires an accurate regulation of gene transcription. A major actor in this regulation is the Mediator complex (MED), serving as a bridge between DNA-bound transcription factors and RNA polymerase II. Whereas the MED complex has a global role in regulating PolII-dependent transcription, some MED subunits display striking specificity. Med19 has the unique property to be required or not for cell viability depending on the cellular context. This suggests an involvement in cell competition, a process resulting in the elimination of developmentally competent Loser cells by apoptosis, through interaction with neighboring Winner cells. We have shown that Med19 mutant cells are indeed eliminated by cell competition but also that Med19 is globally required for cell proliferation and viability. Med19 involvement in these processes can be partially explained by a role in the regulation of the Myc proto-oncogene expression. Our transcriptomic analysis further show a Med19 involvement in apoptosis, oxidative stress and DNA damage response. Our data shed new light on MED complex subunit specificity and mode of action and help to better understand their role in human diseases such as cancers.

448A RNAi screen for novel Hippo tumor suppressor pathway regulators in *Drosophila*

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Abnormal growth of cells, a hallmark of cancer, can be attributed to misregulation of signaling pathways that balance cell proliferation and death. For instance, the conserved Hippo tumor suppressor pathway coordinates the balance of cell proliferation and apoptosis to regulate organ growth. While it has recently been implicated in various human cancers, the mechanisms of tissue-specific Hippo pathway regulation are not well understood.

In the *Drosophila* retina, the Hippo pathway is repurposed to mediate a binary cell-fate decision in post-mitotic photoreceptors resulting in photoreceptors that either express the blue-sensitive Rhodopsin Rh5 or the green-sensitive Rhodopsin Rh6. This constitutes a bi-stable switch: the pathway is either "on", resulting in Rh6-fate (Hippo ON), or "off" , resulting in Rh5-fate (Hippo OFF). This binary readout (Rh5/OFF *vs.* Rh6/ON) provides an ideal context to assess Hippo activity.

To better understand the mechanisms of Hippo pathway regulation in the eye, we aim to identify novel regulators of the pathway. To this end, we performed a differential gene expression (DEG) analysis using transcriptome data from Hippo OFF and Hippo ON photoreceptors, respectively. We identified 225 DEGs; 100 genes are upregulated in the Hippo ON subset and 125 gene upregulated in the Hippo OFF subset. The nexus of the Hippo pathway, the kinase Warts (*wts*), is known to be upregulated in Hippo ON photoreceptors, while the growth regulator Melted (*melt*) is known to be upregulated in Hippo OFF photoreceptors. As expected, our DEG analysis identified *wts* and *melt* to be upregulated in the Hippo ON and Hippo OFF subsets, respectively.

To validate these DEGs and to evaluate their potential role as regulators of the Hippo pathway, we have conducted a high-throughput screen in the *Drosophila* retina. The screen utilizes RNAi-mediated knockdown of individual DEGs with a photoreceptor-specific driver (lGMR-Gal4) in combination with an Rh5>GFP reporter that serves as an *in vivo* readout for the Hippo OFF photoreceptors. We identified candidates that are required for negative and positive regulation of Hippo pathway activity and are currently validating them.

449B Blimp-1 regulates the Hippo pathway in post-mitotic photoreceptors downstream of Ecdysone and Activin Signaling

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The Hippo pathway in its canonical role acts as a tumor suppressor pathway that regulates organ growth, but in R8 color photoreceptors, the pathway is repurposed to regulate subtype specification. In this post-mitotic context, the nexus of the Hippo pathway, the Warts kinase (Wts), is regulated transcriptionally, allowing the Hippo pathway to act as a bi-stable switch: either *wts* is expressed to specify Rh6-fate (Hippo ON), or *wts* is repressed to specify Rh5-fate (Hippo OFF). A transcription factor network intrinsic to R8 photoreceptors allows the Hippo pathway to be repurposed as a bi-stable switch in this context, and extrinsic activin signaling through the activation of the Baboon receptor (Babo) is both necessary and sufficient to switch off the Hippo pathway and promote Hippo OFF fate. The mechanisms by which intrinsic factors integrate extrinsic signaling factors to repress the Hippo pathway are not well understood.

Here, we identify a novel role for Ecdysone signaling and Blimp-1 in specifying Hippo OFF fate. RNAi-mediated knockdown of *EcR*, *Usp*, and the canonical Ecdysone response gene *Blimp-1* each caused a loss of Hippo OFF photoreceptors and a gain of Hippo ON photoreceptors. This suggests that the Ecdysone response pathway is required to specify Hippo OFF fate. Surprisingly, epistasis experiments revealed that EcR/Usp act upstream of Babo activation, while Blimp-1 acts downstream, indicating that *Blimp-1* is activated independently of Ecdysone. We next asked how Blimp-1 can repress the Hippo pathway in one R8 subtype and permit its activity in the other subtype. *Blimp-1* encodes two isoforms that are defined by the inclusion of exon 3 (Blimp-1-A) or the splicing of exon 3 (Blimp-1-B). Strikingly, RNAi-mediated knockdown specifically of *Blimp-1-A* caused a loss of Hippo ON photoreceptors and a gain of Hippo OFF photoreceptors, while the knockdown of all *Blimp-1* isoforms caused the opposite phenotype.

In summary, we propose that Ecdysone acts as a permissive factor for Hippo OFF fate upstream of Babo activation and that two different Blimp-1 isoforms play opposing roles in regulating Hippo pathway activity downstream of Babo activation.

450C Dynamics and regulation of maternal transcript degradation across species of *Drosophila*

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Early development of all animals involves precise coordination between two genomes, that of the mother and that of the zygote. Maternal proteins and RNA are deposited into developing oocytes to carry out the initial stages of embryogenesis because at the time of fertilization, the zygotic genome is not yet activated. As the zygotic genome becomes transcriptionally active, maternal transcripts are degraded and developmental control is handed off from the maternal to the zygotic genome. The maternal to zygotic transition (MZT) is tightly regulated and highly conserved, yet there are differences in how this process occurs across species. There are differences in the transcripts that are maternally deposited across species as well as in the length of time that different species of *Drosophila* take to complete embryogenesis. While several key regulators of maternal transcript degradation are well characterized in *D. melanogaster*, it is not known how or when most maternal transcripts are degraded during development in other species. Here, we investigate the degradation of maternally deposited transcripts through developmental time across four species of *Drosophila* (*D. melanogaster*, *D. persimilis*, *D. virilis*, and *D. yakuba*). These species represent extremes in length of developmental time and have a range of different habitats. Using RNA-sequencing to examine maternal mRNA abundance in 7 distinct developmental stages across embryogenesis, and using crosses between different populations within each species to distinguish maternal from zygotic transcripts, we determined how the dynamics of maternal transcript degradation compare across these species. We sampled a number of developmental timepoints after the MZT, when the dynamics and mechanisms of maternal transcript degradation are less well understood, even in *D. melanogaster*. We also investigated potential regulators that are responsible for the decay of maternal transcripts throughout embryonic development by looking at enrichment of motifs across transcripts and the regulators that may bind these sites. In all, this study provides evidence as to the evolution of dynamic regulation of maternal transcripts during embryogenesis across species.

451A Regulation of *engrailed* and *invected* expression in the *Drosophila* central nervous system

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The neighboring *invected* (*inv*) and *engrailed (en)* genes encode homeodomain proteins important for proper development in *Drosophila. inv* and *en* are co-regulated by at least 15 different enhancers that drive their expression in various cell types throughout development, including a subset of cells in the central nervous system (CNS). We are interested in deciphering the regulation of *inv* and *en* expression in the CNS. Our data show that, in the embryonic CNS, *inv* and *en* are co-expressed in most cells although *en* proceeds *inv* expression in midline cells. In order to study DNA sequences necessary for CNS expression, we are generating deletions in the endogenous locus as well as utilizing a 79kb *HA-en* transgene that includes all the sequences necessary to rescue *inv-en* double mutants to viable and fertile adults. We fluorescently stained a line containing the wild-type *inv-en* domain and an *HA-en* transgene with anti-HA, to examine *en* expression from the transgene, and anti-Inv, so we can compare *HA-en* expression to the endogenous locus. Although many cells in the CNS co-express both Inv and HA-En, we observed a subset of cells mis-expressing HA-En. We hypothesize that the mis-expression from the *HA-en* transgene results from improper maintenance of a repressive Polycomb domain by the transgene. We are inserting various boundaries, such as actively transcribed genes and insulators, on each side of the *HA-en* transgene to see if we can correct transgenic *en* expression. We are also analyzing *inv* expression from an 84kb *HA-inv* transgene that contains CNS enhancers. Recall that Inv and En are normally co-expressed in the CNS. However, in embryos that contain both the 84kb HA-inv transgene and the wild-type *inv-en* domain, we observed a large population of cells in the CNS that have lost expression of endogenous *en*, expressing only *inv*. Interestingly, the number and pattern of CNS cells expressing either Inv or En recapitulate the wild-type pattern of cells that normally co-express *inv* and *en*. We speculate that the increased Inv protein levels due to *inv* expression from both the endogenous locus and the *HA-inv* transgene could lead to negative regulation of endogenous *en* expression. Overall, we are beginning to uncover the complexities of regulating *inv-en* expression in the embryonic CNS.

452B DNA replication promotes zygotic transcription through Zelda-dependent RNA polymerase II clustering

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Conflict between transcription and DNA replication is a fundamental problem that could give rise to DNA damage and genome instability, yet the early wave of zygotic gene expression begins during the rapid syncytial cell cycles in which S phase occupies the entirety of interphase. Numerous studies of the *Drosophila* embryo indicate that DNA replication initiates promptly at mitotic exit, while there is a short lag before active transcription resumes. Nonetheless, the mechanisms that regulate early zygotic transcription and coordinate it with DNA replication remain unknown. Using real-time imaging of endogenously tagged RNA polymerase II (Pol II), we observe abrupt formation of Pol II clusters at the onset of transcription about 3 minutes after mitosis, which subsequently disperse as transcription ramps up. Abrupt inhibition of DNA replication prevents the formation of Pol II clusters throughout the interphase and reduces transcriptional activity, arguing for a strong coupling to coordinate

the two processes. In addition, we show that rapid inactivation of maternally provided Zelda, a key transcriptional activator of zygotic transcription, blocks the formation of Pol II clusters. Our findings suggest a model in which the initiation of DNA replication allows Zelda to reconfigure the chromatin landscapes and nucleate the Pol II clusters at promoter regions, which facilitates the rapid initiation of transcription in order to produce full-length transcripts before next mitosis. Moreover, these observations implicate the potential roles of transcriptional condensates during the processes of zygotic genome activation.

453C Pipsqeuak localization to the histone locus body

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Pipsqueak (Psq) protein is a developmental regulator in *Drosophila* that has pleiotropic effects in oogenesis, adult developmental processes, and more. Studies suggest that the regulatory function occurs due its involvement in regulating gene expression by influencing the chromatin structure. The HTH DNA-binding domain present in Psq targets and binds to sites containing the GAGA repeats. Psq also interacts with polycomb group proteins (Pc-G), which interact with GAGA Factor (GAF). The histone locus (HL) encodes Histones that provide structural support to chromosomes, and it also contains GAGA repeats in the *histone3-histone4* promoter. These observations suggest that Psq targets the HL. To determine if Psq is indeed targeting the HL, I mapped existing Psq ChiP-seq data using Galaxy. I discovered high intensity Psq peaks located in a section of the HL that contains GAGA repeats, the *histone3-histone4* promoter. This is the same location targeted by both GAF and another GAGA-binding protein, CLAMP. How these three proteins interact or compete for HL binding is unknown. In the future, I will confirm localization by staining polytene chromosomes for Psq. I will also map existing independent ChIp-Seq data from other tissues to confirm localization.

454A Repression precedes the stepwise evolution of a highly specific gene expression pattern

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Well-controlled gene expression is critical for the proper development and function of many traits. Highly-specific temporal and spatial expression patterns are often due to the overlapping activities of activator and repressor sequences that form *cis*-regulatory elements called enhancers. While many studies have shown that evolutionary changes in enhancers can result in novel traits, few studies illuminate how enhancers originate, how activator and repressor sequences interact during enhancer evolution, and the order in which they evolve. Here, we traced the evolutionary origin of a recently evolved enhancer that drives the expression of the fatty acyl-CoA elongase, *bond*, specifically in the semicircular wall epithelium (swe) of the *Drosophila* male ejaculatory bulb (EB). We show that this enhancer consists of two activator regions that drive *bond* expression in the entire EB and a repressor region that restricts expression specifically to the EB swe. Interestingly, the repressor region preceded the evolution of the two activator regions. The evolution of the first activator region, consisting of two putative *Abdominal-B* sites, did not drive expression in the EB due to the action of the repressor region. Expression of *bond* in the EB swe requires the evolution of the second activator region, which does not drive expression on its own, but synergizes with the first activator region and the repressor region to produce a highly-specific spatial expression pattern. Our results show that the origin and evolution of a novel enhancer require multiple steps and the evolution of repressor sequences can precede the evolution of activator sequences.

455B Mutual antagonism between Germ cell-less and Torso receptor regulates transcriptional quiescence underlying germline/soma distinction

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Transcriptional quiescence, an evolutionarily conserved trait, distinguishes the embryonic primordial germ cells (PGCs) from their somatic neighbors. In *Drosophila melanogaster*, PGCs from embryos maternally compromised for *germ cell-less* (*gcl*) misexpress somatic genes, possibly resulting in PGC loss. Challenging the role of Gcl during transcriptional silencing in the PGCs, recent studies documented a requirement for Gcl during proteolytic degradation of the terminal patterning determinant, Torso receptor. Here, we demonstrate that the somatic determinant of female fate, *Sex-lethal* (*Sxl),* is a transcriptional target of Gcl and that compromising Sxl levels can partially rescue *gcl* PGC defects. Underscoring the significance of transcriptional silencing mediated by Gcl, ectopic expression of a degradation-resistant form of Torso (Torso^{Deg}) can also activate *Sxl* transcription in PGCs, whereas simultaneous loss of *torso-like* (*tsl*) reinstates the quiescent status of *gcl* PGCs. Intriguingly, like *gcl* mutants, embryos derived from mothers expressing Torso^{Deg} in the germline display aberrant spreading

of pole plasm RNAs. Furthermore, gain-of-function mutations in MEK (downstream target of the canonical Torso signaling pathway) also cause pole plasm spread and ectopic transcription. These observations establish that ectopic stabilization of Torso leads to activation of the ERK-dependent pathway, ultimately resulting in aberrant germ cell specification. Taken together, our data demonstrate that a mutual antagonism between Gcl and Torso ensures the controlled release of germ-plasm and the resulting transcriptional quiescence of PGCs underlying embryonic germline/soma distinction.

456C Dynamic gene control through interallelic interactions in *Drosophila* embryos

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The mechanism of which enhancers interact with the target promoter to initiate transcription has been extensively studied. However, recent studies suggest that the 3D genome organization also plays an important role in gene regulation. We use live-imaging methods and quantitative analysis in living *Drosophila* embryos to show that interallelic interactions can affect transcriptional dynamics. We show that reporter genes at homologous positions interfere with each another, resulting in reduced mRNA production from each allele. Such interference was not observed when the reporter genes were positioned at non-homologous locations.
Interestingly, we find that homologous alleles interfere with each other even when one allele has only a partial transcription unit (enhancer only or a promoter-reporter gene only) and the interference was not observed when the enhancer-promoter interactions are weakened. We propose that the allelic interactions may occur through clusters of transcription factors and mediator+Pol II complexes where transcriptional machineries are shared between two alleles resulting in the reduced transcriptional activity.

457A Examining how differing mechanical mode stimulations elicit proteomic changes in *Drosophila e*mbryos

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Mechanotransduction is essential for proper tissue development and maintenance of normal physiology. There is a growing appreciation that mechanical forces play a critical role during embryogenesis. Embryogenesis in *D. melanogaster* involves numerous morphogenic events controlled by developmental genes capable of inducing mechanical strain. Conversely, mechanical strain also regulates developmental gene expression as demonstrated by the regulation of Twist by ß-catenin signaling. However, it is not known whether additional genes are similarly regulated by mechanical stimulus during development. Moreover, it is unknown whether different mechanical stimulations, such as compression or gravitational loading, elicit stress-specific responses within the embryo. We reasoned that embryos are likely to be sensitive to gravity, given that altered gravity has been shown to influence behavior and gene expression in *Drosophila* adults and larvae.
To identify mechanoresponsive proteins and establish if the early embryo senses altered gravity, we exposed early *Drosophila* embryos to different mechanical stimulation modes, including altered gravity and compression. We then assayed for protein changes using the comparative proteomic technique Difference Gel Electrophoresis (2-D DIGE). Focused proteomic analysis by 2D-DIGE revealed that a set of proteins reproducibly change in expression level or post-translational modification state in response to mechanical stimulation by compression or altered gravity. These results indicate that *Drosophila* embryos respond to different mechanical modes with both altered gravity and compression. The observation that a shared set of proteins respond to different mechanical stimulation modalities suggests that these forces in the early embryo activate common mechanotransduction pathways. This conclusion is supported by the reciprocal nature of changes observed between microgravity and hypergravity. Future work will utilize time-lapse microscopy and biochemical assays to investigate how mechanical perturbation influences developmental processes.

458B GAGA Factor is essential for zygotic genome activation and chromatin accessibility in the early Drosophila embryo

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Following fertilization, the genomes of the germ cells are reprogrammed to form the totipotent embryo. This period of rapid and efficient reprogramming, known as the maternal-to-zygotic transition (MZT), is driven by pioneer factors that initiate widespread transcriptional programs to determine cell fate. Pioneer factors are a class of transcription factors that are capable of facilitating chromatin accessibility, enabling the binding of additional transcription factors that drive gene expression. In several systems, reprogramming during the MZT requires multiple pioneer factors that coordinate to remodel chromatin and activate the genome. In *Drosophila melanogaster*, the pioneer factor Zelda (Zld) functions as an essential global genomic activator during the MZT. However, until recently it was unknown whether additional pioneer factors were required for this transition. Previous data suggested a role for the maternally encoded protein GAGA Factor (GAF) in regulating transcription

during this period. To directly test the role of GAF during the MZT, we overcame previous limitations on depleting GAF by targeting the protein for degradation. This method robustly eliminated GAF in the early embryo. The majority of embryos in which GAF was knocked down died before completing the MZT, and we found that GAF is necessary to activate widespread zygotic transcription and to remodel the chromatin accessibility landscape in the early embryo. These data demonstrate that GAF, along with Zld, is essential for early embryonic reprogramming. Although GAF and Zld both occupy over a thousand shared genomic loci during the MZT, we found that in the absence of GAF or Zld the binding of the other factor is not ablated from co-bound regions. Our analysis indicated that Zld preferentially controls expression of the earliest transcribed genes, while genes expressed during widespread zygotic activation are predominantly dependent on GAF. Together, our data show that GAF and Zld largely function independently to facilitate chromatin accessibility and ultimately activate transcriptional targets. We propose that as development proceeds transcriptional control is gradually transferred from one pioneering factor to the next. Thus, the MZT in *Drosophila* shares with other developmental transitions a requirement for the collaborative action of multiple pioneer factors. Ongoing research will leverage this system to define the mechanisms by which multiple pioneer factors drive reprogramming.

459C Quantitative-enhancer-FACS-seq (QeFS) reveals epistatic interactions among motifs within transcriptional enhancers in developing *Drosophila* tissue

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Understanding the contributions of sequence-specific transcription factor (TF) DNA binding sites to the activities of transcriptional enhancers is a significant challenge. Here, we developed Quantitative enhancer-FACS-Seq (QeFS) technology for highly parallel quantification of enhancer activities from a common chromosomal locus in *Drosophila melanogaster* embryos. Using QeFS, we investigated the contributions of the DNA binding motifs of four poorly characterized TFs – Deaf1, Schlank, CG7928 (ZIPIC), and CG12236PB – to the activities of twelve embryonic mesodermal enhancers across a panel of 88 wild type and mutant enhancers. We found that Deaf1 and CG12236-PB sites lead to context-dependent increases, while Schlank and ZIPIC sites lead to context-dependent decreases, in mesodermal enhancer activity. By measuring the effects of motif mutations both individually and in combination with each other on enhancer activity, we discovered a range of epistatic interactions among the motifs, including both synergistic and alleviating interactions. Altogether, our results indicate that understanding the regulatory consequences of TF binding motifs requires that they be investigated not just individually but also in combination with each other, across a panel of enhancers. Elucidating the context-dependent interplay between TF binding motifs will be important for understanding how *cis*-regulatory information is encoded in transcriptional enhancers.

460A Phosphorylation and alternative interaction motifs guide Hox DNA-binding preferences and promote tissue-specific function

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Hox proteins are transcription factors that specify body segment identity during embryogenesis and many diverse cell types later in development. As monomers, Hox proteins show little difference in DNA-binding preference, yet when bound to their common cofactor Extradenticle (Exd), they exhibit the DNA-binding specificity needed to carry out their unique functions. This interaction is mediated by a conserved “W”-motif in Hox that is bound by a single binding pocket in Exd. Between the Hox W-motif and the N-terminal arm of the DNA-binding domain is a variable “linker” domain that interacts with DNA only when Exd binds Hox; differences in the amino-acid composition of the linker domain contributes to distinct DNA binding preferences between Hox proteins. I found that the linker domains of the *Drosophila* abdominal Hox proteins Abd-A and Abd-B can be phosphorylated during development, and that mutating these phosphorylation sites incurs tissue-specific phenotypes. Further, Abd-A and Abd-B exhibit alternative W-motifs that could position different residues to interact with DNA; mutating these motifs also incurred tissue-specific phenotypes. To investigate the mechanism underlying these phenotypes, I utilized the *in vitro* DNA-binding assay SELEX-seq to study Hox-Exd complexes with site-specific phosphorylation or mutant W-motifs. Complexes under both sets of conditions exhibited altered DNA-binding specificity, revealing that both phosphorylation and alternate W-motifs can guide Hox-Exd’s DNA-binding preferences to potentially specialize Hox activity in a tissue specific manner. Comparative analysis of abdominal Hox proteins indicates that these alternative W-motifs and phosphorylation sites are present in vertebrates, suggesting that this mechanism may be a conserved mechanism for regulating the DNA-binding

specificity of Hox proteins.

461B Enhancer hijacking leads to flies with no thorax

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In Drosophila, the *invected* (*inv*) and *engrailed* (*en*) genes exist within a co-regulated complex and are expressed throughout development. *inv* and *en* encode closely related homeodomain transcription factors important for proper embryonic and adult development. Distinct enhancers drive co-expression of *inv/en* in the head, gut, and CNS of the embryo and in the larval imaginal discs. Although the *inv/en* promoters are separated by ?54 kb, their expression is regulated by the same enhancers distributed across a 70 kb region, showing that these enhancers can activate multiple promoters over long distances. Previous studies have identified a 2 kb regulatory fragment upstream of the *en* promotor, which may serve as a promoter tethering element (PTE) by facilitating interactions between the *en* promoter and distant imaginal disc enhancers. We have generated a transgenic line containing the 2 kb regulatory fragment fused to a *lacZ* reporter gene inserted near the *en* promoter. When coupled with a wild-type chromosome, transgenic organisms expressed ß-Galactosidase and Inv-En only in the posterior compartment of the wing imaginal discs, consistent with appropriate enhancer communication. However, as homozygotes, or over null *en* mutants, transgenic organisms exhibit a reduction in Inv-En expression in thoracic discs while other enhancers appear to function normally. Interference with enhancer-promoter communication in thoracic discs generates flies with a unique and unexpected phenotype, pharate adults with no thorax. We suggest that the endogenous thoracic imaginal disc enhancers have been hijacked by the regulatory fragment within the transgene. Using CRISPR/Cas9 we have inserted an attP landing site just upstream of the *en* promoter. To determine which sequences are required for capturing the thoracic imaginal disc enhancers, we are inserting attB-transgenes containing subsets of the original transgene. Together, these experiments will provide insight into the sequences that facilitate accurate enhancer-promoter communication in Drosophila.

462C Optogenetic rescue of a developmental patterning mutant

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Animal embryos are patterned by a handful of highly conserved inductive signals. Yet in most cases it is unknown which pattern features (i.e., spatial gradients or temporal dynamics) are required to support normal development. An ideal experiment to address this question would be to “paint” arbitrary synthetic signaling patterns on “blank canvas” embryos to dissect their requirements. Here we demonstrate exactly this capability by combining optogenetic control of Ras/Erk signaling with the genetic loss of the receptor tyrosine kinase-driven terminal signaling patterning in early *Drosophila* embryos. Blue light illumination at the embryonic termini for 90 min was sufficient to rescue normal development, generating viable larvae and fertile adults from an otherwise-lethal terminal signaling mutant. Optogenetic rescue was possible even using a simple, all-or-none light input that reduced the gradient of Erk activity and eliminated spatiotemporal differences in terminal gap gene expression. Systematically varying illumination parameters further revealed that at least three distinct developmental programs are triggered at different signaling thresholds, and that the morphogenetic movements of gastrulation are robust to a three-fold variation in the posterior pattern width. These results open the door to controlling tissue organization with simple optical stimuli, providing new tools to probe natural developmental processes, create synthetic tissues with defined organization, or directly correct the patterning errors that underlie developmental defects.

463A The role of Pol II elongation on dynamic gene control

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While enhancer-promoter interactions are key in controlling gene expression, little is known about the role elongation of RNA polymerase II (Pol II) elongation plays in the dynamics of transcriptional regulation. There have been a wide range of reported PolRNA polymerase II (pol II) elongation rates, and in this study, we seek to determine possible factors that influence the rate of Pol II elongation and subsequent rate of mRNA production. To transcription. Using quantitative live imaging, we calculate determine how long Polpol II takes to transverse a gene, we inserted 24x MS2 and 24x PP7 stem loops at the 5’ and 3’ UTR of a *lacZ* reporter gene, respectively. Using quantitative live imaging and measuring the temporal delay between MS2 and PP7 detection, we calculate the rate of Pol II elongation in early *Drosophila* embryos. Upon measuring the Pol II elongation rate in constructs with various regulatory elements, we during *Drosophila* development. We find that enhancers play a role

in affecting elongation rate, with "stronger" enhancers displaying slower rates compared to "weaker" enhancers. Our results also show that during nuclear cycle 12 (NC12), Polpol II is too slow to finish transcribing the entire reporter gene; however, as development continues, transcription is completed in NC13 and the rate of Pol II elongation increases further during NC14 when the zygotic genome is fully activated (1.5~2.5 kb/min). Although these. These results only look at the initial rate of Pol II elongation, further analysispol II, yet our data suggests that once transcription begins elongation rate accelerates within a nuclear cycle. Taken together, Transcription is a major factor in gene expression, and we suggestelucidate the factors that can control the rate of Pol II elongation can vary with developmental time and with different enhancers, and this plays an important role in controlling the total amount of mRNA productionpol II transcription.

464B Functional characterization of human Homeodomain-interacting protein kinases (HIPKs) in *Drosophila* reveals both conserved functions and evidence for direct polycomb regulation

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Homeodomain-interacting protein kinases (Hipks) are a family of conserved proteins that are necessary for development in both invertebrate and vertebrate organisms. Vertebrates have four paralogues, Hipks 1-4. Mice lacking *Hipk1* or *Hipk2* are viable, however loss of both is lethal during early embryonic development, with embryos exhibiting homeotic skeletal transformations and incorrect HOX gene expression. While these results suggest Hipks have a role in regulating HOX genes, a regulatory mechanism has not been characterized, and further comparisons of the roles of Hipks in development has not progressed. One challenge with characterizing developmental regulators in vertebrates is the extensive redundancy of genes. For this reason, we used *Drosophila melanogaster*, which has reduced genetic redundancy, to study the functions of the four human HIPKs (hHIPKs). In *Drosophila*, zygotic loss of the single ortholog *dhipk* results in lethality with distinct eye and head defects. We found that replacing *dhipk* with either *hHIPK1* or *hHIPK2* rescued lethality, while hHIPK3 and hHIPK4 only rescued minor *dhipk* mutant patterning phenotypes. This evidence for conserved functions of hHIPKs in *Drosophila* directed our efforts to identify and compare the developmental potential of hHIPKs by expressing them in well-defined tissue domains and monitoring changes in phenotypes. We observed unique patterns of homeotic transformations in flies expressing hHIPK1, hHIPK2, or hHIPK3 caused by ectopic induction of Hox proteins, including wing-to-haltere transformation, ectopic sex combs, malformed legs, and partial arista-to-leg transformation. These results were indicative of inhibited Polycomb-group complex (PcG) components, suggesting that hHIPKs play a role in regulating its activity. Furthermore, knockdown of PcG components phenocopied hHIPK and dHipk expression phenotypes. Preliminary data suggests that HIPKs colocalize with PcG components in the nucleus as puncta on chromatin, and as blobs off chromatin. Together, this data shows that hHIPKs function in *Drosophila*, where they appear to have variable ability to inhibit PcG, which may reflect their roles in development.

465C Pleiotropic fitness effects of the lncRNA encoding *Unknown host gene 4* in *Drosophila melanogaster*

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Long non-coding RNAs (lncRNAs) contribute to regulation of gene expression, chromatin remodeling, and interactions with other RNAs. In *Drosophila melanogaster*, the lncRNA *Unknown Host Gene 4* (*Uhg4*) is host to seven small nucleolar RNAs (snoRNAs) and is ubiquitously expressed at moderate levels, with especially high expression in the adult ovary. Developmental exposure to ethanol in females of the *Drosophila melanogaster* Genetic Reference Panel (DGRP) results in genetic background dependent coregulation of expression of *Uhg4* and 37 snoRNAs. The vast majority of these snoRNAs belong to the H/ACA class associated with pseudouridylation of ribosomal RNA, suggesting that regulation of snoRNAs may modulate ribosomal function. To further explore the function of *Uhg4* we used CRISPR-*Cas9* to create a series of knockout deletions that encompass approximately 500 bp immediately upstream of *Uhg4* and the first exon of *Uhg4* in multiple DGRP genetic backgrounds. We found that each independent deletion that rendered *Uhg4* dysfunctional resulted in sterility as well as background-dependent changes in viability. The sterility was due to inability of female flies to lay eggs. Thus, *Uhg4* has pleiotropic effects; it is indispensable for reproduction and represents an example of a lncRNA essential for fitness. Supported by NIH grant GM128974.

466A Enzyme activity regulation is complicated: expanding the interacting NADP(H) network

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Enzyme activity regulation is complicated. Malic enzyme (MEN), a metabolic enzyme and member of the NADP(H) network, has interesting activity variation in response to environmental change and changes in the activity of other network members, variation that is sensitive to genetic background. *Men* expression is also regulated, in part, by transvection, *trans*-regulation of

gene expression in which regulatory elements on one chromosome influence elements on a paired homologous chromosome. We explored the effects of genetic background on both MEN activity and the amount of transvection by performing Genome Wide Association Studies (GWAS) using the the *Drosophila* Genetic Reference (DGRP). We found considerable variation in the amount of MEN activity across 159 DGRP lines, with activity varying by 4.6- fold, and significantly associating genetic variation in the GWAS. The top hits included an unannotated peptide, *CG43244*, transcription factor *Dp*, SNPs in the *Men* regulatory region, and *tara*, a gene shown to influence transvection.

We also explored the effects of genetic background on transvection at *Men* by crossing 150 DGRP lines with a suite of *Men* excision alleles and performing a GWAS of MEN activity in the offspring. Relatively few studies have explored how transvection is affected by distal genetic variation, perhaps because transvection is strongly influenced by local regulatory elements and chromosomal architecture. We found both local and distal genetic variation associated significantly in the GWAS with transvection at *Men*. The specific genetic variation identified was dependent on the excision allele used, highlighting the complex genetic interactions influencing transvection. The most strongly associated genetic variation was found just upstream of the *Men* transcriptional start site, however several top hits in the GWAS mapped to other areas of the genome. These areas included the long non-coding RNA, *Iab8*, several transcription factors (*Cnc*, *Fru*), and RNA binding proteins (*Bru-2*, *CG10418*).

These results identify several candidate genes to further explore in the understanding of regulation at *Men,* other members of the NADP(H) network, and in other genes regulated by transvection. Overall, these findings highlight the complexity of the interactions involved in gene regulation, even in phenotypes, such as transvection, that were traditionally considered to be primarily influenced by local genetic variation.

467B Three distinct mechanisms, Notch instructive, permissive, and independent, regulate the expression of two different pericardial genes to specify cardiac cell subtypes

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The development of a complex organ involves the specification and differentiation of diverse cell types constituting that organ. Two major cell subtypes, contractile cardial cells (CCs) and nephrocytic pericardial cells (PCs), comprise the *Drosophila* heart. Binding sites for Suppressor of Hairless [Su(H)], an integral transcription factor in the Notch signaling pathway, are enriched in the enhancers of PC-specific genes. Here we show three distinct mechanisms regulating the expression of two different PC-specific genes, *Holes in muscle* (*Him*), and *Zn finger homeodomain 1* (*zfh1*). *Him* transcription is activated in PCs in a permissive manner by Notch signaling: 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. In contrast, *zfh1* is transcribed by a Notch-instructive mechanism in most PCs, where mere alleviation of repression by preventing the binding of Su(H)-co-repressor complex is not sufficient to activate transcription. Our results suggest that upon activation of Notch signaling, the Notch intracellular domain associates with Su(H) to form an activator complex that binds to the *zfh1* enhancer, and that this activator complex is necessary for bringing about *zfh1* transcription in these PCs. Finally, a third, Notch-independent mechanism activates *zfh1* transcription in the remaining, *even skipped*-expressing, PCs. Collectively, our data show how the same feature, enrichment of Su(H) binding sites in PC-specific gene enhancers, is utilized by two very distinct mechanisms, one permissive, the other instructive, to contribute to the same overall goal: the specification and differentiation of a cardiac cell subtype by activation of the pericardial gene program. Furthermore, our results demonstrate that the *zfh1*enhancer drives expression in two different domains using distinct Notch-instructive and Notch-independent mechanisms.

468C Quantitative imaging of transcription in living *Drosophila* embryos reveals the impact of core promoter motifs on promoter state dynamics

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Developmentally regulated genes are expressed in a “bursty” manner with alternating intervals of transcriptionally active and inactive states characterized by their duration, frequency, and level of mRNA production. We investigate two key *Drosophila* developmental promoter motifs, the TATA box and the Initiator (INR), using natural and synthetic promoter

sequences to determine the impact of the core promoter "code" on transcriptional regulation. Using live imaging and novel model-independent computational methods, we demonstrate that transcriptional bursting operates over multiple timescales ranging from seconds to minutes. TATA-containing promoters and INR-containing promoters exhibit distinct dynamics, with one or two separate rate-limiting steps respectively. We discover that the TATA box is associated with longer active periods, rapid RNA Polymerase II (Pol II) initiation rates, and rare short off durations. In contrast, the INR motif is associated with three distinct promoter states with differing durations and probabilities associated with each state. Intriguingly, we are able to associate the emergence of a third promoter state with an increase in promoter-proximal Pol II pausing, and demonstrate that reducing pausing results in loss of this third promoter state. Our mathematical modeling also proposes that Pol II pausing is not required for each polymerase initiation event, but instead is stochastically imposed at only a subset of successful initiations. Collectively, our platform enables quantitative, model-independent investigation of promoter dynamics in living embryos and offers new insight into the mechanisms underlying transcriptional regulation.

469A Context-specific regulation of gene expression by tethered Retinoblastoma paralogs in developing wing tissue

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Retinoblastoma (Rb) family proteins are transcriptional corepressors that localize to promoter-proximal regions of diverse classes of genes. Uncovering the mechanism of transcriptional repression by Rb proteins and their context specificity during Drosophila development is challenging due to the pleiotropic effects of global knockdown or overexpression of the factors. An *in vivo* approach to directly compare the activities of the fly Rb proteins on specific loci would provide the necessary setting to understand repressor selectivity and mechanism. To address this, we engineered flies expressing a nuclease dead Cas9 enzyme (dCas9) fused to the Drosophila Rbf1 and Rbf2 proteins and deployed them to diverse gene promoters in the developing wing tissue using guide RNAs. We found that Rb proteins can function in gene-specific ways when tethered to dCas9. When targeting the *E2F2* promoter, wild type Rb proteins can mediate potent gene repression, while specific mutant versions have a more limited ability. Notably, a form of Rbf1 that lacks an "instability element" previously shown to be critical for activity of the endogenous protein is functional when tethered, pointing to the domain's role in recruitment, rather than repression. In contrast, targeting the promoter of *InR*, the Insulin receptor, suggests an activating, or de-repressing, role by the Rbs. Using this dCas9-mediated approach, we will determine how genomic, temporal, and tissue-specific contexts impact the activities of these paralogs in the fly. The molecular analysis of promoter-specific regulation by diverse Retinoblastoma proteins will enhance our understanding of these conserved regulatory proteins in development and disease.
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470B The mode of expression divergence in *Drosophila* fat body is infection-specific

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Abstract
Transcription is controlled by the interactions of *cis*-acting DNA elements with diffusible *trans*-acting factors. Changes in *cis* or *trans* factors can drive expression divergence within and between species, and the relative prevalence of each can reveal the evolutionary history and pressures that drive expression variation. Previous work delineating the mode of expression divergence in animals has largely used whole body expression measurements in a single condition. Since *cis*-acting elements often drive expression in a subset of cell types or conditions, these measurements may not capture the complete contribution of *cis*-acting changes. Here, we quantify the mode of expression divergence in the *Drosophila* fat body, the primary immune organ, in several conditions. We performed allele-specific expression analysis using two geographically distinct lines of *D. melanogaster* and their F1 hybrids. We measured expression in the absence of infection and in separate infections with Gram-negative *S. marcescens* or Gram-positive *E. faecalis* bacteria, which trigger the two primary signaling pathways in the *Drosophila* innate immune response. The mode of expression divergence strongly depends on the condition, with *trans*-acting effects dominating in response to Gram-positive infection and *cis*-acting effects dominating in Gram-negative and pre-infection conditions. Expression divergence in several receptor proteins may underlie the infection-specific *trans* effects. Before infection, when the fat body has a metabolic role, there are many compensatory effects, changes in *cis* and *trans* that counteract each other to maintain expression levels. This work demonstrates that within a single tissue, the mode of expression divergence varies between conditions and suggests that these differences reflect the diverse evolutionary histories of host-pathogen interactions.

471C Investigating interactions that coordinate histone expression

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Accurate gene expression is crucial for proper organismal development, and is dependent on regulatory factors binding to their target DNA sequences. The *Drosophila* histone locus is an excellent model to study the relationship between DNA and regulatory factors because the replication-dependent histone genes exhibit unique regulation. Their expression is tightly coupled to the cell cycle, so that newly synthesized histones can be incorporated into replicating DNA. Additionally, transcription of the five histone genes (H1, H2A, H2B, H3, and H4) must be coordinated to achieve the precise stoichiometry found in nucleosomes. Furthermore, histone transcripts are not polyadenylated and thus require specialized factors for their processing. To meet these regulatory criteria, a collection of transcription and processing factors, known as the histone locus body (HLB), forms at the histone locus. Although several components and their function are known, the full composition of the HLB remains a mystery, and the molecular interactions within the HLB are not well characterized. To address these unknowns, we are investigating both DNA-protein and protein-protein interactions at the histone locus. We will first examine DNA-protein interactions by using an artificial tethering transgenic system. Our previous results using this system confirmed that the zinc finger transcription factor CLAMP is critical for HLB formation and histone gene expression. Our planned experiments include 1) artificially tethering HLB components to an ectopic histone locus, and 2) manipulating the positioning of key binding motifs within the ectopic locus, to determine how this interaction affects HLB formation and histone expression. To investigate protein-protein interactions, we are combining two modern technologies: nuclease-dead Cas9 targeting and *in vivo* proximity labeling. We are creating a transgene consisting of a promiscuous biotin ligase fused to dCas9, which we will target to the histone locus. The biotin ligase will biotinylate proximal HLB proteins, which we will identify via mass spectrometry. This unbiased approach will allow us to determine the changing composition of the HLB in the context of a developing embryo. Overall, our work will investigate the molecular interactions at the *Drosophila* histone locus, providing a comprehensive picture into the regulation of a unique set of genes.

472A Contributions of HP1 proteins to transcription start site activity

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The Heterochromatin Protein 1 (HP1) family is a group of highly conserved non-histone chromosomal proteins with diverse functions in chromatin structure, DNA repair, and regulation of gene expression. In *Drosophila melanogaster*, there are three HP1 paralogs that are expressed constitutively in somatic tissue, and they can be distinguished by their genomic distributions. While all three proteins can be found in heterochromatin and euchromatin, HP1a is enriched in heterochromatin where it has essential functions, while HP1B and HP1C are enriched in euchromatin. All three proteins bind to transcription start sites (TSSs) throughout heterochromatin and euchromatin. Many of these binding sites are shared by multiple HP1 family members. The significance of this shared binding is unknown, and it is unknown to which extent the overlap in binding profiles represents HP1 proteins simultaneously targeting shared binding sites. Here, we characterize binding patterns of HP1 family members in six biological sources including three tissues and three cell cultures. We find that HP1 binding at TSSs is cell-type specific. We document simultaneous binding of HP1 proteins at gene promoters using sequential ChIP of S2 cell culture. Through an integrated analysis of epigenomic and transcriptomic data, we observe that HP1 binding targets are actively transcribed and display signatures of increased promoter proximal pausing. These data suggest that HP1 proteins may function synergistically to promote transcription. To test this hypothesis, we are using dCas9 mediated recruitment of HP1 proteins to promoters to examine impacts on nascent transcription. Our analysis to date highlights the importance of considering context of protein-protein interaction when inferring function of chromosomal proteins.

473B Sex chromosome-specific variation in R-loop formation in *Drosophila melanogaster*

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R-loops are three-stranded nucleotide structures consisting of a DNA:RNA hybrid and a displaced ssDNA non-template strand. R-loop formation alleviates topological stress caused by multiple processes including transcription-induced negative superhelicity, playing important roles in gene expression and genomic stability. Although R-loops are conserved across cell types in mammals, little is known about natural variation in R-loop formation between individuals. Using DNA:RNA immunoprecipitation followed by high-throughput sequencing (DRIP-seq), we have mapped the R-loop profiles of two *D. melanogaster* individuals from the Drosophila Genetic Reference Panel (DGRP) in both adult males and females. In both individuals, the male X chromosome is significantly depleted of R-loops compared to the female X. In cultured *Drosophila* S2 cells, which are also male, the X chromosome is depleted of R-loops and is less negatively supercoiled compared to autosomes. Moreover, inhibition of DNA topoisomerase I exhibits smaller changes in superhelicity of the X chromosome versus autosomes. These results run counterintuitive to our understanding of R-loops and torsion and may represent a unique feature of the dosage compensated X chromosome in *Drosophila* males. To address this, our current efforts aim to assess how

pharmacological induction of topological stress elicits changes in R-loop formation.

474C Elements of a *decapentaplegic* (*dpp*) enhancer involved in adult head capsule morphogenesis

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The *Drosophila* BMP gene, *decapentaplegic* (*dpp*), contains five promoters that direct transcription of five distinct mRNAs, all encoding an identical polypeptide. *dpp* is employed in almost every stage of development for a large number of processes which are regulated by extensive proximal and distal cis-regulatory regions, including many enhancer elements. We studied a 3.5 kb enhancer at the 5' (distal) end of the gene that controls morphogenesis of the ventral adult head through expression limited to the lateral peripodial epithelium of the eye-antennal disc. It has long been assumed that the five *dpp* promoters respond interchangeably to the enhancers within the *dpp* gene, however, work on the function of this enhancer has uncovered elements that provide enhancer barrier function and may direct expression specifically to the most distal promoter. Here we describe our analysis on the different regulatory elements of this enhancer, a possible mechanism for isolation of this enhancer from others, and a possible interaction with the most distal promoter of the five mRNA transcripts.

475A Determining how noisy transcription controls stochastic fate specification in the developing fly eye

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How gene expression noise is harnessed to generate cellular diversity during development is poorly understood. To determine how cells randomly choose between fates, we studied the random mosaic of two color-detecting photoreceptor subtypes in the fly eye. The on/off expression of the transcription factor Spineless (Ss) controls R7 photoreceptor subtype fate, resulting in 65% Ss^{on} R7s and 35% Ss^{off} R7s. Patterning is controlled by a two-step mechanism. In step 1, *ss* is transcribed in a pulse in R7 precursors. In step 2, the transcriptional pulse ceases, cells differentiate, and Ss turns back on in a random subset of terminal R7s. Manipulating regulatory DNA elements and transcription factors tunes the strength of *ss* expression in the early pulse, leading to changes in the on/off ratio in terminal R7s. These findings suggested that the level of *ss* expression in an individual R7 precursor determines the on or off state as the cell differentiates into a terminal R7. To assess transcriptional variability in the early pulse, we developed a three-color RNA FISH approach to visualize the 5', middle, and 3' region of the nascent *ss* transcript. This quantitative approach showed that the early pulse of transcription is noisy and that this variability is independent of developmental timing. To monitor transcription in real time, we utilized the MS2/MCP system. We inserted MS2 stem loops into different positions and found that upstream insertions into the 5' UTR and the first intron are expressed in more cells than downstream insertions in a middle intron and the 3' UTR, indicating that elongation regulates transcriptional variability in the early pulse. Our current work aims to use live imaging to link noisy transcription to terminal cell fate.
In addition to *ss* gene expression, we found that upstream *cis*-regulatory regions are transcribed. Transcription was observed in both the sense and antisense directions, suggesting an alternative role for transcription in these regions. Counterintuitively, individual enhancers are not transcribed in cells in which they induce ss expression. Additionally, transcription of upstream regions is dependent on the *ss* promoter. Future work aims to characterize how transcription of regulatory DNA regions controls *ss* expression and stochastic fate specification.

476B Molecular competition can shape enhancer-driven expression in the *Drosophila* embryo

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Transgenic reporters that allow the assessment of a DNA sequence's activity *in vivo* have long been incredibly useful tools in furthering our understanding of how regulatory regions of DNA control gene expression in time and space. Despite the great utility of transgenic reporters, little work has been done to study the potential effect of these reporters on other transgenic reporters or endogenous genes. A full understanding of how transgenic reporters interact with one another and the endogenous systems in which they are placed is required not only for accurate interpretations of transgenic reporter data, but also for a fuller understanding of the biological mechanisms that regulate the activity of these reporters and endogenous sequences. Here, we investigate the impact such transgenic reporters have on the expression of other transgenic reporters as well as endogenous genes. By measuring the expression of *Kruppel* (*Kr*) enhancer reporters in living *Drosophila* embryos containing either one or two copies of identical reporters, we find reporters have an inhibitory effect on one another's expression. We present evidence that this inhibition is due to reporter competition for endogenous factors required for transcription. To test the hypothesis that reporters are competing for activating transcription factors (TFs), we measure the expression of the *Kr* enhancer reporters in the presence of TF binding site arrays. An array containing as few as six Bicoid (Bcd) binding sites, but lacking a promoter or reporter element, reduces the activity of Bcd-regulated enhancer reporters. Further,

we show that expression of endogenous genes decreases in a distance-dependent manner when a *Kr* enhancer transgene is present. Growing evidence shows that the nucleus is not a homogeneous environment but that instead, TFs and other molecules are often concentrated in so-called "hubs" within the nucleus. We suspect that the reporter-induced competition we observe stems from limited levels of TFs within these hubs surrounding the sites of reporter transcription, as opposed to globally limited TF levels. A simple thermodynamic model shows that the probability of enhancer-TF binding is unaffected by the addition of hundreds of TF binding sites at the level of the whole genome but is significantly changed by the addition of even a few TF binding sites at the level of a sub-nuclear hub. Further, at both the genome and sub-nuclear hub scale, the predicted enhancer expression pattern is sharpened by the addition of competing TF binding sites. Our findings have important implications for the use of transgenic reporters as well as for our understanding of how endogenous expression patterns may be shaped by endogenous binding sites competing for a limited TF pool.

477C Interchromosomal Effect and Recombination Rate Plasticity in *Drosophila pseudoobscura*

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Environmental factors influence recombination, impacting the ability of the population to respond to selection pressures. Similar to recombination rate plasticity, chromosomal inversions act as global modifiers of recombination by increasing the crossover rate with the interchromosomal effect, (ICE). While plasticity and ICE have been studied independently, this is the first experiment to examine their interaction. For plasticity, we used temperature to induce recombination rate variation. For ICE, we crossed the flies with inversion differences: Treeline and Arrowhead chromosomal arrangements. Visual markers spanning the X chromosome: *scalloped (sd), yellow (y), and sepia (se)* were used to measure recombination rates. In a fully factorial experimental design, first, homokaryotic F1 females with the treeline arrangement were reared in high temperature, 26˚C, and control of 21˚C, and these conditions were repeated for heterokaryotic flies with both treeline and arrowhead formations to examine the interaction of plasticity and ICE. In homokaryotypes temperature increases the recombination rate by around 6%, yet in heterokaryotypes, we see a "subtractive" effect where it is 22.6% lower (p=2e-16). Ongoing work is combining both ICE and plasticity due to heat stress in the same experiment to confirm these results and investigate possible mechanisms that lead to variation in responses. In a combinatorial approach, our study is the first to report results for the interaction between plasticity and ICE, providing a better reflection of natural populations.

478A Structure-specific endonucleases SLX1 and SLX4 mediate DNA damage tolerance responses in Drosophila

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Genome integrity and stability rely upon continuous, high-fidelity DNA replication, but exogenous DNA-damaging agents can disrupt replication by distorting the shape of the helix or adding bulky adducts. These lesions can inhibit the processivity of replicative polymerases and stall the replication forks. Collapse of stalled forks can lead to double strand breaks, genome instability, and cell death. Our objective is to understand how cells deal with fork-stalling damage, using *D. melanogaster* as a metazoan model.

Specifically, we are interested in the relationship between the REV1 protein and structure specific endonucleases, like SLX4 and SLX1, at stalled forks. REV1 recruits translesion polymerases via its C-terminal domain (CTD) for translesion synthesis. We have shown that the REV1 translesion polymerase is important for tolerance to alkylation damage, but less so for hydroxyurea (which does not add bulky adducts), suggesting a critical role for REV1 in lesion bypass. The null *rev1* mutants demonstrate increased sensitivity to MMS in addition to increased damage checkpoint activation compared to the *rev1ΔCTD* mutants lacking the CTD, implicating an additional role for REV1 in a non-translesion synthesis pathway of damage tolerance. We postulate REV1 may also stabilize reversed forks.

To test the model that action by structure-specific endonucleases at reversed forks may result in maladaptive cleavage reactions and cell death, we created double mutants lacking *REV1* and structure-specific endonucleases. In a *rev1*-null background, loss of *SLX4* leads to a partial rescue of MMS-induced lethality, but no such rescue occurred in *rev1-slx1* mutants. We conclude that the rescue seen in *rev1-slx4* flies may be due to either SLX4's scaffolding function for multiple structure-specific endonucleases or an additional role in damage tolerance. A quadruple mutant (*mus81xpf; rev1-slx1)* is currently being created to distinguish between these possibilities. Interestingly, the *rev1ΔCTD-slx1* flies are hypersensitive to methyl methanesulfonate (MMS) treatment when compared to *rev1ΔCTD* flies. The *rev1ΔCTD-slx1* flies also demonstrate increased staining for damage checkpoint compared to *rev1ΔCTD* flies. We speculate that SLX1 is needed to cleave repair intermediates that form in the absence of translesion synthesis. Experiments with additional DNA damaging-agents and structure-specific-endonuclease mutant combinations are underway to further test these models.

479B Mapping Protein-Protein Interaction of Bloom Syndrome Helicase and Topoisomerase 3-alpha

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Bloom Syndrome is a rare autosomal recessive disorder caused by mutation of the *BLM* gene that leads to increased genome instability and cancer. The *BLM* gene codes for a helicase (Blm) that works together with Topoisomerase 3-alpha (Top3α) in homology directed repair of DNA. Top3α assists by directly binding to Blm and helping to release the torsional stress on DNA as Blm helicase unwinds recombination intermediates. The proteins preserve genome stability and operate together via two main DNA repair pathways, Synthesis-Dependent Strand Annealing and double-Holliday Junction dissolution. To investigate the Blm-Top3α interaction, we performed a yeast 2-hybrid assay using the *Drosophila* proteins. We found that the interaction was specific to certain regions of Blm and strongest for conserved region 5 (aa 1381-1487) with another possible region involved. Continued experimentation could be conducted through *in vivo* testing of the interaction using CRISPR or examining other Blm fragments not included in this study. By effectively characterizing this interaction, we can better understand detrimental effects of *BLM* mutations.

480C Mapping and characterization of the *mus302* gene

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Repair of damaged DNA is required for the viability of all organisms. Studies in *Drosophila melanogaster*, driven by the power of genetic screens, pioneered the discovery and characterization of many genes and pathways involved in DNA repair in animals. However, fewer than half of the alleles identified in these screens have been mapped to a specific gene, leaving a potential for new discoveries in this field. Here we show that the previously uncharacterized mutagen sensitive gene *mus302,* first isolated by Boyd in 1981, codes for the *Drosophila melanogaster* ortholog of the E3 ubiquitin ligase RING finger and WD domain protein 3 (RFWD3). In human cells, RFWD3 promotes ubiquitylation of RPA and RAD51 to facilitate repair of collapsed replication forks and double-strand breaks through homologous recombination. Despite the high similarity in sequence to the human ortholog, flies deficient in *mus302* are not involved in homologous recombination, and we suggest this may be linked to a new N-terminal region of the protein that is only present in mammals. Flies deficient in *mu302* are hypersensitive to the DNA alkylation and crosslinking. Since nucleotide excision repair is responsible for the repair of these types of damage, we are currently testing the involvement of Mus302 in this repair pathway, and whether *Drosophila* Mus302 may retain the ancestral function of the protein.

481A Late replication is necessary but not sufficient for tissue-specific Underreplication in Drosophila

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Complete and accurate duplication of the genome is essential to maintain genome stability during mitotic cell divisions. Throughout development, however, the regulation of genome duplication can be modified to accommodate cell-type specific functions. One example of this developmental regulation of genome duplication is the repression of DNA replication within heterochromatin and specific euchromatic regions in Drosophila polyploid cells - a process known as underreplication (UR).

Underreplicated regions are synonymous with common fragile sites. They are late replicating, associated with DNA damage and show cell-type specificity. In Drosophila, UR is an active process, dependent on both *SuUR* and *Rif1*. What is less clear, however, is how specific regions of the genome are selected to be underreplicted and the exact mechanism that SUUR and Rif1 employ to promote underreplication.

To begin to address these issues, we sought to determine the relationship between replication timing (RT) and underreplication. To this end, we utilized a sequence-coverage based technique called TIGER to generate replication timing profiles from the whole genome sequencing of large polyploid cells without the need for FACS. This technological advance has allowed us to measure replication timing on a genome-wide scale for polyploid cells of both the larval salivary gland and fat body. After validating the ability of this new method to generate RT profiles, we proceeded to determine the relationship between UR and RT across tissues. Our data shows that late replication is necessary, but not sufficient, to promote underreplication. Furthermore, tissue-specific changes in RT can explain tissue-specific differences in UR. Finally, we were able to determine how both SUUR and Rif1 affect RT in the larval salivary gland. This analysis has revealed that SUUR and Rif1 have differing effects on RT and underreplication, suggesting that changes in replication timing and inhibition of replication fork progression could work independently to control underreplication.

482B Defining mitotic crossover mechanisms in CRISPR and DNA damage using mismatch repair knockout

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Genome stability is key to the longevity of multicellular organisms and avoidance of disease. Despite being challenged daily with numerous sources of DNA damage that threaten this stability, cells regularly repair their DNA and maintain organismal resilience. Sometimes however, improper repair or repair misregulation causes accumulation of "scars" in the form of detrimental mutations within the genome, eventually leading to genome instability, cancer, and other disease. Homology directed repair (HDR) of DNA double strand breaks is one DNA repair pathway that, if improperly regulated, can lead to accumulation of mutations via mitotic (somatic) crossovers and loss of heterozygosity. Therefore, better understanding mitotic crossover mechanisms is critical to genome stability and prevention of cancer and other genetic disease. CRISPR/Cas9 has also become increasingly reliant on accurate HDR to integrate desired mutations or corrections in genome editing, but the precise CRISPR/Cas9 HDR mechanisms remain elusive. Through use of total mismatch repair (MMR) knockout that is only possible in *Drosophila* (both canonical and backup short-patch pathways), it is possible to now analyze resulting HDR products in a CRISPR/Cas9 context using Sanger sequencing. I will use this key tool will accurately define mitotic crossover mechanisms for the first time in a multicellular organism. I hypothesize that the double Holliday junction resolution model will prevail as the primary CRISPR/Cas9 mitotic crossover mechanism. I will also use this unique MMR knockout tool to define mitotic crossover mechanisms in the context of gamma irradiation-induced DNA double strand breaks using whole genome sequencing. I will compare these crossovers with CRISPR/Cas9 mitotic crossovers and begin to establish a catalog of mitotic crossover signatures for specific damaging agents. This work will enhance understanding of how DNA is repaired in both CRISPR and damage-induced contexts, expanding understanding of how mitotic crossovers and DNA damage lead to genome instability and how to beneficially utilize mitotic crossover mechanisms in genome editing.

483C Imaging chromosome inversion breakpoints in Drosophila

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Heterozygous inversions disrupt the meiotic crossover (CO) distribution by suppressing COs locally both within and outside of the inversion, and also triggering the interchromosomal (IC) effect, where the CO frequency on structurally normal chromosomes dramatically increases. The increased COs mostly occur in the centromere-proximal and sub-telomeric regions, thus causing a shift in the genome-wide distribution of COs in addition to changing the frequency. We previously showed that these changes in the CO distribution are mediated by CO patterning mechanisms that alter the CO/NCO decision. We are currently analyzing the recombination mechanics of DSB repair outside of inversion breakpoints by using the *rosy* assay to recover heteroduplex DNA associated with recombination events. Additionally, we will present preliminary data using the CRISPRainbow system that we have adapted for use in the Drosophila germline.

484A Molecular mechanisms of the inter chromosomal effect

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Chromosome structural variants (SVs) are the primary source of polymorphism in the human population, yet they are also a major cause of human infertility because they disrupt chromosome inheritance during meiosis. Studies in multiple model organisms have shown that the presence of SVs triggers a genome-wide response in recombination, termed the interchromosomal effect. We previously showed that this genome-wide response is mediated by changing the relative proportion of crossovers (COs) to total recombination events, but the molecular mechanisms that lead to the shift in the recombination landscape are unknown. We are currently testing the hypothesis that the developmental delay in prophase caused by heterozygous inversions allows the meiotic machinery to bypass normal CO patterning mechanisms, leading to un-patterned, mitotic-like CO distributions. We will present cytological data on the kinetics of CO formation in the presence in heterozygous inversions and genetic data determining whether these COs are truly meiotic or are mitotic-like. Together, these data will provide mechanistic insight into how chromosome structural variants impact meiotic recombination.

485B Meiotic Crossover Patterning in *Drosophila*

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Crossing over is a critical part of meiosis that prevents aneuploidy by ensuring proper segregation of homologous chromosomes. Crossovers are not randomly distributed along the chromosome, but instead show complex patterning; they do not occur close to one another (crossover interference) and are inhibited in centromeric and telomeric regions (crossover suppression). Erroneous crossover patterning may lead to chromosomal disorders such as Down syndrome, Turner syndrome,

and even miscarriage. My goal is to investigate the poorly understood mechanisms behind crossover suppression around the centromere, also known as the centromere effect. We hypothesize that one major reason behind crossover exclusion in centromere-proximal regions could be due to an absence of double-strand breaks (DSBs) in the pericentromeric satellite heterochromatin. To test this, I plan to study differences that arise in the number and positioning of DSBs in flies mutant for heterochromatin generation and packing, such as *Su(var)* or HisH3^{K9R} flies. Recent work from our lab has also suggested that the centromere effect in adjacent euchromatin is dependent on distance to the centromere. I hope to further build on this result by looking at the effects of changing the distance of phenotypic markers from the centromere in both the *X* and the second chromosome. We predict that reducing distance to centromere will increase crossover suppression in adjacent beta-heterochromatin and euchromatin. Based on studies in triploid flies, we also hypothesize that the strength of the centromere effect is dependent on the total number of centromeres. To investigate this, I will measure crossover rates in flies that have a reduced number of centromeres, using compound chromosomes. Through these and other methods, my overarching goal is to gain more understanding of the mechanisms at work behind crossover patterning, particularly the suppression of crossovers near the centromere.

486C Suppressor of Hairy Wing Is Required for Genome Integrity

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The ability of a cell to sense and respond to various forms of stress is essential to maintain integrity of the genome. Numerous pathways have been implicated in cellular responses to environmental and genotoxic stresses, often involving proteins and complexes that bind DNA directly to orchestrate changes in transcription and genome organization. Chromatin insulators describe a class of protein complex that bind specific sequences in the genome and work through two classically described functions: to restrict communication between enhancers and promoters through physical separation into different genomic domains and to prevent the spread of heterochromatin into euchromatic regions of the genome. Insulator sites also demarcate the boundaries between topologically associated domain (TAD) boundaries. *Drosophila melanogaster* has an array of different insulator complexes, with each complex being recruited to different sequences. Here, we demonstrate an interaction between proteins that associate with the *gypsy* insulator and the phosphorylated histone variant H2Av (γH2Av), a marker of DNA double strand breaks. The *gypsy* retrotransposon contains binding sites for Suppressor of Hair Wing (Su(Hw)), which interacts with and recruits Mod(mdg4)67.2 and CP190. Components of this insulator complex colocalize with γH2Av throughout the genome and at specific *gypsy* loci. Mutation of insulator components prevents stable H2Av phosphorylation in polytene chromatin. Deficiency for Su(Hw) in particular is also associated with chromosomal aberrations in actively dividing larval neuroblasts. PP2A phosphatase inhibition strengthens the association between insulator components and γH2Av and rescues γH2Av localization in insulator mutants. Likewise, mutation of *His2Av* affects binding of *gypsy* insulator components in polytene chromatin. We also show that γH2Av is a component of insulator stress bodies, and that phosphatase activity is required for insulator body dissolution after recovery from stress. We propose a model in which H2Av and its phosphorylation are functionally linked with the activity of insulator binding proteins. Together, our results add to the growing body of evidence linking genome organization to genome stability and provide potential clues to one such mechanism in flies.

487A Identifying and characterizing novel satellite DNA-binding proteins using proximity biotinylation

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Up to 50% of eukaryotic genomes can consist of satellite DNA, which are simple tandem repeats that are predominantly located in centromeric and pericentromeric heterochromatin. Unlike centromeric satellite DNA repeats, which are a platform for kinetochore formation and can function in chromosome segregation, the much more abundant pericentromeric satellite DNA repeats are typically considered to be 'junk DNA' due to a lack of protein coding potential and poor sequence conservation between species. Recently, work from our lab has characterized sequence-specific satellite DNA-binding proteins, which mediate the clustering of their cognate satellite DNA repeats into chromocenters in *Drosophila* and mouse cells and form a physical network that mediates the encapsulation of the entire genome in a single nucleus. Chromocenter disruption by depletion of satellite DNA-binding proteins led to micronuclei, genomic fragments that are encapsulated separately from the primary nucleus, which formed by budding during interphase. Micronuclei formation was accompanied by DNA damage, nuclear envelope defects and loss of cell viability. Despite these advances, proteins that bind 15 out of the 17 satellite DNA repeats that make up the *D.melanogaster* genome remain unknown and/or uncharacterized. Therefore, our overarching aim is the identification and characterization of novel satellite DNA binding proteins using proximity biotinylation. This method takes advantage of the fact that chromocenters formed by distinct satellite DNA-binding proteins are typically associated with

each other within nuclei. We will use the APEX2 biotinylation enzyme fused to the two known satellite DNA-binding proteins, D1 and Prod, to biotinylate novel repeat-binding proteins and identify them by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Candidate proteins will be validated using *in situ* hybridization as well as chromatin profiling methods. Together, we propose that studying novel satellite DNA-binding proteins will elucidate the largely uncharacterized biology of pericentromeric satellite DNA.

488B Whole genome approaches to understanding meiotic recombination

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During meiosis, crossovers between homologous chromosomes ensure proper chromosome segregation and prevent chromosomal abnormalities in offspring. Crossovers are formed by repairing double-strand DNA breaks (DSBs) via homologous recombination. To ensure proper homolog segregation, the number and spatial arrangement of crossovers is tightly regulated in a phenomenon known as "crossover patterning." Pathway choices within homologous recombination are traceable in products via heteroduplex DNA (hDNA), DNA in which the strands come from different parental chromosomes. The classic meiotic HR model indicates that a crossover is formed via a double Holliday junction (dHJ), a structure in which two DNA molecules are linked via criss-crossing of their strands at two adjacent sites. In this classic model, ligated dHJs give rise to all crossovers by being cleaved in one of two patterns, generating two possible hDNA signatures. The model predicts that both patterns are equally likely, yet only one of the hDNA signatures has been observed. This hDNA signature bias indicates that the meiotic recombination model must be revised. Our lab has mapped hDNA at recombinants of a test locus in *Drosophila melanogaster,* but redefining the meiotic recombination model requires much more extensive analysis of hDNA than is possible with this methodology. To overcome this obstacle, I am pioneering "hetSeq", a whole-genome sequencing technique to detect hDNA from meiotic products, to continue redefining this model. A further gap in our understanding of crossover regulation is that although crossover patterning has been observed since the early 1900s, its relationship to homologous recombination mechanism remains unclear. Many meiotic proteins have a known function in homologous recombination, and their depletion leads to crossover patterning defects. I am devising a mathematical model of recombination to test hypotheses about these proteins. I will test predictions by altering aspects of crossover patterning within the model and compare the output to previously obtained experimental data from mutants lacking these proteins. I am additionally using this model to develop a simulation of recombination using whole-genome sequencing data. Illuminating the interplay between meiotic recombination and crossover regulation is essential to understanding the mechanisms behind chromosome segregation and propagation of sexually reproducing organisms.

489C Changes in Meiotic Crossover Distribution and Frequency in Response to Chromosome Structural Variants

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Meiosis is an important mechanism that generates genetic variation for sexually reproducing organisms through recombination. In order for an organism to successfully propagate its genetic material from one generation to the next, its genome must be properly delivered as gametes. Meiotic crossing over of DNA ensures proper homolog segregation by repairing programmed double-stranded breaks. Meiotic defects caused by chromosome structural variants are detrimental to reproduction. During the interchromosomal effect, heterozygous inversions suppress crossing over between affected chromosomes while increasing crossing over between normal chromosome pairs. These defects in chromosomal dynamics trigger the pachytene checkpoint, leading to a delay in prophase progression. It has been suggested that this delay in prophase causes the interchromosomal effect on recombination. However, whether the interchromosomal effect on crossing over is caused directly by defects in chromosome dynamics or indirectly by the delay in prophase remains unclear. We are distinguishing between these two hypotheses by investigating the distributions and frequencies of crossovers in Drosophila mutants when prophase is extended by utilizing maelstrom mutants that trigger the pachytene checkpoint independently of chromosome defects. We are analyzing the changes in crossover distribution and frequencies in these mutants using recessive markers on unaffected chromosome. We are also collecting confocal imaging data of the mutants' germarium to visualize the effects of maelstrom mutations on meiosis. Our data will provide insights into the mechanisms of the interchromosomal effect and reveal whether or not the interchromosomal effect is directly mediated by a delay in pachytene or, alternatively, mediated by disrupted crossover control mechanisms.

490A The role of DmCtIP in homologous recombination during DNA double-strand break repair

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DNA double-strand breaks (DSBs) are a particularly genotoxic type of DNA damage that can result in chromosomal aberrations. Thus, proper repair of DSBs is essential to maintaining genome integrity. DSBs can be repaired by non-homologous end joining (NHEJ), which often results in a mutagenic event and is considered error prone. Alternatively, DSBs can be repaired by homologous recombination (HR), where a homologous donor sequence is utilized to guide homology-directed repair. DNA end resection is essential to initiating homology-directed repair. In human cell cultures, CtIP has been shown to play a role in DNA end resection through its interactions with CDK, BRCA1, DNA2, and the MRN complex. To elucidate the role of CtIP in the multicellular context, we used CRISPR/Cas9 genome editing to create a *DmCtIPΔ* null mutant in *Drosophila*. Using the DSB repair reporter assay, DR-*white,* we characterized the frequency of HR in the *DmCtIPΔ* mutants. We found a two-fold decrease in HR in *DmCtIPΔ* mutants when compared to heterozygous controls ($P<0.00001$). However, preliminary results analyzing gene conversion tracts (GCTs) suggests DmCtIP plays a minimal role in determining GCT length. Moreover, to assess the function of *DmCtIP* on both short (500 bp) and long (~3.5 kb) end resection, we implemented modified homology-directed single-strand annealing DSB repair assays. Through these analyses, we will affirm the importance of end resection on DSB repair pathway choice in multicellular systems, as well as describe the function of *DmCtIP* in short and extensive DNA end resection during homologous recombination.

491B Genome-wide association studies identified RNA-binding protein Syncrip in regulating Starvation-Induced Hyperactivity in adult *Drosophila*

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Animals living in the wild often face periods of starvation. How to physiologically and behaviorally responds to starvation is essential for survival. One prominent behavioral response is Starvation-Induced Hyperactivity (SIH). SIH is paradoxical as it promotes food-seeking but also increases energy expenditure. Despite its importance in regulating fitness, genetic contributions to SIH as a behavioral trait have not been explored. Here, we examined SIH in the *Drosophila melanogaster* Genetic Reference Panel (DGRP) and performed genome-wide association studies. We identified 23 significant loci, corresponding to 14 genes, significantly associated with SIH in adult *Drosophila*. Gene enrichment analyses indicated that gene encoding mRNA binding proteins (RBPs) were most enriched in SIH. Using RNA interference, we validated the role of *Syp* in regulating SIH. *Syp* encodes Syncrip, and RBP. While ubiquitous knockdown of *Syp* led to lethality during development, adult flies with neuron-specific *Syp* knockdown were viable and exhibited decreased SIH. Using the Temporal and Regional Gene Expression Targeting (TARGET) system, we further confirmed the role of *Syp* in adult neurons in regulating SIH. Using RNA sequencing, we found that *Syp* was alternatively spliced under starvation while its expression level was unchanged. Lastly, exon-specific knockout using CRISPR/Cas9 revealed that two exons in *Syp* are critical to SIH. Together, this study not only demonstrates genetic contributions to SIH as an important behavioral trait but also highlights the significance of RBPs and post-transcriptional processes in the brain in regulating behavioral responses to starvation.

492C Identifying genes and proteins that respond to Vitamin A deprivation in the eye

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Vitamin A is essential for vision and must be obtained through our diet. It forms retinal, a component of the light-sensing Rhodopsin. Vitamin A deprivation (VAD) severely disrupts the morphology of the photoreceptors, causes loss of the light-sensing pigments, and is the leading cause of preventable childhood blindness. However, it is poorly understood how the eye responds to VAD at the molecular level. Using the *Drosophila melanogaster* retina as model, we aim to obtain fundamental insights into the molecular changes in the vitamin A deprived eye and identify factors that respond to vitamin A deprivation to stabilize damaged photoreceptors.
We raised flies on vitamin A-rich food (vitA+, contains beta-carotene as vitamin A source) or vitamin A-depleted food (vitA-). Using immunohistochemistry and confocal microscopy, we observed that VAD causes significant reduction in the size of the photoreceptors and defective opsin expression. However, we did not observe any photoreceptor death. We therefore hypothesized that protective factors are upregulated during VAD to stabilize *Drosophila* photoreceptors. As a complementary approach to our previous identification of VAD-responsive proteins using mass-spectrometry, we performed RNA sequencing of total RNA from heads of wild-type flies. EdgeR analysis yielded 68 differentially expressed genes (DEGs) between vitA+ and vitA- conditions. Our first goal was to identify protective factors and consistent with the proteome analysis, we detected a six-fold increase in the transcript levels of the novel gene *mps* upon VAD. Our validation experiments with *mps* null mutant, revealed that the transmembrane protein Mps is upregulated in damaged photoreceptors and stabilizes them. Secondly, we wanted to determine feedback mechanisms on vitamin A responsive DEGs which might promote proper structure and function

of photoreceptors upon VAD. Gene Ontology analysis on the DEGs identified 'phototransduction' as an enriched category and included genes that encode factors that inhibit Rhodopsin signaling (*Arr1, Arr2*), Rhodopsin processing enzymes (*ninaB, ninaG),* cation channels (*trpl*), and a novel lipid phosphate phosphatase (*CG11426*). This suggest that there are novel vitamin A-dependent feedback mechanisms that maintain proper Rhodopsin signaling.
Taken together, we found that in addition to upregulation of novel protective molecules during VAD, important genes related to the phototransduction pathway are also regulated via transcriptional feedback to overcome vitamin A deprivation stress.

493A *Drosophila* Hox genes induce melanized pseudo-tumors when misexpressed in hemocytes

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Hox genes are early determinants of cell identity along the anterior-posterior body axis across bilaterians. Several late non-homeotic functions of Hox genes have emerged in a variety of processes involved in organogenesis in several organisms, including mammals. Several studies have reported the misexpression of Hox genes in a variety of malignancies including acute myeloid leukemia. The Hox genes *Dfd, Ubx, abd-A* and *Abd-B* were overexpressed via the UAS-Gal4 system using *Cg-Gal4, Lsp2-Gal4, He-Gal4* and *HmlD3-Gal4* as specific drivers. Genetic interaction was tested by bringing overexpression lines in heterozygous mutant backgrounds of Polycomb and trithorax group factors. Larvae were visually scored for melanized bodies. Circulating hemocytes were quantified and tested for differentiation. Pupal lethality was assessed. Expression of *Dfd, Ubx* and *abd-A,* but not *Abd-B* in the hematopoietic compartment of *Drosophila* led to the appearance of circulating melanized bodies, an increase in cell number, cell-autonomous proliferation, and differentiation of hemocytes. Pupal lethality and melanized pseudo-tumors were suppressed in Psc^{1} and esc^{2} backgrounds while polycomb group member mutations Pc^{1} and $Su(z)12^{3}$ and trithorax group member mutation $TrlR^{85}$ enhanced the phenotype. *Dfd, Ubx* and *abd-A* are leukemogenic. Mutations in Polycomb and trithorax group members modulate the leukemogenic phenotype. Our RNAseq of *Cg-Gal4>UAS-abd-A* hemocytes may contain genes important to Hox gene induced leukemias.

494B Population variation of piRNA expression among *D. melanogaster* strains reveals rapid divergence in piRNA cluster activity

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Transposable elements (TEs) pose a significant threat to host genome integrity, especially in the animal germline. Consequently, host organisms have evolved mechanisms to regulate TE activity. The piRNA pathway in the animal germline regulates TEs transcriptionally and post-transcriptionally by small RNAs produced from discrete loci in the genome called piRNA clusters. This pathway is proposed to be adaptive in response to the changing landscape of TE threats. Adaptive signatures in the piRNA pathway effector proteins have been well documented. Another proposed adaptive mechanism is bias in piRNA amplification toward active TE families. While the former has been a topic of extensive research in *Drosophila melanogaster*, the latter is under-studied due to inaccessibility of highly repetitive piRNA clusters. Here we use the long-read genome assemblies of 10 highly inbred *D. melanogaster* strains of distinct geographical origins and sequenced small RNA libraries from their ovaries. We identify piRNA clusters *de novo* for each strain and characterize piRNA expression, sequence, and structural variation among TEs residing in and out of piRNA clusters. This revealed a rich description of strain-specific TE insertions and piRNA expression profiles. We also found that ovarian piRNA clusters exhibit significant variation in their genomic location, TE content, and expression between strains at the family level. Such plasticity in expression of piRNA clusters had not previously been characterized. We hypothesize that such strain-specific piRNA cluster activity is driven by the strain-specific TE insertion landscape, and speculate that differences in TE expression among strains results in piRNA cluster divergence among *D. melanogaster* strains. We suggest specifically that differential TE expression among strains leads to a strain-specific Ago3-bound sense piRNA pool, which leads to differences in processivity of piRNA precursors, thereby altering the mature anti-sense piRNA pool. This altered anti-sense piRNA pool, when maternally inherited, leads to increased expression of *de novo* piRNA clusters and decreased expression of ancestral clusters. Such Ago3-guided tuning of piRNA cluster expression may serve as an adaptive response to the ever-changing TE expression landscape in the animal germline.

495C Breakpoint Evolution in Inversions of *Drosophila pseudoobscura*

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Inversions are caused by breaking chromosomes in two locations and then rejoining the central region of the chromosomes in reverse order. Inversions have been shown to cause disease in humans when the inversion disrupts genes, but there are documented cases of naturally occurring inversions in humans without deleterious effects. In *Drosophila*, inversions do not have these negative effects because males do not recombine during gamete formation and in females' aberrant meiotic

products are lost in polar bodies. As a result, fruit fly populations can harbor extensive inversion variation within populations. Recent studies have shown that chromosomes have a stereotypic 3D architecture in the nucleus organized into membrane-less chromatin compartments. In *Drosophila pseudoobscura* it is unknown whether the inversion breakpoints occur within or between chromatin compartments. One of the challenges of testing this hypothesis is that the presence of repeats has prevented the genome assembly of sequences in breakpoint regions. We are using long-read sequencing approaches to determine breakpoint sequences in *D. pseudoobscura* because this species has numerous inversions on the third chromosome that makes it a great model system for looking at breakpoints relative to chromatin structure. Understanding the sequences within the breakpoints will provide a framework for the functional genetics of *D. pseudoobscura*. We used Oxford nanopore technology to assemble the Tree Line sequence. Tree Line is four inversion steps from the Arrowhead reference genome sequence and a comparison of Tree Line to Arrowhead will allow us to capture four pairs of inversion breakpoints relative to the chromatin compartments. Long read technology allows us to sequence through the repeats so that we can understand the mechanisms that generate inversions and the constraints on breakpoint location imposed by 3D chromatin architecture. This research will allow us to understand the repeat structure in breakpoint regions and to determine if the inversion breakpoints occur within or between chromatin compartments.

496A A novel role for CRTC linking age-related cardiac dysfunction and remodeling to metabolic syndrome in Drosophila heart model.

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Age-related pathological cardiac remodeling impacts quality of life by promoting cardiac arrhythmias. The cellular and molecular links between cardiac remodeling and arrhythmia are unknown. Drosophila melanogaster is used as an evolutionarily conserved genetic model system to identify novel genetic pathways involved in vertebrate heart development as well as genetic causes of cardiac disease and aging. For example, flies with sei/hERG K+ channel mutants or cardiac-specific KD exhibit arrhythmias that worsen with age. In humans, hERG is a target for cardiac therapeutic interventions. In flies, sei/hERG deficiency also causes extensive myofibrillar structural remodeling and fibrosis. Hence, despite the physiological and structural differences between fly and human hearts, gene networks regulating cardiac structure and function remain highly conserved. I propose to use this fly model of cardiac arrhythmia to identify and characterize sei/hERG genetic interactors. I hypothesize that one such gene, CREB Regulated Transcription Coactivator (CRTC), a nutrient sensor, is a potential link between metabolic dysfunction and hypertrophic/fibrotic cardiomyopathies. CRTC is a key regulator of metabolism in the mammalian liver and appears to be permanently active in diabetes. CRTC's role in the heart remains largely unknown. In hepatocytes, insulin inhibits CRTC by sequestrating it in the cytoplasm via binding to 14-3-3 proteins. hERG has also been shown to bind 14-3-3 proteins. Our preliminary data from the fly show dramatic effects of systemic and cardiac-specific CRTC KD on heart size, contractility and cardiac fibrosis. I have recently identified two new hERG interacting proteins, Zyxin and Ajuba, both from the same family and involved in Hippo pathway regulation. Loss of Ajuba and Zyxin causes excessive cardiac fibrosis and severe myofibrillar disorganization. I hypothesize that these proteins interact providing a potential link between metabolic dysfunction and hypertrophic/fibrotic cardiomyopathies. I propose to characterize these interactions by cardiac-specific genetic manipulation of these genes expression in "sensitized" sei/hERG or CRTC heterozygous backgrounds. I will use a high-throughput assay employing a cardiac-specific fluorescent reporter to conduct in situ and in vivo cardiac functional analyses in Drosophila. Progeny will be filmed at 2 and 4 weeks to identify those interactions that affect cardiac function in an aging-dependent manner. Flies will be also dissected to visualize effects on sarcomeric and fibrotic structural proteins. Using Zebrafish I will validate the potential hERG interactors, identified in Drosophila, in a vertebrate model system. My studies will identify connections between cardiomyopathies and metabolic dysfunction to identify new potential drug targets and therapies for heart diseases.

497B Identification of candidate Atrial Fibrillation gene interactions using a multi-model system approach

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Atrial fibrillation (AF), the most common heart rhythm disorder, affects more than 33 million individuals. Although progress has been made in understanding the genetics underlying AF, this asymptomatic disorder continues to serve as the leading cause of heart failure and stroke in aging humans. Meta-analyses of GWAS have identified over 150 common and rare genetic variants that increase AF susceptibility, suggesting that the underlying cause is multifactorial, involving networks of interacting genes. A barrier to understanding AF mechanisms is the lack of experimental platforms for rapid large-scale exploration of gene function in cardiac tissue. To overcome this, we utilize a multi-platform approach encompassing the genetically tractable *Drosophila melanogaster* cardiac-aging model and hIPSC-atrial-like cardiomyocyte (ACM) model. The fly model allows for high-throughput quantification of aging and diet effects in conjunction with genetic insult, whereas the ACM model allows for high-throughput

combinatorial gene knockdown. The ACM system allows for the validation of *Drosophila* phenotypes, solidifying a connection between fly and human genetics. High-speed imaging of ACMs and fly hearts permit quantification of cardiac parameters such as action potential duration and contraction rhythm, as well as diastolic/systolic intervals and arrhythmia, respectively. I performed an initial screen of 20 AF candidate genes in the fly and ACM model systems, identifying a novel network centered around KCNA5, an atria-specific potassium ion channel. I hypothesize that a subset of genes in this KCNA5 network interact to produce arrhythmia that are exacerbated by diet and/or age. We have phenotyped cardiac function across multiple ages and high caloric diets to observe how genes within this subnetwork interact to promote arrhythmia or mitigate cardiac dysfunction. Additionally, we also characterized potential morphological (muscle organization and fibrosis) and metabolic (triglycerides and protein content) responses to the genetic and dietary insults. The novelty and significance of this experiment lies in the ability to functionally assess specific genetic interactions between AF candidate genes in an aging-dependent manner across multiple platforms, with the intention to delineate conserved arrhythmogenic cardiac signaling pathways, identifying specific genetic/molecular/metabolic interactions underlying age-related cardiac arrhythmias.

498C The Forkhead/Fox transcription factor Jumeau mediates specific cardiac progenitor cell divisions by regulating the expression of the kinesin Nebbish

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Forkhead (Fkh/Fox) domain transcription factors (TFs) mediate multiple cardiogenic processes in both mammals and *Drosophila*. We showed previously that the *Drosophila* Fox gene *jumeau* (*jumu*) controls three categories of cardiac progenitor cell division—asymmetric, symmetric, and cell division at an earlier stage—by regulating Polo kinase activity, and mediates the latter two categories in concert with the TF Myb. Those observations raised the question of whether other *jumu*-regulated genes also mediate all three categories of cardiac progenitor cell division or a subset thereof. By comparing microarray-based expression profiles of wild-type and *jumu* loss-of-function mesodermal cells, we identified *nebbish* (*neb*), a kinesin-encoding gene activated by *jumu*. Phenotypic analysis shows that *neb* is required for only two categories of *jumu*-regulated cardiac progenitor cell division: symmetric and cell division at an earlier stage. Synergistic genetic interactions between *neb*, *jumu*, *Myb*, and *polo* and the rescue of a subset of *jumu* mutant phenotypes by ectopic cardiac mesoderm-specific expression of *neb* demonstrate that *neb* is an integral component of a *jumu*-regulated subnetwork mediating cardiac progenitor cell divisions. Our results emphasize the central role of Fox TFs in cardiogenesis and illustrate how a single TF can utilize different combinations of other regulators and downstream effectors to control distinct developmental processes.

499A Model system functional analysis of RPS15A suggests a growth-related role for ribosomal genes in Congenital Heart Disease

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Hypoplastic Left Heart Syndrome (HLHS) represents the most lethal Congenital Heart Disease (CHD) and is characterized by a severely underdeveloped left ventricle. HLHS is likely of oligogenic origin, however, the causal genes and disease mechanisms are poorly understood.

Whole genome sequencing was performed in a familial HLHS case and a cohort of 25 HLHS proband-parent trios with poor clinical outcome. Segregation analysis in the familial case identified a rare promoter variant affecting the ribosomal protein (RP) *RPS15A*. Supporting a biological impact, patient-derived iPSC-cardiomyocyte (CM) proliferation and *RPS15A* expression was reduced compared to the parents. Candidate genes identified by Mendelian modeling in the 25 trios were subject to gene network enrichment analysis, revealing an over-representation of RP genes.

Functional testing identified RPs as regulators of cardiac growth and cardiomyocyte proliferation, thus potentially contributing to hypoplasticity in HLHS. Knockdown of RPs reduced proliferation in generic hiPSC-CMs and impaired cardiac differentiation in *Drosophila* resulting in a partial or 'no heart' phenotype. Functional validation in zebrafish showed that *rps15a* knockdown causes reduced cardiomyocyte numbers, diminished heart looping and contractility, without affecting overall embryonic development.

Testing for cardiac-specific RP functions, we found synergistic interactions between *RPS15A* and *tinman* or *Nkx2-7* in flies and zebrafish, respectively. Furthermore, *RPS15A* knockdown-induced defects were significantly reversed by *p53* knockdown in

hiPSC-CMs and zebrafish and by Hippo activation in flies. Based on these findings, we conclude that RP genes play a critical role in cardiogenesis and are candidates for a novel class of genetic effectors in CHDs, such as HLHS.

500B Forkhead/Fox transcription factors mediate proper positioning of cardiac cells by restricting the expression of ECM genes

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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/Fox 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. Statistical analysis demonstrated that these positioning defects cannot be completely explained by steric constraints caused by differing number of cardial cells in contralateral hemisgments due to cell division defects. In order to discover the other cause underlying positioning defects, we compared genome-wide transcription expression profiles of purified mesodermal cells from wild-type embryos and Fox mutants to identify Fox-regulated targets. Among the 2,131 target genes we identified, genes encoding extracellular matrix (ECM) proteins were overrepresented among genes repressed by the Fox TFs. In particular, the basement membrane (BM) proteins Pericardin, Multiplexin, Viking, Collagen type IV alpha 1, Terribly reduced optic lobes and Glutactin were all overexpressed in Fox mutants. Our preliminary phenotypic analysis of a subset of these specific targets suggests that the Fox TFs bring about the correct positioning of cardiac cell types by restricting their expression: ectopic overexpression of each of these BM genes in the mesoderm phenocopies the cardiac cell positioning defects observed in *CHES-1-like* and *jumu* loss-of-function mutants.

501C Characterization of membrane markers for localization-based super-resolution imaging of filopodia at the neuromuscular junction in *Drosophila*

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The precision of partner recognition is crucial to the formation of synapses in a specific manner for normal neurodevelopment. The stereotypical connectivity of the neuromuscular junction (NMJ) in *Drosophila* presents great opportunities for studying synaptic development. Throughout synaptogenesis at the NMJ, the filopodia of the growth cone interact with those of the muscle and go through distinct morphological transitions. To investigate these transitions, we use localization-based super-resolution imaging. In this study, we identify useful fluorescent probes and evaluate approaches for membrane labeling, fixation, refractive index matching in dissected embryos for optimal 2-color super-resolution imaging. With these tools, we can precisely characterize morphological changes in the synaptic partners throughout development. Altogether, our tools create opportunities for further studies of the different molecular machinery of the neurons, muscles, or NMJ during different stages of growth by 2-color localization-based super-resolution microscopy.

502A Cell-type-specific, multi-color labeling of endogenous proteins with split fluorescent protein tags in *Drosophila*

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The impact of the *Drosophila* experimental system on studies of modern biology cannot be overstated. The ability to tag endogenously expressed proteins is essential to maximize the utility of this model organism. Here, we describe a method for labeling endogenous proteins with self-complementing split fluorescent proteins (split FPs) in a cell-type-specific manner in *Drosophila*. A short fragment of FP coding sequence is inserted into a specific genomic locus whereas, the remainder of the FP is expressed using an available GAL4 driver line. In consequence, complementation fluorescence allows examination of protein localization in particular cells. Furthermore, when inserting a tandem array of multiple copies of the short FP fragment at the same genomic locus, we can substantially enhance the fluorescence signal. The enhanced signal is of great value in live-cell imaging at the sub-cellular level. A multiplexed labeling system can be accomplished with orthogonal split FPs. However, besides split GFP, other orthogonal split FPs do not function for *in vivo* imaging. Through protein engineering and *in vivo* functional tests, we report the first red split FP that can be used for multiplexed visualization of endogenous proteins in complex *Drosophila* tissues. By using the two orthogonal split FP systems, we have simultaneously imaged two proteins

that reside in distinct subsynaptic compartments. Our approach allows for the first time to study the proximity between and localization of multiple proteins endogenously expressed in essentially any cell-type in *Drosophila*.

503B Transcriptional Mechanisms Controlling Immune Priming in *Drosophila melanogaster*

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Studying the way organisms fight off infections is a universally useful endeavor: anything that is alive has the possibility of getting sick. The current paradigm in immunology focuses on the binary distinction between the innate and adaptive immune systems. However, this binary convention has been recently challenged by the reporting of a primed innate immune response in organisms both with and without an adaptive immune system. This so termed "immune priming" helps organisms fight off a second infection after survival of an initial infection. Despite the wide use of immune priming in diverse organisms, including plants, insects, and mammals, the bulk of the mechanistic work on this phenomenon used cell-based mammalian studies. We work with the fruit fly *Drosophila melanogaster* to create a powerful*, in vivo* model for studying epigenetic control of immune priming. By infecting flies with an insect-derived strain of the Gram-positive bacterium *Enterococcus faecalis*, we modeled infection response and discovered enhanced survival in immune-primed cohorts. We also tracked bacterial load over time and found preliminary evidence showing that the enhanced survival in primed cohorts does not correlate with differences in bacterial load. Using RNA-seq, we have tracked transcriptomic changes associated with immune priming in the primary immune organ of *D. melanogaster*, the fat body. Using differential expression analysis, we classified families of genes that remain activated throughout immune response, activate faster upon re-infection, or are qualitatively unique to a primed immune response. We then paired these gene expression patterns with our survival and bacterial load data to get a complete picture of how the animal is changing its infection response after priming. Future experiments will then focus on epigenetic elements driving these gene expression trends by probing the fat body with ATAC-seq and scanning areas of the genome that undergo chromatin accessibility changes. By eventually integrating gene expression with sites in these remodeling events, we will begin shortlisting regulatory elements that may be driving primed immune response. In this way, we see a concerted mechanism explaining immune priming in the fly.

504C Investigating the immediate effects of high sugar diets on immune defense in *Drosophila*

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The nutritional status of an organism has a direct impact on its resistance to infection. *Drosophila melanogaster* reared on high sugar diets experience adverse outcomes from bacterial infection including higher bacterial burdens and higher mortality. Despite clear knowledge that nutrition is important for immune defense, we have little understanding of the mechanisms by which nutrition impacts infection outcome. Prior studies that have investigated the impact of high sugar diets on infection outcome have generally been performed on flies developed from an embryo to adult on a high sugar medium, which makes it difficult to distinguish the developmental effects of diet from the immediate effects of diet on metabolic physiology. Here, we assess the immediate effects of a high sugar diet on immune defense in flies. We hypothesized that when transferred to a high sugar diet as an adult, flies will experience higher mortality and higher pathogen burden compared to flies fed on low sugar diets. We tested this by first rearing flies on a standard diet, and, upon eclosion, transferring them to one of four experimental diets that varied in their caloric content, protein:carbohydrate ratios, and concentration of sucrose. After 3-5 days of being fed on one of the four experimental diets, we infected the flies with *Serratia marcescens* and measured infection survival over five days and pathogen burden at 18 hours post-infection. Flies fed on the diet with the lowest sugar relative to protein had the highest survivorship and lowest pathogen burdens. These results suggest that immediate metabolic physiology is a major determinant of dietary impact on resistance to infection and lay the foundation for our future work on understanding the mechanisms by which high sugar diets impact infection outcome.

505A Progenitor-specific IMD activity promotes intestinal growth and secretory cell differentiation

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Upon recognition of bacterial peptidoglycan, the immune deficiency (IMD) pathway activates NF-κB-family transcription factor Relish and is required for effective antimicrobial response in the *Drosophila* intestine. In addition to immune functions, IMD and Relish have important cell-type specific roles in intestinal homeostasis. For instance, IMD/Relish is required in enterocytes for delamination in response to infectious microbes and genetic hyperactivation of IMD in progenitors promotes intestinal dysplasia and tumorigenesis. Given the close proximity of progenitors to microbial agents in the intestine, and that proliferation is required to survive most bacterial infections, progenitor-specific IMD is a potential mechanism to adapt

to bacterial stimuli in the intestine. However, it is still unclear if IMD is activated in progenitors in response to bacteria and whether physiological levels of progenitor-specific IMD or Relish activity impact intestinal proliferation or differentiation. To determine if IMD is active in progenitors we characterized expression of IMD components and target genes in progenitors in response to commensal bacteria. We found that in wild-type intestines, expression of IMD components such as Relish are enriched in progenitors compared to other intestinal cell types, and that commensal bacteria induce expression of downstream IMD target genes in progenitors. These findings suggest that the microbiome activates IMD in progenitors. To determine if IMD is required for progenitor function, we inhibited IMD specifically in progenitors and measured intestinal growth and differentiation. We found that IMD inhibition or knockdown of Relish in progenitors reduces intestinal proliferation and inhibits age-dependant dysplasia. In addition, progenitor-specific IMD inhibition increases enterocyte precursor numbers and decreases enteroendocrine cell numbers. Together, these data suggest that commensal bacteria induce IMD activity in progenitors to promote proliferation and secretory cell differentiation in the intestine. Our observations highlight the importance of immune signalling within progenitor cells under homeostasis and provide insight into how chronic immune activation contributes to intestinal dysplasia and tumorigenesis.

506B A single mating triggers persistent suppression of *Drosophila melanogaster* female immune defense

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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 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 could eventually recover virgin levels of immunity after much longer times. 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 therefore conclude that mating has a permanent suppressive effect on the female immune system. Knowing that females mate multiply, we hypothesized that a second mating might have compounding negative impact on immune performance and that females who mated twice might become more susceptible to infection than females who mated only once. However, we found that females mated either once or twice before infection survived at equal proportions, both significantly lower than virgin females. Thus, a single mating is sufficient to fully suppress the immune response to a persistent lower state. During mating, the male transfers seminal fluid proteins that change female physiology and behavior, including Sex Peptide. Sex Peptide induces the female to produce Juvenile Hormone (JH), which is immunosuppressive and promotes egg development through stimulating production of Yolk Proteins. Our ongoing experiments include deletion of *yolk protein* genes via CRISPR-Cas9-mediated genome editing to test whether the costs of producing Yolk Protein directly limit immune capacity.

507C Natural Genetic Variation in *Drosophila melanogaster* Reveals Genes Associated with *Coxiella burnetii* Infection

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The gram-negative bacterium *Coxiella burnetii* is the causative agent of Query (Q) fever in humans and coxiellosis in livestock. Host genetics are associated with *Coxiella burnetii* pathogenesis both in humans and animals; however, it remains unknown if specific genes are associated with severity of infection. We employed the *Drosophila* Genetics Reference Panel to perform a genome-wide association study to identify host genetic variants that affect host survival to *Coxiella burnetii* infection. The genome-wide association study identified 64 unique variants ($P < 10^{-5}$) associated with 25 candidate genes. We examined the role each candidate gene contributes to host survival during *Coxiella burnetii* infection using flies carrying a null mutation or RNAi knockdown of each candidate. We validated 15 of the 25 candidate genes using at least one method. This is the first report establishing involvement of many of these genes or their homologs with *Coxiella burnetii* susceptibility in any system. Among the validated genes, *FER* and *tara* play roles in the JAK/STAT, JNK, and decapentaplegic/TGF-β signaling pathways which are components of known innate immune responses to *Coxiella burnetii* infection. *CG42673* and *DIP-ε* play roles in bacterial infection and synaptic signaling but have no previous association with *Coxiella burnetii* pathogenesis. Furthermore, since the mammalian ortholog of *CG13404* (*PLGRKT*) is an important regulator of macrophage function, *CG13404* could play

a role in host susceptibility to *Coxiella burnetii* through hemocyte regulation. These insights provide a foundation for further investigation regarding the genetics of *Coxiella burnetii* susceptibility across a wide variety of hosts.

508A The BaramicinA gene is required at several steps of the host defense against *Enterococcus faecalis* and *Metarhizium robertsii* in a septic wound infection model in *Drosophila melanogaster*

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The host defense against several Gram-positive bacterial and fungal infections is mostly mediated by the Toll pathway in *Drosophila*, which regulates the expression of multiple genes including effectors of the innate immune response. One such potential effector is IMPPP/BaraA, a precursor protein that is processed at furin cleavage sites into an armamentarium of small DIM peptides that display a high degree of sequence similarity. We report here the generation of multiple mutants affecting this gene. Our phenotypic analysis revealed a specific sensitivity to pathogens belonging to distinct kingdoms, the Gram-positive bacterium *Enterococcus faecalis* and the entomopathogenic fungus *Metarhizium robertsii*, only in septic injury models of infection. Unexpectedly, we failed to reveal a consistently increased microbial burden in the mutant flies infected with either of these microorganisms, opening the possibility for a role of BaraA in resilience rather than in resistance, which we were however unable to confirm. We also found that some BaraA-derived DIM peptides display an antimicrobial activity at millimolar concentrations *in vitro*. BaraA is additionally required for an efficient cleavage of pro-phenol oxidase into an active enzyme. BaraA is also involved in the cellular host defense, but through distinct mechanisms: it needs to be expressed in hemocytes for an efficient response solely to *E. faecalis* infection whereas it is required for the uptake by plasmatocytes of *M. robertsii* conidia. We propose a model whereby BaraA secreted by hemocytes may act at a very short range and protect host tissues or organs targeted specifically by *E. faecalis*. This study thus reveals an unexpected functional complexity of a potential effector of the Toll pathway in the host defense against specific infections.

509B Elucidating the biological roles of ADAR RNA editing/modification enzymes

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Mutations in the *ADAR* gene encoding ADAR1 cause the virus infection mimic, autoinflammatory disorder Aicardi Goutières syndrome 6, with aberrant expression of type-I IFN and IFN stimulated gene (ISGs) transcripts. In *Adar* mutant mice the antiviral dsRNA sensor Mda5 is aberrantly activated by unedited endogenous dsRNA. *Adar, Mavs* double mutant mice lacking the adaptor for Mda5-mediated interferon activation, avoid *Adar* mutant aberrant interferon expression and embryonic lethality. In ADAR1 projects,;*Adar, Mavs* double mutants which still die either as fetuses or within days or weeks of birth and we are testing whether adding a third mutation removing the dsRNA-activated protein kinase R (PKR) completely prevents *Adar, Mavs* fetal and pupal lethality. In ADAR2 projects, we are currently investigating infant epilepsy mutations in *ADARB1* to understand their effects on ADAR2 RNA editing in CNS.

In *Drosophila* the single *Adar* gene on the X-chromosome encodes an orthologue of vertebrate *ADAR2*. *Adar* null mutant flies are locomotion-defective and have synaptic vesicle accumulations leading to an age-dependent vacuolar neurodegeneration. We screened for mutations that improve *Adar* null mutant viability at eclosion and found that reduced *Tor* dosage or increased canonical autophagy or endosomal microautophagy rescue all *Adar* mutant defects. We also recently showed that *Adar* mutant flies have an aberrant innate immune response in heads which is partly editing-independent. Flies lack homologs of the mammalian RIG-I-like antiviral cytoplasmic dsRNA sensor helicases (RIG-I-like receptors, RLRs) that mediate aberrant interferon expression and induction of interferon stimulated genes (ISGs) in *Adar1* mutant mice. The aberrant innate immune induction in *Adar* mutant flies is mediated through Dicer-2, which has a helicase domain related to the RLRs ; the Dicer-2 and RIG-I-like helicases probably act as translocases that scan along dsRNA to discriminate between imperfect or modified self dsRNA and foreign, viral dsRNA with perfect strand pairing.

510C 2'3'-cGAMP triggers a STING and NF-κB dependent broad antiviral response in Drosophila

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Stimulator of interferon genes (STING) is a conserved cyclic dinucleotide sensor, fundamental for triggering the mammalian interferon response against DNA viruses. In mammals, STING is activated by the cyclic dinucleotide 2'3'-cGAMP produced by cGAS, which acts as a receptor for cytosolic DNA. We previously shown that in *Drosophila melanogaster*, which is devoid of interferons, the ortholog of STING (dSTING), regulates infection by picorna-like viruses. However, it is still unknown how dSTING is activated in vivo and what is the transcriptional program it regulates. Here, we show that injection of 2'3'-cGAMP in flies is sufficient to trigger the expression of previously identified dSTING-regulated genes and to induce a broad antiviral protection against a panel of RNA and DNA virus. This 2'3'-cGAMP-mediated protection is independent of the autophagy and small interfering RNA pathways. By contrast, it is abrogated in flies mutant for the NF-κB transcription factor Relish. Analysis of the transcriptome of 2'3'-cGAMP injected flies revealed more than 400 induced transcripts, of which 80% have putative NF-κB *cis*-regulatory elements, in a complex pattern of response, with early and late induced genes. cGAMP induced antiviral protection is also conserved across multiple drosophila species. Our results reveal that dSTING regulates an ancient NF-κB -dependent antiviral program predating the emergence of interferons in vertebrates.

511A The *Drosophila* antimicrobial peptide, Diptericin A, plays a role in both immunity and microbiome composition

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The innate immune system is rapidly triggered by an array of pathogens, making it an effective first line of defense in animals. However, it must strike a delicate balance between host defense and maintenance of microbiome composition. Too strong an immune response in the gut could wipe out beneficial microbes, while too weak a response could fail to suppress harmful gut microbes or systemic infection. In *Drosophila*, innate immune effector genes called antimicrobial peptides mediate both immune response to systemic infection and microbiome composition. Here, we characterize the *Drosophila* antimicrobial peptide Diptericin A (DptA) and its roles in host defense and microbiome maintenance. In *D. melanogaster*, a single amino acid polymorphism in mature DptA (S92R) substantially affects immune phenotype: flies homozygous for the serine allele (SS) exhibit higher survival following systemic infection with the bacterium *Providencia rettgeri* than those homozygous for the arginine allele (RR). Interestingly, this polymorphism appears to be under balancing selection. We hypothesize that the arginine and serine alleles play important roles in gut immunity and systemic immunity, respectively. To investigate, we cultured microbes from whole-fly homogenate from CRISPR genome edited flies (SS, RR, SR) to compare the number of colony forming units and bacterial species associated with each genotype. We observed an association between DptA genotype and microbiome composition. In addition, we used bacterial isolates from conventionally reared flies to determine the effects of these bacteria in gnotobiotic flies. We found bacterial load 10 days post feeding of bacteria is dependent on DptA genotype. We also analyzed the microbiomes of CRISPR genome edited lab-reared flies and wild-caught flies of each genotype (SS, RR, SR) using 16S metagenomic sequencing. Finally, we compared the longevity of axenically and conventionally reared flies of each genotype to evaluate how interactions between DptA and the microbiome affect lifespan. Interestingly homozygous arginine flies survive significantly longer than homozygous serine flies (in the absence of infection), but this difference disappears when flies are reared axenically. These results will help determine if life history tradeoffs between effective systemic immune response and maintenance of the gut microbiome play a role in maintaining genetic variation in immune genes.

512B Suppression of glial immune system-mediated neurodegeneration suppresses seizures in *prickle* mutants

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Epilepsy is a neurological disorder characterized by seizures and affects ~1% of the population. Our laboratory has previously shown that mutations in *prickle* (*pk*), a planar cell polarity gene, cause myoclonic-like seizures and ataxia in *Drosophila*, similar to what is observed in humans carrying mutations in orthologous *PRICKLE* genes. We have previously reported that transcriptome and gene ontology (GO) analysis of *pk* mutant brains revealed a significant upregulation of innate immune response genes when compared to controls, and that *pk* mutants exhibit a continual accumulation of degenerating neurons as well as exacerbation of the seizure activity across adulthood. Using genetic, behavioral and immunohistochemical methods, we also reported that genetic suppression of the innate immune response specifically in the glial cells of the *pk* mutant brains leads to a significant reduction in both neurodegeneration as well as seizure activity, directly demonstrating for the first time that the glial-based innate immune response plays a crucial role in seizure generation. Given that the neurodegeneration is a

downstream event of the robust innate immune system activation in our *pk* mutants, we next sought to determine whether the neurodegeneration was the specific cause of the seizure exacerbation. We now show that neuronal overexpression of *DIAP1* (an inhibitor of apoptosis) in the *pk* mutants not only leads to a significant reduction in neurodegeneration, but also promotes strong suppression of seizure activity. Collectively these results uncover a new pathway to seizure generation whereby dysfunctional neuronal function leads to glial innate immune response activation, thus promoting apoptosis that in turn leads to an increase in seizure activity. This work is supported by NIH/NINDS R01NS098590.

513C Exploring the role of thoracic injury in infection response and outcomes in *Drosophila melanogaster*

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Thoracic infections and abdominal infections have been reported to result in very different outcomes in *D. melanogaster*. Flies infected in the thorax succumb to infections in greater proportion than those infected in the abdomen. An aseptic injury to the thorax in combination with injection of bacteria into the abdomen is sufficient to cause mortality equivalent to a thoracic infection, although a sterile thoracic injury alone causes no mortality. This indicates that death is due to the combination of circulating bacteria and injury to the thorax. Several muscle genes such as *Actin88F, Tropomyocin2, held up* are upregulated in flies that sustain injuries specifically to the thorax. Down-regulation of *Hep*, which encodes JNK kinase, prevents upregulation of the muscle genes after thoracic injury. We hypothesize that the JNK pathway is activated when thoracic muscle is damaged, but not during abdominal injury, and that JNK activation contributes to host mortality. We are testing this hypothesis by measuring expression of genes such as *puc*, a negative regulator of JNK signaling and *Kenny,* a subunit of the *AP1* transcription factor of the JNK pathway, and by determining whether genetic manipulation of the JNK pathway improves or worsens outcomes of thoracic infections.

514A The limits of chronic infection induced protection during secondary infection in *Drosophila melanogaster*

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Chronic infections induced by injection of bacteria into *Drosophila melanogaster* are generally protective against future lethal bacterial infections across diverse combinations of infections. Sometimes this protection is dramatic and in particular the protective effect of *S. marcescens* is especially strong. For example, while typically 100% of flies infected with *Providencia sneebia* die two-days post-infection even at very small infectious doses, we see survival of 30% of flies infected with *P. sneebia* if they were carrying a *S. marcescens* chronic infection.
Chronically infected flies also exhibit upregulation of antimicrobial peptide gene expression and we hypothesize that this is responsible for the improved ability to control normally lethal infections. In other bacterial infections in *D. melanogaster*, antimicrobial peptide gene expression is correlated with the number of bacteria present. In addition, the number of bacteria present during a chronic infection is correlated with the infectious dose used to induce the chronic infection. Therefore we hypothesized that the protective effect of chronic infection during secondary infection would diminish as the dose used to induce chronic infection decreased. Supporting our hypothesis, initial data indicates that there is a minimum dose required to establish a protective chronic infection. In fact, any dose below this minimum dose results in worse survival for the doubly infected. This provides indirect support for the idea that it is antimicrobial peptides that are responsible for protection during secondary infection. Future work will modulate the strength of antimicrobial peptide induction using other techniques to further test the causal relationship between antimicrobial peptide expression and protection during secondary infection.

515B Systemic *Aspergillus fumigatus* infection reveals an unexpected, noncanonical, function of the Toll pathway in host defense against toxins

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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. As reported previously, *A. fumigatus* does kill *MyD88*, Toll pathway immunodeficient, flies but not wild-type flies. However, we observed in our injection model that the fungal burden hardly increases in the mutant flies, even upon death, in contrast to other fungal infections. Some 5 conidia suffice to kill *MyD88* flies in the absence of invasion of most fly tissues,

but still end up approximately with the same load when the flies died. 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, even though classical read-outs of Toll pathway activation are not induced. Therefore, the Toll pathway is not only a major pathway in the resistance against fungal infections but is also required for resilience against such infections by protecting the host from the action of pathogen-secreted toxins. This discovery highlights that evolution has selected mechanisms to elude, neutralize, or repair the damages exacted by toxins and has placed them under the control of a major innate immunity signaling pathway. Interestingly, only the Spz/Toll/MyD88/tube/pelle signaling cassette is required in host defense against *A. fumigatus*. We have identified effectors that partially mediate the Toll-dependent protection against toxin actions.

516C Essential functions of two Golgi phosphatidylinositol 4-kinases in *Drosophila*

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Phosphatidylinositol 4-kinases (PI4Ks) synthesize phosphatidylinositol 4-phosphate (PI4P), which binds and recruits factors required for post-Golgi trafficking. Mammals have four distinct PI4Ks, two type II (PI4KIIα and PI4KIIβ) and two type III (PI4KIIIα and PI4KIIIβ) enzymes, whereas flies and budding yeast each have a single type II enzyme and two type III enzymes. Budding yeast PI4KIIIβ (Pik1p) localizes to the Golgi and is essential for secretory trafficking. In contrast, PI4KII (Lsb6p) is non-essential and plays a role in endosome motility. We previously discovered that both PI4KIIIβ (Fwd) and PI4KII are non-essential in *Drosophila*, as the null mutants are viable. Fwd is required for spermatocyte cytokinesis and PI4KII is needed in salivary glands for maturation of regulated secretory granules. This led us to hypothesize that these enzymes might have an overlapping essential function in post-Golgi trafficking. Thus, the main objective of this study was to investigate potential redundancy of Fwd and PI4KII in *Drosophila*.
Here, we show that GFP-Fwd and endogenous or mCherry-tagged PI4KII localize near each other at the Golgi. *fwd PI4KII* double mutants are delayed in their development and die shortly after pupariation. In *fwd PI4KII* double mutant salivary glands, Golgi distribution of the PI4P binding effectors Rotini (GOLPH3) and oxysterol-binding protein (OSBP) is dramatically affected. Also, Golgi volume and post-Golgi trafficking are perturbed. *fwd PI4KII* double mutants show defects in insulin signaling due to Dilp2 retention in insulin-producing cells in the larval brain. The developmental delay is accompanied by reduced ecdysone signaling in prothoracic and salivary glands and can be rescued by feeding larvae with 20-hydroxyecdysone. Moreover, percent pupariation can be rescued by supplementation with 7-dehydrocholesterol, suggesting cholesterol deficiency is a primary defect in *fwd PI4KII* double mutants. Taken together, our results indicate that Fwd and PI4KII are redundant for synthesizing Golgi PI4P, which is essential for Golgi integrity and secretory trafficking in *Drosophila*.

517A The EMC is required for the biogenesis of the Tail Anchored Proteins Xport-A and fan

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The endoplasmic reticulum (ER) is the cellular site for biogenesis of membrane proteins, which contain transmembrane domains (TMDs) that are inserted into the lipid bilayer. Most membrane proteins are inserted co-translationally via the Sec61 translocon, but TMDs present an extensive variety of topological features that require multiple insertion pathways that remain to be fully defined.
The ER membrane complex (EMC) has recently been identified as an insertase for TMDs of a subset of tail anchored (TA) and polytopic membrane proteins, containing TMDs of low hydrophobicity. In order to identify EMC client proteins, we performed a prediction of TA proteins in the Drosophila proteome and identified around 250 TA proteins, from which we screened a subset of candidates and identified 2 novel EMC clients: fan, which controls sperm individualization and Xport-A. Interestingly, Xport-A is essential for the biogenesis of both rhodopsin-1 and TRP, whose biogenesis is also affected in EMC mutant cells. We made mutants of XportA that bypass EMC requirement and are able to rescue Rh1 and TRP defects in EMC mutants. Our results show that EMC may affect the biogenesis of polytopic membrane proteins indirectly, by controlling the biogenesis and insertion of an essential TA protein co-factor, such as Xport-A.

518B Osbp drives PI4KII-mediated retrograde transport to provide membrane lipids needed for secretory granule maturation

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Secretory granules (SGs) are important for normal animal physiology due to their role in regulated exocytosis of biologically active cargoes such as hormones, digestive enzymes and mucus. SG membranes are enriched in phosphatidylinositol 4-phosphate (PI4P) and cholesterol. However, the molecular details of how PI4P and cholesterol contribute to SG biogenesis remain poorly understood. Here, we demonstrate that oxysterol binding protein (Osbp), which binds PI4P, is required for normal SG maturation. Loss of Osbp leads to small SGs, whereas overexpression of Osbp results in abnormally large SGs. Genetic analysis reveals that the ability of overexpressed Osbp to increase SG size depends on type II phosphatidylinositol 4-kinase (PI4KII), which synthesizes PI4P. Overexpression of Osbp results in an increase in PI4P on both SGs and endosomes. In contrast, loss of Osbp results in a reduction of PI4P on SG membranes and an increase in PI4P on endosomes. We show that Osbp is needed for formation of cholesterol-rich endosomal tubules that are positive for PI4KII. Indeed, *Osbp* mutant salivary gland cells exhibit enlarged endosomes that are PI4KII positive but relatively poor in cholesterol. Filipin staining reveals that overexpression of Osbp results in an increase in cholesterol on SG membranes. In addition, feeding larvae with food supplemented with cholesterol precursor leads to partial suppression of the small SGs and enlarged PI4KII-positive endosomes in *Osbp* mutants. Our results indicate Osbp is required for cholesterol accumulation at endosomes, which in turn allows formation of PIKII-positive and cholesterol-rich membrane tubules. Formation of these tubules is important for accumulation of PI4P and cholesterol on SG membranes, leading to SG maturation.

519C Dynamics of clathrin-mediated endocytosis and actin in the native tissue context

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Clathrin-mediated endocytosis (CME) is an essential eukaryotic process, which has been extensively studied using cultured cells. These studies have shown that the assembly dynamics of endocytic events and the required proteins vary considerably within cells and between cell types. For example, the life time of individual endocytic events and the requirement for actin polymerization have been reported to vary significantly.
Cultured cells, however, lack the tissue context, which influences their mechanical properties and developmental state. It remains unclear how the variability and heterogeneity of endocytosis in cultured cells relates to endocytosis in cells within their natural environment.

To understand how CME functions in the native tissue context, we studied it in *Drosophila melanogaster,* and used CRISPR to create fly lines with endogenously tagged endocytic proteins. We then used fluorescence microscopy to image CME in pupae, which are non-motile and have all major organs developed. Due to the exquisite contrast of fluorescent knock-ins and optimized imaging, we were able to image individual endocytic events with a similarly high spatiotemporal resolution as in cell culture.

We focused on the epithelium at the surface of the pupal notum, where specialized mechanosensory bristles are formed along a highly stereotypic developmental trajectory. We used these cells as model system to image CME throughout cell differentiation.
We found that endocytic dynamics are highly regular in bristle cells, contrasting many reports from cultured cells, and change as bristles develop. From imaging the clathrin adaptor protein AP-2, we found that coat assembly and disassembly phases remain stereotypic, but a variable delay phase in between changes during cell growth, which we hypothesize is linked to changing requirements for actin polymerization in CME.
Bristles have a highly organized actin cytoskeleton composed of long and thick actin bundles at the plasma membrane, and local and dynamic actin assemblies between the bundles. To study how these actin structures are involved in endocytosis throughout bristle development, we simultaneously imaged AP-2 and the actin binding protein cortactin. We found that endocytosis is corralled between the bundles, and associated with highly dynamic actin networks.

In summary, we have found evidence that CME is reprogrammed during development, likely in response to changing cellular function and architecture.

520A The Adaptor Protein Complex 1 controls E-cadherin dynamics during epithelial morphogenesis

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Intracellular trafficking regulates the distribution of transmembrane proteins, including the critical determinants of epithelial

polarity and adhesion like E-cadherin. The Adaptor Protein 1 (AP-1) complex is the key regulator of vesicle sorting, binding a large number of specific cargos and shuttling them between the Trans Golgi and endosomal compartments. We examined roles of the AP-1 complex in epithelial morphogenesis, using the *Drosophila melanogaster* developing wing as a paradigm. We revealed that AP-1 knockdown leads to a complex tissue phenotype which includes abnormal folding attributed to a defect in integrin trafficking. Concurrently, cells increase their apical cell area and cell death is induced in an integrin-independent manner. We discovered a distinct pool of AP-1 localized outside the Golgi and endosomal compartments at the apical Adherens Junctions. There, the membrane levels of E-cadherin drop as the protein accumulates intracellularly upon AP-1 knockdown. This imbalance between cytoplasmic and membrane levels of E-cadherin triggers cell death by an uncharacterised mechanism with a tumour-suppressing potential, as blocking apoptosis leads to hyperplastic tissue growth. However, we found that as E-cadherin intracellular levels increase, cells are able of recovering its membrane presentation using a transcriptional feedback. This further layer of regulation of E-cadherin at the membrane allows the cell to maintain the cell-cell adhesions and increase the resilience of epithelial tissue and function. In line with recent discoveries, our findings highlight the pivotal role of the AP-1complex in preventing tumour development and enabling correct epithelial morphogenesis.

521B Regulation of adult lipid homeostasis by *Drosophila Estrogen-Related Receptor*.

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Metabolism and development must be closely coupled to meet the changing physiological needs of each stage in the life cycle. The molecular mechanisms that link these pathways, however, remain poorly understood. Here we show that the *Drosophila* estrogen-related receptor (*dERR*) directs a transcriptional switch in mid-pupae that promotes glucose oxidation and lipogenesis in young adults. *dERR* mutant adults are viable but display reduced locomotor activity, susceptibility to starvation, elevated glucose, and an almost complete lack of stored triglycerides. Molecular profiling by RNA-seq, ChIP-seq, and metabolomics revealed that glycolytic and pentose phosphate pathway genes are induced by dERR, and their reduced expression in mutants is accompanied by elevated glycolytic intermediates, reduced TCA cycle intermediates, and reduced levels of long chain fatty acids. Unexpectedly, we found that the central pathways of energy metabolism, including glycolysis, the tricarboxylic acid cycle, and electron transport chain, are coordinately induced at the transcriptional level in mid-pupae and maintained into adulthood, and this response is partially dependent on *dERR*, leading to the metabolic defects observed in mutants. Our data support the model that *dERR* contributes to a transcriptional switch during pupal development that establishes the metabolic state of the adult fly. Current studies are aimed at identifying the role of dERR and glycolysis in the adult *Drosophila* renal system, where *dERR* is highly expressed.

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522C Sex determination gene *transformer* regulates the sex difference in *Drosophila* triglycerides storage and breakdown

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Sex differences exist in triglycerides storage and breakdown in *Drosophila*. While key triglycerides metabolism genes have been identified that impact the male-female differences in fat storage and breakdown, the upstream genes that determine a male- or female-specific profile of triglycerides metabolism remain unclear. Here, we show that the sex determination gene, *transformer* (*tra*) regulates the sex differences in triglycerides storage and breakdown. Normally, a functional Tra protein is expressed only in females, where the presence of Tra determines most aspects of female development and behavior. Our examination of triglycerides metabolism in males and females reveals a new role for Tra protein in regulating triglycerides metabolism. In females, lack of a functional Tra protein significantly reduced triglycerides storage and increased triglycerides breakdown compared with control females. In males, both Tra overexpression and expression of physiological Tra levels augmented triglycerides storage and delayed triglycerides breakdown compared with control males. Importantly, these gain- and loss-of-function studies with Tra expression significantly reduced the sex differences in triglycerides storage and triglycerides breakdown, revealing a previously unrecognized role for sex determination gene *tra* in regulating triglycerides metabolism.

523A Elucidating the role of Spenito, an RNA-binding protein, in the sexual dimorphism of *Drosophila* metabolism

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Genetic studies of obesity have revealed major hereditary contributions to variation in body weight, but the vast majority of these remain unidentified. The large number of genes that contribute to obesity means that a treatment plan that works for one individual may not work for another. In order to develop individualized treatments for obesity we need a mechanistic understanding of the pathways in which obesity-linked genes normally function. An additional complication is that males and females are fundamentally different with regards to metabolism, but the underlying molecular mechanisms are incompletely understood. The research presented here shows the role of Spenito (Nito), an RNA-binding protein in *Drosophila melanogaster,* in the regulation of fat storage, in a sex dimorphic manner. Preliminary data suggests a role for Nito in the fat body (FB, equivalent to liver and adipose) in a sex-specific manner to potentially modulate splicing and gene expression via m^6A methylation, as was previously shown for sex determination. To test this molecular mechanism, I will utilize the robust genetic, cellular and molecular tools of *Drosophila* paired with metabolomics, and transcriptomic analysis. Ultimately, my work will elucidate how differences in male and female metabolism are established and regulated at the molecular and cellular level.

524B Hopscotch overexpression in adult female fat body decreases survivability and alters metabolic parameters in the high fat diet obesity model of *Drosophila melanogaster*

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There is a molecular and physiological connection between obesity and diabetes. Obesity-associated aberrant cell signaling can lead to insulin resistance and includes altered Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway activity in humans, although this impact is often tissue and nutrition context specific. *Drosophila melanogaster* and humans share analogous metabolic and energy homeostasis systems. An obesity-like state can be mimicked in *Drosophila* by providing media with high saturated fat content, such as coconut oil. In *Drosophila* hopscotch (hop) encodes a kinase similar to mammalian JAK, which acts to phosphorylate STAT92E, the sole STAT transcription factor in flies.
The present study utilizes a 10XStat92E-GFP (BDSC#26197) to expresses fluorescence (GFP) in proportion to STAT92E DNA binding. Virgin female flies were placed on a high fat diet (HFD) (20% w/v coconut oil) or a normal diet (NMD). The flies exhibited increased triglyceride content on a HFD relative to a NMD, and exhibited an increase in fluorescence in various tissues on a HFD. w[1118] (BDSC#5905) HFD-fed flies also exhibited decreased performance during negative geotaxis (NG) assay. *Drosophila* fat body tissue was further studied because it is analogous to mammalian liver and adipose tissue. To activate the Hop-Stat92E pathway in the fat body we utilized the GAL4-UAS system. The yolk-GAL4 driver (BDSC#58814), induces expression in female adult fat bodies and was backcrossed into the *w*[1118] background to match that of a line carrying UAS-hop^{tum}. Hop^{tum} is a hyperactive form of hopscotch. Virgin female flies utilizing a yolk-GAL4 driver to overexpress hopscotch in the adult female fat body show a decrease in lifespan on both HFD and NMD, and they reach near full cohort mortality in 1-2 weeks. Flies expressing hopscotch also show elevated glycogen levels on HFD that is not seen in controls. Additionally, they show an increase in feeding quantity on HFD that is not seen for control. While HFD increases starvation resistance relative to NMD, flies expressing hopscotch in the fat body show a reduction in survival during starvation relative to controls. Finally, flies expressing hop in fat body showed alteration in the ability to mobilize and uptake glucose from hemolymph. These results are indicative of the feasibility of the *Drosophila* obesity model in studying the connection between elevated JAK/STAT signaling and peripheral insulin resistance.

525C Dietary Stress Affects Structure and Function of the Eye

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The function of the eye can be impaired by suboptimal diets that deprive it of essential nutrients. For instance, vitamin A (vitA) is essential for vision because it is converted to retinal which is required for Rhodopsin maturation and function. Short-term vitA deprivation can cause night blindness, which will eventually lead to complete blindness upon prolonged deprivation. Like human photoreceptors, the light-sensing compartments (rhabdomeres) of *Drosophila melanogaster* outer photoreceptors (functionally equivalent to human rods) and inner photoreceptors (equivalent to human cones) are severely damaged upon vitA deprivation. Moreover, polyunsaturated fatty acids (PUFAs) are essential for photoreceptor membrane integrity and the functioning of the phototransduction cascade. Our goal is to analyze how defined diets that lack, or oversupply nutrients affect the morphology and function of the eye.
To study the effects of vitA deprivation or an excess of unsaturated or saturated fatty acids on photoreceptor structure and

function. Moreover, we seek to determine whether different (rod- and cone-equivalent) photoreceptor types show different sensitivity to specific dietary stresses. Consistent with previous studies, we found that vitA deprivation caused a cross-sectional shrinkage of the outer photoreceptor rhabdomeres by more than 50%. Strikingly, an oversupply of unsaturated fatty acids also caused a 40% reduction in outer photoreceptor rhabdomere diameter. In contrast, only vitA deprivation caused a significant reduction (by 50%) of the inner photoreceptor rhabdomeres; oversupplying unsaturated or saturated fatty acids did not affect the size of the inner photoreceptor rhabdomeres.
Since we detected severe rhabdomere defects in the case of vitA deprivation or excessive supply of unsaturated fatty acids, we asked how these dietary manipulations affected photoreceptor function. As expected, the optomotor response in response to a striped-drum stimulus (l=24 degrees), which is mediated by the outer photoreceptors, was dramatically impaired by vitA deprivation. However, despite the comparable reduction in rhabdomere size, excessive unsaturation did not significantly affect motion vision under the same experimental conditions. Taken together, vitA deprivation and an excess of unsaturated fatty acids in the diet differentially affect different photoreceptor types.

526A Metabolic measurements of *Drosophila* larval hemocytes

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Energy metabolism, the process of generating ATP from nutrients, is a fundamental reaction in cell biology. However, the metabolic tendency of Drosophila hemocytes remains poorly understood. Here, we utilize a Seahorse XFe96 Analyzer to measure metabolic activities of Drosophila hemocytes and identified that ATP production in third-instar-larval hemocytes largely depends on mitochondrial oxidative phosphorylation. Corresponding well with previous studies, larval hemocytes under normal growing conditions exhibit minimal metabolic activity. Interestingly, hemocytes in earlier stages show higher metabolic rates than in late instars, and innate immune challenge significantly increases metabolic activities. Overall, our observations first measure the metabolic profiles of larval hemocytes and establish dynamic transformations in hemocyte metabolism during development and immunity.
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527B Nuclear translocation ability of Lipin differentially affects gene expression and survival in fed and fasting *Drosophila*

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Proteins of the lipin family play essential roles in energy homeostasis in flies and mammals. Lipins act as enzymes in the cytoplasm that produce diacylglycerol as a precursor for neutral storage fats and membrane phospholipids. Under conditions of nutrient deprivation, they can migrate into the cell nucleus and act as transcriptional co-regulators. Translocation into the nucleus is controlled by the nutrient-sensitive TOR signaling pathway. Whereas functions of lipins in the cytoplasm are well understood, the functional significance of nuclear translocation is less clear. To address this question, we created a *Drosophila Lipin* mutant expressing Lipin protein lacking its nuclear translocation signal (NLS), a mutant that lacks enzymatic activity ($Lipin^{D812E}$), and a *Lipin* null mutant ($Lipin^{KO}$). As predicted by the partially lethal phenotype of a hypomorphic *Lipin* mutant, $Lipin^{KO}$ animals were not viable. Lethality could be fully rescued by ubiquitous expression of wild-type *Lipin* from a transgene, but not by fat body-restricted expression, indicating that Lipin has essential functions outside the fat body. The $Lipin^{D812E}$ mutant was not viable as well, indicating that the enzymatic activity of the protein is essential. In contrast, we found that $Lipin^{\Delta NLS}$ animals were viable when kept under normal feeding conditions. Strikingly, they were not only viable, but their lifespan was greatly increased compared to control flies. In contrast, when subjected to starvation, these animals died much faster than control flies. To identify genes controlled by nuclear Lipin that may explain these phenotypes, we carried out RNA sequencing with male and female $Lipin^{\Delta NLS}$ and control flies that had been subjected to starvation or normally fed. The results revealed that interference with Lipin's ability to migrate into the nucleus not only interfered with the normal expression of metabolic genes involved in lipid and carbohydrate metabolism, but also of genes that control feeding behavior and the immune response. Consistent with the altered regulation of metabolic genes, the metabolic rate of $Lipin^{\Delta NLS}$ flies was decreased, which might explain their extended lifespan. Our results suggest that lipins have a much broader role in animal physiology than previously thought and provide the basis for testable hypotheses about novel functions of lipins in behavior and immunity.

528C Peroxisomal dysfunction disrupts mitochondrial dynamics through mTORC2

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Inter-organelle communication is known to play important roles in maintaining cellular homeostasis. In this project, we focus on the communication between peroxisomes and mitochondria in Drosophila oenocytes, homolog of mammalian liver. We found that knockdown of Pex5, the adaptor protein for the peroxisomal matrix protein transport, resulted in dysregulated mitochondrial dynamic and enlargement of mitochondria in fly oenocytes. Interestingly, we observed that Pex5 knockdown induced the phosphorylation of AKT, suggesting an activation of mammalian target of rapamycin complex 2 (mTORC2) upon peroxisomal dysfunction. In additional, subcellular localization of rictor, the key subunit of mTORC2, was also altered by Pex5 knockdown (KD). Lastly, we showed that overexpression of rictor attenuated Pex5 KD-induced mitochondrial enlargement, whereas knockdown of rictor enhanced the mitochondrial dysfunction upon Pex5 KD. Together, our results suggest that activation of mTORC2 might act as an adaptive response to protect mitochondria from cellular damages associated with peroxisomal dysfunction.

529A Hexosamine biosynthetic pathway integrates circadian and metabolic signals to regulate daily rhythms in protein O-linked N-acetylglucosaminylation

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The integration of circadian and metabolic signals is essential for maintaining robust circadian rhythms and ensuring efficient metabolism and energy use. Using *Drosophila* as an animal model, we showed that cellular protein O-linked N-acetylglucosaminylation (O-GlcNAcylation) exhibits robust 24-hour rhythm and represents a key post-translational mechanism that regulates circadian physiology. We observed strong correlation between protein O-GlcNAcylation rhythms and clock-controlled feeding-fasting cycles, suggesting that O-GlcNAcylation rhythms are primarily driven by nutrient input. Interestingly, daily O-GlcNAcylation rhythms were severely dampened when we subjected flies to time-restricted feeding (TRF) at unnatural feeding time. This suggests the presence of a clock-regulated buffering mechanism that prevents excessive O-GlcNAcylation at non-optimal times of the day-night cycle. We found that this buffering mechanism is mediated by glutamine-fructose-6-phosphate amidotransferase (GFAT) activity, which is regulated through integration of circadian and metabolic signals. Finally, we generated a mathematical model to describe the key factors that regulate daily O-GlcNAcylation rhythm. In summary, our results provide insights into the mechanisms by which metabolic input coordinates with metabolic enzyme activity to regulate circadian physiology at the post-translational level and shed light on the health benefits of TRF at natural eating time and deleterious effects of non-optimal meal times.

530B Transcriptome Response to a Habanero Pepper-Containing Diet in *Drosophila melanogaster*

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Diabetes, obesity, and metabolic syndrome are becoming epidemic both in developed and developing countries in recent years. Chili peppers represent an important crop worldwide due to the beneficial properties of their phytochemicals including carotenoids, capsaicinoids, phenolic compounds, vitamins, and minerals. These compounds have been associated with the control of obesity, the reduction in the risk for coronary disorders, diabetes, cancer, osteoporosis, and neurodegenerative diseases. However, focused research in Drosophila has not yet fully addressed. Thus, this study aims to analyze the effects of pepper containing diet in the transcriptome response of *Drosophila melanogaster* and identify genes that may relate to pathogenesis accompanying an obesity-like state. We used the wild type *D. melanogaster* Berlin-K strain reared on control and 7.5% (w/w) pepper-containing diets. Experiments were initiated by placing approximately 10 males and 10 females onto vials containing the different diets. Adults were allowed to lay eggs for 72 h before being removed. The larvae were fed and once the adult stage was achieved, these flies were selected for bodyweight, triglyceride, and glucose level determination and RNAseq analysis. We observed a significant weight reduction in female flies on a pepper diet compared with those reared on control diet. Similarly, triglycerides level showed a high reduction at both sexes under pepper treatment. Although glucose levels did not show a significant difference, we noticed a slight reduction in male and female flies. RNA-seq revealed 539 differentially expressed genes between control and pepper diets. Moreover, genes involved in fatty acid and antioxidant metabolism were down-regulated. Likewise, a gene with function glucocerebrosidase 1a (*Gba1a*) associated with Parkinson's disease was down-regulated, meanwhile, genes related to obesity such as adipokinetic hormone (*Akh*) and scratch, isoform B (*Kah*) were up-regulated. This transcriptome study provided a comprehensive understanding of various molecular mechanisms

underlying pepper diet effects in Drosophila.

531C An enzyme catalog for *Drosophila melanogaster*

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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 have remained unidentified or poorly classified within biological databases, hampering research progress and inter-species comparisons. In order to address these shortcomings, we have systematically reviewed *D. melanogaster* enzyme data obtained from several key databases and the primary literature. We have now completed our review of the 6 major enzyme classes: oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. All verified activities have been annotated using appropriate Gene Ontology (GO) and Enzyme Commission (EC) terms while incorrect annotations have been corrected, providing feedback to the source databases as necessary. In addition, we have compiled convenient ‹Gene Group› reports within FlyBase for each enzyme class. We will present an overview of this work, demonstrating how the improved data can be accessed within FlyBase and other databases, and how it will aid studies of fly metabolism in particular.

532A Effects of the mitochondrial alternative oxidase expression on *Drosophila* development: the good, the bad and the ugly

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The expression of the mitochondrial alternative oxidase AOX from tunicates has provided clear beneficial effects in a variety of mitochondrial disease models. Because of its terminal oxidase activity, AOX can bypass oxidative phosphorylation (OXPHOS) complexes III and IV, alleviating possible overload of electrons upon mitochondrial dysfunction. Significant detrimental outcomes have also been reported, raising concerns regarding its putative deployment as a therapy enzyme for human diseases. In *Drosophila*, AOX expression is developmentally advantageous at low temperatures when the flies are cultured on a standard, rich diet (SD), but it dramatically compromises adult eclosion when the flies are cultured on a low-nutrient diet (LN), at 25ºC or above. We show here that mitochondria of AOX-expressing larvae cultured on SD have a functional rearrangement of the OXPHOS, in which oxygen consumption driven by the mitochondrial glycerol-3-phosphate dehydrogenase (mGPDH) is downregulated, whereas complex I (CI)-driven respiration is upregulated, independent of temperature. However, at 12ºC, only mGPDH-driven respiration is sensitive to AOX inhibition. Because mGPDH and AOX do not contribute to the proton-motive force that drives mitochondrial ATP synthesis, we expect that the sequential electron transfer through these enzymes leads to thermogenesis, which is consistent with the body temperature of AOX-expressing larvae being higher than controls at 12ºC. Moreover, increased CI-driven respiration suggests higher activity of the TCA cycle, increased cataplerosis, and consequently an elevated larval growth, which is consistent with AOX-expressing larvae cultured at 12ºC accumulating more body mass. Conversely, the interaction between LN and AOX expression causes a ~40% decrease in larval biomass at 25ºC, accompanied by a general alteration of larval amino acid metabolism and a clear starvation signature in pupae, as inferred by transcriptomics and metabolomics data. The elevated levels of lactate dehydrogenase, lactate and 2-hydroxyglutarate in AOX-expressing flies, independent of diet, combined with mGPDH downregulation, point to an important role for these redox-regulating enzymes in adjusting larval physiology upon AOX expression. As temperature and diet are two of the most important external factors that influence metabolism, our work provides important insights into how AOX expression could be safely accomplished, with minimal impact for higher animals.

533B Respiratory chain supercomplexes and their functional and evolutionary interaction with alternative enzymes

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Conditions that affect the functioning of the mitochondrial oxidative phosphorylation (OXPHOS) may cause depletion of cellular ATP levels and/or excessive formation of reactive oxygen species (ROS). The xenotopic expression of respiratory chain alternative enzymes (AEs) in model organisms allows the electron transport bypass of OXPHOS complexes I (CI) and/or III/IV (CIII/IV) and reestablishment of oxygen consumption in defective mitochondria, and thus has been considered a possible therapy to prevent excess ROS formation in pathological conditions. The countless promising results in *Drosophila* and mouse appear contradictory to the fact that the genome of vertebrates and insects have independently lost the genes for AEs. To get evolutionary and functional insights into the differences between animal OXPHOS that naturally have and do not have AEs, we used public databases and bioinformatics tools to identify all genes coding for subunits of all OXPHOS complexes in AE-bearing tunicate species of the genus *Ciona*, and compare them to their orthologs in *D. melanogaster*, *D. similans*, chimps and humans.

We hypothesized that the presence of AEs would be reflected as relaxed selection pressure in the genes coding for CI, III and IV subunits in *Ciona*, but the calculated Ka/Ks ratios did not support this. Interestingly, we found that a significant number of CI, III and IV genes is missing from the genomes of *Ciona* species. Most of these subunits are important for the formation of supercomplexes (SCs), which are supramolecular assemblies of CI, III and IV that may streamline electron transfer in mammals and in flies. Our results suggest that *Ciona* species might not form SCs, or, if they do, their formation is accomplished via a different mechanism. The idea that *Ciona* species have AEs but not SCs is in agreement with our data that points to a preferential electron transfer between *D. melanogaster* mitochondrial glycerol-3-phosphate dehydrogenase (an OXPHOS enzyme that does not participate in SCs) and a *Ciona intestinalis* AE called alternative oxidase (AOX), which we ectopically expressed in the fly. We are in the process of characterizing strains of *D. melanogaster* with varying expression levels of both enzymes, hoping to show that AOX does not function efficiently with SCs, which would provide an explanation for the putative lack of these structures in *Ciona*. Our results would also provide insights into how to fine-tune the expression of AOX in a therapeutic context. Funding: FAPESP (2017/04372-0, 2014/02253-6, 2017/13743-2), CNPq (424562/2018-9, 306974/2017-7).

534C Lactate and glycerol-3-phosphate metabolism cooperatively regulate growth and carbohydrate metabolism during *Drosophila melanogaster* larval development

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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 *Ldh* together, exhibit developmental delays, synthetic lethality and aberrant carbohydrate metabolism. Finally, we demonstrate that *Ldh* and *Gpdh1* are capable of influencing larval growth in a cell nonautonomous manner, indicating that the cooperative relationship between them is complex and capable of regulating larval growth factor signaling.

535A Annotation of the Pentose Phosphate Pathway gene Zw/G6PD in *Drosophila* species

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Glucose-6-phosphate dehydrogenase (G6PD) is the most common enzyme deficiency in humans, affecting 400 million people worldwide. The *Drosophila melanogaster* Zw (Zwischenferment) gene codes for the conserved G6PD enzyme that is the rate limiting step in the pentose phosphate pathway (PPP). The PPP provides NADPH required for lipid biosynthesis under high nutrient conditions. In both humans and *Drosophila melanogaster, Zw/*G6PD is alternatively spliced in the 5' UTR (untranslated region) to produce two different proteins that vary in length and initial N terminal sequence. Using *melanogaster* as the reference genome, we annotated *Zw* coding sequences in *erecta, pseudoobscura* and *mojavensis* to further investigate the conservation of the products of alternative splicing. All four species were similar in their 5› UTR alternative splicing pattern and protein product lengths. A Clustal Omega alignment indicated 96%, 91% and 80% amino acid identity between *melanogaster* and *erecta, pseudoobscura* and *mojavensis* respectively. Interestingly, *pseudoobscura* and *mojavensis* contain an additional *Zw* exon and vary in the length of the first intron compared to *melanogaster* and *erecta*. We hypothesize that the alternative splicing of Zw/G6PD results in different proteins that vary in enzyme activity. To investigate this, a qPCR method (quantitative Polymerase Chain Reaction) was developed to detect the two major isoforms in *melanogaster* under fed and starvation conditions. Our results suggest that the longer isoform A is predominantly produced under fed conditions but starvation conditions favor the formation of isoform B. Future experiments will test G6PD enzyme activity under fed and starved conditions.

536B *orsai*, an essential regulator of lipid metabolism

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Every stage in the *Drosophila* life cycle is highly regulated by hormones and in relation with metabolic consumption. During embryogenesis, the metabolism is highly dependent of maternal lipids deposited during oogenesis, as evidence by the ß-oxidation of fatty acids as the main source of energy during this stage. In the larval stages, a metabolic switch occurs where ß-oxidation stops and aerobic glycolysis takes the lead, with the accumulation of pyruvate being used in the synthesis of nucleotides and amino acids necessary for organismal growth. At the end of the larval stage and the beginning of the pupal stage, the metabolic program suffers a new switch and lipid reserves generated in the previous stage are used to sustain metamorphosis by means of ß-oxidation. Despite the different energy requirements of *Drosophila* throughout development have been explored and specific metabolic programs have been singled out, it is still not very clear how these changes in metabolic programs are coordinated. We have identified a lethal mutation associated to a novel gene (*CG6115*), that we have renamed *orsai* (*osi*). Animals depleted of *osi* are not able to develop beyond first instar and die within 72 h after egg lying with a very small size and completely depleted from lipid reserves in the fat body. Sequence analysis suggests OSI could be an orthologue of human ETFRF1/LYRm5, a poorly characterized protein associated to the regulation of ETF, an entry point to β-oxidation products into the electron transport chain. Interestingly, *osi* depletion leads to reduced lipid storage, reduced organismal size, and decreased mitochondrial health. Overexpression of human ETFRF1/LYRm5 partially rescues the lethality observed upon *osi* loss of function, strongly suggesting that ORSAI could work as a *Drosophila* orthologue of human ETFRF1/LYRm5. Our results lend support to a new model where *orsai* could be an important regulator of the metabolic switch that coordinates lipid storage and consumption at the appropriated times during development.

537C The identification and characterization of lipogenic proteins contributing to cardiac lipotoxicity

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Overnutrition, induced by a high calorie diet, is associated with obesity, type two diabetes, and cardiovascular disease in a myriad of organisms including flies and humans, although the causal relationship remains to be elucidated. There is an indication that high calorie diets influence metabolic pathways and lead to an influx of lipid concentration, known as lipotoxicity. However, the mechanism of lipotoxicity remains unknown. In recent studies, investigators have proposed the idea of maximum adipose expandability, where they suggest that habitual consumption of glucose can lead to a complete occupation of triglycerides in fat storage, leaving newly synthesized free fatty acids and their derivatives to toxically accumulate in peripheral tissues, such as the heart (Unger et al., 2010, Virtue et al, 2010). Our previous data has supported a Drosophila model where cardiac arrhythmia is linked with overnutrition through lipotoxicity via a reduction in triglycerides, the fat storage fatty acids, and an increase of free fatty acids and their derivatives following high sugar feeding (Tuthill, B, in review). In order to identify pathways that are involved in lipotoxicity, we conducted a genetic screen for heart function using tissue specific RNAi knockdowns of genes involved at the interface of the maximum expandability model and identified genes contributing to heart function. These genes role in lipotoxicity will then be mechanistically defined through an evaluation in animal physiology and organelle morphology. Specifically, changes in whole animal metabolites, cardiac physiology, and lipid pool composition will aid in the identification of phenotypes essential to proper cardiac function after overnutrition. Organelle morphology, such as peroxisome abundance and extracellular matrix composition, will provide mechanistic information to how cardiac function is being ameliorated as well as providing potential targets for drug therapeutics.

538A Intergenerational effects of high dietary sugar

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While food is crucial for an organism's survival, certain nutrients in the wrong proportions deter its ability to protect bodies against chronic diseases. A major shift in the dietary environment during the past 5 decades shows a startling increase in calorie consumption among which diets rich in sugar (among other highly palatable foods) remains one of the characteristic components of Western food. Over the last few years, it has been shown that high dietary sugar drives significant metabolic and feeding behavior changes - a crucial path leading to overeating and obesity. Using *Drosophila*, these behavioral changes have been shown to result from epigenetic reprogramming that remodels neurodevelopmental transcriptional pathways on chromatin of taste cells. However, despite the knowledge that epigenetic patterns are known to be carried over through mitotic cell divisions, it's heritability through generations is far less understood. Given the recent findings that a high sugar diet is involved in rewiring the adult brain, has led me to ask what are its effects on a developing organism, a phase which is far more receptive to environmental changes. Characterization of epigenetic factors consequent on offspring physiological and behavioral changes is therefore paramount towards exploring the mechanistic link between parental diet and offspring brain development and behavior, one which is largely underexplored. To investigate first part of the question, we subjected

wild-type *Drosophila melanogaster* to a high sugar diet environment and collected eggs at the end of the dietary regime. We find that parents on a high sugar diet (SD) suffer from loss of perception to sweet taste, overeating, increased levels of glucose and triglycerides in the body. To identify changes in the metabolic state of the offspring from parents on SD, we carried out metabolomics analysis of embryos and found significant changes in offspring embryonic metabolite levels. Most metabolites were depleted in the embryos from SD fed parents compared to embryos derived from control diet fed parents. Metabolites from most classes such as amino acids, nucleotides, carbohydrates, lipids, cofactors and vitamins exhibited strikingly different profiles between parental diet types. Embryo RNA sequencing also revealed changes in levels of few neurodevelopmental genes. These experiments are helping us characterize inherited nutritional state of offspring when parents reproduce being on a high sugar dietary environment. Further work probing on the embryonic neurodevelopmental changes are underway to provide insights into the effects of parental dietary environment and lead us to evaluate if and how parental high sugar diet during reproduction could potentially prime the offspring to a wide array of diseases.

539B Investigating the alternative splicing of metabolic genes

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Obesity is characterized by an excessive storage of triglycerides and affects over one-third of the adult population in the United States, leading to an increase in related conditions such as heart disease, stroke and type 2 diabetes. Previous studies have shown that splicing is important for proper triglyceride storage yet the signals that activate splicing factors required for triglyceride storage are not completely understood. In humans, a master regulator, called mTOR, promotes lipid synthesis through the splicing factor activating kinase SRPK2. mTOR integrates signals from nutrients and growth factors, such as insulin, to increase cellular growth and lipid synthesis. In *Drosophila melanogaster,* similar insulin and mTor (called Tor) pathways are found. We hypothesize that splicing factors are activated downstream of insulin signaling through the TOR pathway in *Drosophila*. To investigate this, we attempted Western blots to examine the phosphorylation of the splicing factor U1-70K in a SRPK knockdown background. Samples were generated from dissected fat bodies of adult females but Western blots were not successful and optimal protein levels in fat body samples remain to be determined. An additional potential target of SRPK is the SR protein splicing factor 9G8. Although 9G8 is critical for proper triglyceride levels in larvae and adults, its splicing targets are not well known. In an effort to identify potential 9G8 splicing targets, we took a bioinformatics approach and classified the alternative splicing patterns of gene models available on FlyBase. Alternative splicing patterns were investigated for key genes in different metabolic processes including lipid biosynthesis, glycolysis, insulin/TOR signaling and the pentose phosphate pathway. We found that 60% of these genes were alternatively spliced and the most common splicing pattern involved the 5' UTR. Potential 9G8 binding sites in the 5' UTR of these genes will be investigated.

540C The C-terminal region of Pex14 promotes lipid storage in Drosophila melanogaster

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Peroxisomes are thought to associate and coordinate with lipid droplets (LDs) to regulate β-oxidation of fatty acids and promote synthesis of plasmalogens and docohexanoic acids. When Drosophila embryonic Schneider 2 (S2) cells are cultured in medium containing excess oleic acid (C18:0) an increase in lipid droplets but not peroxisomes is observed. Differential RNA-Seq on S2 cells cultured in normal and +oleic acid conditions showed elevated levels of mRNAs encoding two Peroxin proteins (Pex) Pex13 and Pex14, known to contribute in peroxisome biogenesis. These were the only Pex genes to be upregulated and there was no corresponding increase in peroxisome count. Further analysis showed that of 13 Pex proteins, only three, Pex3, Pex13, and Pex14, were seen co-localizing with lipid droplets, suggesting that this event occurred independently of mature peroxisomes.

During peroxisome biogenesis, Pex14 and Pex13 are translated on the free ribosomes and post-translationally inserted within the membrane of peroxisomes or vesicles that contribute to peroxisomes. Pex14 is a multi-functional, single-pass transmembrane protein that is the main component of the transposon complex that imports proteins into the peroxisome matrix. The N-terminal region contains a Pex14 domain that directs protein import. It has also been shown to bind tubulin and is implicated in peroxisome motility. However, the role of C-terminal region remains poorly characterized. To investigate how Pex14 is recruited to LDs, we expressed Myc-tagged, truncated, N- and C-terminal Pex14 with or without transmembrane domain (TMD). We found that TMD is required to localize Pex14 to LD and C-terminal region can direct localization to LDs but not to peroxisomes.

The Drosophila larval fat body stores lipids in large LDs and releases them during later development or during starvation

conditions. A dsRNA mediated knockdown (KD) of Pex14 in the fat body resulted in reduced total volume of LDs and negatively affected survival in larvae fed with lard-supplemented food. Pex14 KD in S2 cells caused more numerous and smaller LDs compared to S2 cells cultured under normal conditions. Transgenic over-expression of Pex14 in S2 cells caused higher number of LDs suggesting that it participates in lipid storage at LDs. When overexpressed in the same cell, the C-terminal Pex14 behaved similarly while N-terminal Pex14 was found to cause similar changes to similar LD morphology as Pex14 KD. Further functional characterizations of Pex14 identified a contribution to regulation of lipolysis at LDs. When S2 cells was transferred from oleate-rich to minimal medium, Pex14 was shown to prevent lipolysis by inhibiting localization of Drosophila Hsl. Overall, this suggests a previously undiscovered role for Pex14 in promoting formation and inhibiting lipolysis at LDs.

541A Zw/dG6PD is a potential 9G8 target to regulate glycogen metabolism in the *Drosophila* fat body

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With the increase in abundance of food in Western cultures, the storage of these excess nutrients can lead to obesity and other metabolic diseases. In animals, excess nutrients from food are stored as triglycerides, mostly as lipid droplets found in adipose tissue. Genome-wide RNAi screens in *Drosophila* cells have identified several groups of genes involved in triglyceride metabolism and storage. One such group of interest to our lab includes RNA splicing factors. Previous studies from our lab have characterized the metabolic roles of a group of splicing factors called SR proteins that function to identify intron/exon borders. One SR protein that we have shown to regulate nutrient storage is 9G8. Decreasing 9G8 function causes an increase in triglyceride and glycogen storage and alters the processing of the lipid breakdown gene, CPT1. To better understand the mechanisms whereby 9G8 causes these changes in triglyceride and glycogen storage, we performed RNA sequencing on flies with decreased 9G8 levels in the adult *Drosophila* fat body. Not surprisingly, differential expression and pathway analysis of the RNA sequencing data showed a down regulation of genes related to various metabolic pathways, including lipid and glucose metabolism. One down regulated gene from this dataset involved in glucose metabolism was Zwischenferment (*Zw*), the *Drosophila* homolog of human glucose 6-phosphate dehydrogenase (G6PD). G6PD regulates the entry of glucose 6-phosphate (G6P) into the pentose phosphate pathway. G6P is also a substrate for glycogen synthesis, so it is possible that decreasing G6P flux into the pentose phosphate pathway would shift flux towards glycogen synthesis. To test this hypothesis, RNAi knockdown of *Zw* was performed in the adult *Drosophila* fat body. Zw-RNAi flies had an increased glycogen phenotype, similar to that found in flies with RNAi knockdown of 9G8 suggesting a role for Zw in the 9G8-mediated glycogen accumulation phenotype. Together, these findings suggest that 9G8 regulates several metabolic genes and may control the function of *Zw* to specifically control glycogen storage in the *Drosophila* fat body.

542B Neuronal E93 regulates metabolic homeostasis

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Organisms need to adapt to environmental challenges to sustain energy reserves. The central nervous system serves to integrate environmental and internal cues to drive physiological and behavior responses to energy deficits. The transcriptional regulator, E93 (*Eip93F*) is essential for metamorphosis, however its neuronal function has largely gone unstudied. We hypothesized that neuronal E93 might play a central role in coordinating fuel reserved required for survival.
As knockout flies are lethal, we decided to use the Gal4/UAS system to drive RNAi in neurons and assess the flies for starvation resistance. Male knockdown flies survived about 20% longer than controls. Assessments of whole-body glucose, glycogen and triglyceride levels indicated a sexual dichotomy in their fuel stores, with males having increased triglyceride stores while virgin females show increased glycogen stores. In order to identify the neuroendocrine cells required for the alterations in lipid and triglyceride levels, we used 17 Gal4 lines targeting subsets of neurons and endocrine cells. We determined *Eip93F* in MIP and AKH expressing cells are required for maintaining proper energy stores.
These results indicate an important neuronal function for E93 in controlling metabolism, and may assist in understanding neural regulation of fat and carbohydrate metabolism.

543C Dynamic changes in mitochondria-associated proteasome activity drive mitochondrial remodeling during quiescence

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Cellular quiescence is a dormant state where cells display low levels of transcription, translation, and metabolic activity. Quiescence is a common feature of oocytes and adult stem cells in many systems. When needed, these quiescent cells

are reactivated and drive growth. Therefore, entering, maintaining, and exiting cellular quiescence are steps that drive development, reproduction, regeneration, and aging. In our previous work, we discovered that as *Drosophila* oocytes enter quiescence, mitochondria are remodeled, and oxidative metabolism is suppressed in a process called mitochondrial respiratory quiescence (MRQ). To understand the mechanisms that drive this remodeling of mitochondria, we conducted an in-depth biochemical characterization of the physiological changes that drive cellular quiescence.
Using this system, we have discovered that there is a significant 3-fold increase in cellular proteasome activity during the onset of quiescence. Interestingly, much of this increased activity is located on the mitochondria's surface, where it promotes the onset of MRQ. We have found that GSK3, a key regulator for MRQ, functions to drive an increased level of proteasome activity at the outer mitochondrial membrane during quiescence. Using a GSK3-APEX transgene, we combine proximity labeling and cellular fractionation to systematically identify all of the mitochondria-specific targets of GSK3 in quiescent cells. In particular, our data shows that GSK3 functions through 2 mechanisms to induce MRQ. First, GSK3 promotes the assembled 26S proteasome's recruitment to the mitochondrial outer membrane by phosphorylating VDAC, a small outer mitochondrial membrane ion channel. Second, we have found that GSK3 induces proteasome recruitment to the mitochondria by inhibiting fatty acid oxidation (FAO). RNAi-mediated silencing of MTPα and ETFα, two critical components of the FAO pathway, triggers a premature onset of MRQ and significantly higher proteasome activity at the outer mitochondrial membrane. Overall, our work shows that proteasome function is regulated both temporally and spatially during oogenesis to drive mitochondrial remodeling during cellular quiescence. Moreover, we have found that mitochondrial metabolic pathways directly regulate proteasome function. Overall, these studies highlight an intimate relationship between cellular proteostasis and mitochondrial metabolism that supports quiescence cells' competence during development and reproduction.

544A Transcriptomic response during Spiroplasma -Drosophila-wasp interaction

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Spiroplasma poulsonii, a heritable bacterial symbiont of *Drosophila melanogaster*, inhibits the development of certain species of wasps that parasitize *Drosophila* spp. larvae. Production of toxins by *Spiroplasma* has been proposed as the possible mechanism of protection, based on the presence of Ribosome Inactivating Proteins (RIPs) in *Spiroplasma* genomes, but also on evidence of RIP-induced damage (ribosomal RNA depurination) in *Spiroplasma*-susceptible wasps. Alternative hypotheses such as enhancement of fly immune response during the *Spiroplasma*-wasp interaction has been less explored.
Using a metatranscriptomic approach, we asked if *Spiroplasma*-wasp interaction influences gene expression of *D. melanogaster* larvae, especially immune related genes. We evaluated treatments parasitized by the *Spiroplasma*-susceptible wasp *Leptopilina heterotoma* or the *Spiroplasma*-resistant wasp *Ganaspis hookeri*. The results show no expression of immune related genes during the *Spiroplasma*-*L. heterotoma* interaction. However, the presence of the symbiont seems to restore gene expression of two groups of genes, when comparing with parasitism in the absence of *Spiroplasma*. Some of these genes are associated with developmental aspects.
A second objective evaluated RIP gene expression in two strains of *Spiroplasma*. Based on qPCR, we show that neither wasp treatment (i.e., no wasp vs. *L. heterotoma* vs. *G. hookeri*) nor *Spiroplasma* strain affect the pattern of RIP gene expression. However, one of the RIP genes, RIP2, was consistently more highly expressed than the other four RIP genes in all treatments, suggesting that if RIPs are indeed involved in the wasp-killing mechanism, RIP2 may be the most important one.
Consistent with the action of RIPs, the metatranscriptome revealed depurination of the ribosomal RNA of the susceptible wasp (*L. heterotoma*), but not of the resistant wasp (*G. hookeri*), implying that *G. hookeri* avoids RIP-induced damage through an unknown mechanism.

545B *Drosophila* gut bacteria regulate the growth of invasive microbes both in culture and in the host gut environment

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The study of microbiomes, comprised of the microbial communities and the associated chemistry of particular sites on multicellular organisms, has broadened our understanding of how commensal microorganisms co-exist with an animal host. Examination of the human gut microbiome has uncovered functions that beneficial microbes play in maintenance of homeostasis. It is well-known that depletion of the gut microbiota following a course of antibiotic therapy leaves one vulnerable to infection with opportunistic pathogens. Probiotic therapy is a means to block such infections by replenishing the perturbed microbiome. To study the dynamic interactions between the gut microbiota, pathogens, and probiotic bacteria, we use the model organism *Drosophila melanogaster*. *D. melanogaster* is an excellent host for microbiome research because of the structural and functional similarities between the intestinal tracts of flies and mammals. The microbial communities of lab-raised flies can be eliminated by bleach dechorionation of embryos, which enables us to feed back a defined community

to these axenic flies and observe the contributions of individual microbes. *In vitro* experiments in which pairs of microbes are co-cultured on solid media demonstrate that the gut commensal *Lactobacillus plantarum* (*Lp*) inhibits the growth of the gut pathogen *Pectobacterium carotovora* (*Ecc15*) and the human probiotic bacterium *Escherichia coli* Nissle 1917 (*EcN*). This inhibition requires *Lp* is given a head-start to grow as it appears dependent on the secretion of inhibitory metabolites. We have also observed that both *EcN* and *Ecc15* are each capable of inhibiting eachothers' growth in a similar time-dependent manner. Within the host gut environment, we have conducted similar experiments by feeding back different combinations of *EcN, Ecc15,* and microbiome members to axenic *D. melanogaster* adults. The inhibitory effect of *Lp* on non-resident microbes observed in the *in vitro* analysis is largely recapitulated *in vivo*. We are actively exploring the chemical basis underlying these interactions by screening the genomes of each organism for potential biosynthetic gene clusters using the program antiSMASH. We are also taking an LC-MS approach to identify secondary metabolites secreted by co-cultures of bacteria. Elucidating the chemistry of these interactions will broaden our understanding of how gut bacteria respond to invasive microbes and protect the micro-environment from perturbation and disruption of homeostasis.

546C Investigation of *Wolbachia*-induced changes in the *Drosophila* brain and their impact on host behavior

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Infection with parasites and intracellular microbes often leads to behavioral changes within the host. The "Behavioral Manipulation Hypothesis" claims these pathogens evolved the ability to initiate these behaviors in order to facilitate successful transmission from host to host. However, there is much debate over the validity of this hypothesis, prompting the need for in-depth studies of mechanisms underlying microbe influence on host behavior. The relationship between *Drosophila* species and the obligate intracellular symbiont *Wolbachia* can be used to test this phenomenon, as multiple host effects have been observed upon infection, including changes in behavior, mating, and fitness. In order to elucidate mechanisms of Wolbachia-host interaction on a molecular level, we used two-dimensional difference gel electrophoresis (2D-DIGE) to observe proteomic differences between infected and uninfected flies. Using this method, we have discovered post-translational modification (PTM) changes of various proteins found within the *Drosophila* brain upon *Wolbachia* infection. One of these modified proteins is glutamic acid decarboxylase (GAD), the enzyme responsible for the synthesis of the neurotransmitter GABA. It is known that several *Drosophila* behaviors are directly controlled by GABAergic circuits within the brain, such as locomotion, mating, olfaction, sleep, and circadian rhythms. Current work is aimed at investigating changes in GABA levels upon infection in addition to analyzing GABAergic behaviors in Wolbachia-infected flies.

547A A candidate niche in the *Drosophila* proventriculus mediates specific associations with *Lactobacillus plantarum* and *Acetobacter indonesiensis*

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Each individual animal is colonized by a unique gut microbiome. Why a specific microbial composition is assembled and persists, what role the host or microbes play in maintaining that microbiome and how exactly it affects the health and fitness of the animal is the subject of much inquiry. It is difficult to study the microbiome because of the high diversity, large inter-individual variation, and lack of powerful genetic tools. Furthermore, the vast diversity of bacteria in vertebrate guts presents many technical challenges. Here we develop a *Drosophila* model to study the role of microbial interactions in colonization. We first demonstrate that a wild fly isolate of *Lactobacillus plantarum* (*LpWF*) establishes a strain-specific, spatial association with the cardia region of the fly's foregut, using a combination of microscopy, microsurgery, and culture-based assays. We find that primary colonizers exclude secondary colonizers of the same strain. A separate *Drosophila* bacterium, *Acetobacter indonesiensis* (*Ai*) shows a similar colonization phenotype, localizing to the cardia and excluding secondary *Ai* colonists. We hypothesized that *LpWF* and *Ai* would also exclude one another. However, we found that *LpWF* facilitates *Ai* colonization. In examining the cardia of colonized flies, we discovered an engorgement of the tissue when bacteria colonize, making more space available to susequent colonizers and suggesting habitat expansion as the mechanism of facilitation. Integrated experiments and modeling revealed a spatially-structured metapopulation that drives priority effects in microbiome assembly. Our results establish that a simplified microbiome in Drosophila can be used to construct tractable experiments and offers a system for bottom up assembly of gut microbial communities.

548B Measuring Genetic Influences on the Microbiome of *Drosophila melanogaster* using CRISPR/Cas9

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The Chaston lab has made several important scientific contributions relating to the *Drosophila melanogaster* microbiome. Life history traits such as lifespan and fecundity have been linked to the presence of different types of bacteria found in the *D. melanogaster* microbiome (Chaston, 2014). In my project, I aim to expand on those findings by applying CRISPR to study a genetic question: how the microbial composition of the *D. melanogaster* microbiome is affected by the modification of specific genes in flies from Florida and Maine. For each of the selected genes, the Florida fly allele will be put into the Maine fly genome and the Maine fly allele will be put into the Florida fly genome. The microbiome composition of these two new flies will be compared against the original lines in a factorial design. Embryos will be injected with the necessary plasmids for a double-stranded cut to take place. After injection, homology-dependent repair will incorporate the new allele. Sanger sequencing will be used to screen for successful knock-in of each allele. Finally, the concentrations of each bacteria strain found in the microbiota of the flies will be measured and compared against the flies from which the allele came from.

549C Uncovering mycobiome genetic composition that promotes host proteostasis

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The protein homeostasis (proteostasis) network is a group of stress response pathways that maintain proteome functionality. It protects cells from cellular damage when environmental stresses occur, such as due to heat stress. Disrupted proteostasis has been implicated in an increasing number of diseases, including cystic fibrosis, lysosomal storage disorders, cancers, and assorted neurodegenerative diseases. Therefore, it is pressing to search for molecular pathways to enhance proteostasis capacity. To tackle this challenge, this research aims to identify bioactive molecules that facilitate proteostasis under stress by modulating mycobiota-host interactions, and characterize their underlying regulatory mechanisms. Mycobiota are the fungal communities that live in and on a host organism. Similar to humans, fruit flies harbor the yeast *Saccharomyces cerevisiae* in their mycobiota that are distinct from their environmental mycobiota. In this study, we found flies showed a significant decline in heat stress survivorship when treated with the wild-type haploid yeast (BY4741), as compared to the flies under germ-free conditions ($P<0.001$, Student's *t*-test). Through screening a genome-wide yeast knockout library, we identified 44 out of 2,083 yeast single-gene knockout strains that are capable of restoring fly heat resistance and proteostasis in three independent experiments. Many of them are involved in protein modification, response to unfolded proteins, protein transport, cytoskeleton and structural integrity, and polysaccharide metabolism. Our results reveal the novel link between fungal genetic composition and host proteostasis capacity. It is the first systematic analysis of proteostasis-promoting fungal genetic factors in the host. It will facilitate the discovery of innovative nutraceutical targets and new strategies of probiotic therapies for treating protein-homeostasis governing diseases.

550A The autism-associated chromatin modifier, Chromodomain Helicase DNA Binding Protein 8, affects gastrointestinal phenotypes in *Drosophila melanogaster*

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INTRODUCTION: Mutations in *Chromodomain Helicase DNA Binding Protein 8 (CHD8)* are the most common *de novo* mutations associated with autism spectrum disorder (ASD). CHD8 is a chromatin modifier that influences the transcription of many other ASD-risk genes; thus, it is regarded as a master regulator and defines a common ASD subtype characterized by macrocephaly and gastrointestinal (GI) problems. The cellular phenotypes caused by *CHD8* mutations have not been completely explored. In particular, how CHD8 impacts GI phenotypes at the cellular level, and if changes to the gut play a role in behavioral phenotypes associated with ASD.

AIMS: Our goal was to determine if *CHD8* affects two specific GI phenotypes—rate of digestion and gut microbiota composition.

METHODS AND RESULTS: We examined *Drosophila melanogaster* with a null allele of *kismet (kis),* the fruit fly ortholog of *CHD8*. To study the rate of digestion, food containing a dye tracer was fed to wild-type and *CHD8/kis* mutant flies. We measured the time from consumption of food to excretion. Consistent with studies of *CHD8* in vertebrate models, we found that *kis* mutant flies had a significantly reduced rate of digestion compared to wild-type flies. To compare the gut microbiomes of *CHD8/kis* and wild-type *Drosophila,* we isolated DNA from dissected midguts and used metagenomic sequencing. Our results indicate that the microbial composition of the guts differ across the two genotypes at the species level. This is the first research to our knowledge in which the gut microbiome of *Drosophila* carrying an autism risk gene has been characterized.

CONCLUSIONS: Our data shows that loss of *kis* impairs digestion; thus, *kis* plays critical role in gut physiology and is functionally conserved with its vertebrate ortholog. We also show that *kis* impacts the gut microbiome. While our current data does not indicate if these two phenotypes are connected, one possibility is that the reduced digestion rate is directly impacting microbial colonization.

FUTURE DIRECTIONS: Studies in mice have begun determining how gut microbiota modulate gut function and behaviors associated with ASD. We will conduct similar experiments in *Drosophila* by altering the gut microbiomes using antibiotics in each genotype and measuring consequent behavioral phenotypes. We will also determine if alterations to the microbiome can affect the rate of digestion.

551B Effect of *L. plantarum* Abundance on the Climbing Ability of a *Drosophila melanogaster* Model of Parkinson's disease

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Parkinson's disease (PD), the second most common neurodegenerative disease in the United States, is commonly associated with predominantly motor and neurological symptoms. Recently, the development of non-motor symptoms have begun to be increasingly correlated with PD, with the most common symptoms being gastrointestinal (GI) disturbances such as gut dysbiosis and constipation. In fact, nearly all PD patients will display one or more GI symptoms, and will often do so years before any neurological symptoms develop. Due to the high prevalence of these GI symptoms in PD patients, it is thought that there is a mechanism of communication between the gut and brain. A recent study demonstrated that the gut microbiome has the ability to alter motor function of a mouse PD model. In a previous experiment by our laboratory, developmental effects were observed due to microbiome alterations in PD model (*park*^{25}) *Drosophila melanogaster* through a fecal transfer technique. The *park*^{25} flies are an excellent PD model as they possess many similar phenotypes to PD, such as decreased lifespan, dopaminergic neuron loss, and impaired motor function. To further test this relationship between the microbiome and its ability to affect motor ability, we inoculated axenic (germ-free) *park*^{25} and control (*w*^{1118}) *Drosophila* embryos with four different standard bacterial stocks including *Lactobacillus brevis, Lactiplantibacillus plantarum, Acetobacter pomorum*, and *Acetobacter tropicalis*. The embryos were either mono-associated with one bacterial strain or were given a combination of all four strains and motor function was tested via climbing assay once the adult flies reached 6-7 days post-eclosion. In PD model flies that were mono-associated with *L. plantarum* a beneficial effect on climbing was observed in some climbing metrics. However, when the flies received a combination inoculation with all four strains, that beneficial effect was subsequently lost. This result suggested the potential need for a certain abundance of *L. plantarum* to be reached in order for the beneficial climbing effect to occur. To test this, axenic PD and control embryos were inoculated with *L. plantarum* at various CFU concentrations including 10^2, 10^4, 10^6 and 10^8 CFUs. Climbing was measured and the flies were homogenized and bacterial colonies were cultured and counted to determine the average CFUs/fly. Initial results indicate that the control flies had a dose-dependent increase in CFU load, while the *park*^{25} flies were not as clearly dose-dependent and had the highest CFU load at 10^6 CFUs. The associated climbing data revealed that the 10^6 CFU *park*^{25} flies also displayed the most benefits in climbing. Our initial results suggest that in order for the PD flies to receive the climbing benefits from *L. plantarum*, a minimal CFU load must be reached. Further studies will be performed and presented at the meeting.

552C Modeling of *Drosophila melanogaster* gut microbiome growth interactions using reconstructed genome-scale metabolic networks

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Most if not all multicellular organisms are inhabited by a vast number of bacteria, the so-called microbiome. The bacteria present in the gut are the most abundant and important for various aspects of the hosts' physiology. On top of making food components accessible, microbiome members produce micronutrients required by the host as well as metabolites, which affect several of the hosts' signaling pathways. The importance of the gut microbiome becomes most evident in a dysbiosis – a disturbed composition of the microbiome, which was associated with multiple disease states including persistent infections, inflammatory bowel disease, diabetes, obesity or autism.
A lot of knowledge exists concerning varying microbiome compositions. The interplay between the gut bacteria, however, is still poorly understood. In mammals, this is at least in part based on the sheer complexity of the microbiome with hundreds to thousands of different species. Thus, model organisms are commonly used to investigate mechanistic questions. The gut

microbiome of *Drosophila melanogaster*, for example, turned out to be an exquisite model based on its low complexity of only about 10 leading bacterial species in laboratory flies.
Studying the interplay of gut microbiome members is often limited by difficulties of an *ex vivo* culturing or a specific detection of the different bacterial species. Modeling-based approaches bear the potential to overcome these obstacles and to investigate e.g. the metabolic interdependency of co-occurring bacteria. Here, we isolated abundant gut bacteria from laboratory-reared Drosophila, sequenced their respective genomes and used this information for the reconstruction of genome-scale metabolic network models. With these, we simulated growth in mono- and co-culture conditions and different media including a synthetic diet designed to grow *Drosophila melanogaster*. Our simulations support a synergistic growth of some but not all gut microbiome members. This growth promoting effect is based on the exchange of distinct metabolites. Our simulations thus provide an entry point to design "metabolite probiotics", which can be used to promote the growth of beneficial bacteria.

553A Host acquisition on the effects of the geography specific microbiota of *Drosophila melanogaster*

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The genotype of an organism can act in collaboration with the microorganisms that are present within a host organism to determine the organism's locally adapted phenotypes. These microorganisms are referred to as the microbiota. The microbiota of the gut plays a significant role in the development of the host along with its nutritional makeup, which can include tradeoffs between fecundity and lifespan. Studies have shown that host genotype can significantly alter the microbiota composition of wild flies (Dobson, 2015; Chaston, 2016). However, the way that host genotype selects for the microbiota is not fully understood. The goal of this project is to understand how the feeding preferences of *Drosophila melanogaster*, a model organism for analyzing microbiota, can help determine which microorganisms are associated with the flies. This will be done by setting up a choice versus no choice assay where the effects on microbiota variation will be measured when flies can or cannot choose between microbes in diet. This work fits into a broader set of questions by contributing to understanding the source of variation in microbiota composition.

554B Investigating the role of a bacterial secreted protein in *Drosophila* epithelial stem cell proliferation

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The Drosophila gut is home to a diverse collection of microorganisms collectively called the gut microbiota. To better understand the contributions of the gut microbiota to overall host health and development, flies can be reared to adulthood under axenic or germ free conditions. Germ free flies have a lower rate of intestinal epithelial stem cell proliferation compared to conventionally reared flies, that are raised with their microbiota intact. In previous investigations with germ free zebrafish and native symbiont Aeromonas, our laboratory found a secreted factor from Aeromonas, GlcNAc binding protein A (GbpA), is sufficient to increase intestinal epithelial proliferation. Our research has shown that GbpA is sufficient to increase intestinal epithelial stem cell proliferation in *Drosophila*. GbpA was originally characterized as a virulence factor in *Vibrio cholerae* that binds to GlcNAc and cleaves sugars through its lytic polysaccharide monooxygenase activity. GlcNAc is a sugar that composes chitin and mucin, two substances found in animal guts that provide a layer of protection between host gut epithelium and the gut microbiota. With this information, we propose that GbpA is increasing gut epithelial stem cell proliferation in *Drosophila* through its enzymatic action on GlcNAc polymers lining the gut. Here we show that GbpA can be fed to germ free adult flies as part of either a crude mixture with other bacterial secreted proteins or as a purified protein and both will increase gut epithelial stem cell proliferation. GbpA can also be ectopically expressed in fly intestinal epithelial stem cells and will increase intestinal epithelial stem cell proliferation in germ free flies. To investigate the topology of GbpA's mechanism of action, we plan to ectopically express GbpA in tissues in the gut that have differing levels of access to the GlcNAc polymers of the peritrophic matrix in the gut lumen. In tissues that contact the peritrophic matrix, we predict there will be an increase in intestinal epithelial stem cell proliferation in the presence of GbpA, while there will be no increase in intestinal epithelial stem cell proliferation in the presence of GbpA driven in cell types that do not contact the peritrophic matrix. We plan to visualize GbpA in the gut using immunohistochemistry to confirm whether GbpA accesses the peritrophic matrix as predicted. This research will begin to characterize the mechanism by which bacterial secreted proteins modulate intestinal epithelial stem cell proliferation.

555C Circadian Rhythms in Gut Microbes of *Drosophila melanogaster*

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Circadian rhythms are driven by internal clocks that govern daily patterns in physiology and behavior. The gut microbiome in an organism consists of bacteria, fungi, and viruses. These commensal microorganisms help organisms digest food and secrete molecules that affect host physiology and behavior. In mammals, the gut microbiome shows bidirectional interaction with host circadian rhythms. Host rhythms set up rhythmic fluctuations in relative abundance of bacterial genera, which in turn results in rhythmic secretion of molecules that can modulate host circadian rhythms.
The objective of this research is to determine whether the *Drosophila* gut microbiome exhibits daily rhythms in relative abundance of bacterial genera, and if those rhythms are responsive to the host circadian clock. Dissected guts were collected every 6 hours from flies kept under either 12:12 hours of light:dark or 24 hours constant light. Circadian rhythms in flies do not to function under constant light. Microbial DNA was purified using the QiaAMP Fast DNA Stool Mini Kit and the 16S rDNA was amplified with PCR using universal 16S primers. The 16S rDNA amplicons were sequenced using the Illumina platform and the genera were identified from these data using the EzBioCloud microbiome taxonomic profiling pipeline. Circadian rhythmicity in normalized counts was assessed using JTK_Cycle. We found significant differences in bacterial abundance between light and dark phases, particularly of *Gilliamella* and *Burkholderia* ($P<0.069$ and 0.007 respectively). This variation in abundance was abolished under constant light. Additionally, abundances of *Paenibacillaceae*, *Bradyrhizobiaceae*, and *Burkholderiaceae* were found to cycle significantly ($P< 0.0052$, 0.047, and 0.047, respectively). However, the conclusions are limited by an overabundance of *Wolbachia* sequences interfering with detecting other genera that are likely present in lower quantities. Current studies are investigating bacterial cycling in *Wolbachia*-free flies.

556A Identification and characterization of putative *de novo* evolved genes essential for *Drosophila* male fertility

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De novo evolved genes arise from previously non-protein-coding DNA regions. These genes are often expressed in the male reproductive tract. We performed an RNAi screen to identify putatively *de novo* evolved, testis-expressed genes that are essential for male fertility in *Drosophila melanogaster*. This screen identified several genes with major effects on fertility; here, we focus on two called *atlas* and *katherine johnson (kj)*, whose fertility phenotypes were confirmed with CRISPR-mediated knockout (KO). *Atlas* KO males show few sperm in their seminal vesicles due to incomplete spermatid individualization. Both an *atlas*-GFP knock-in line and an *atlas*-HA genomic rescue construct fully restored fertility in *atlas* null flies. Using these tools, we observed Atlas protein in the nucleus during the late canoe stage of spermatid nuclear condensation, but not in mature sperm, suggesting *atlas* may encode a transition protein that facilitates the transition from histones to protamines. Fertility assays on males knocked down or knocked out for *kj* showed that mutants produced progeny, but at significantly reduced levels. Dissection of mutant testes revealed high numbers of mature sperm, so functional analysis has focused on how mutant sperm behave upon transfer to the female. We are currently using the protamine-GFP sperm nuclear marker to count the number of sperm transferred to females at mating, the number of sperm stored in the female seminal receptacle after mating, and the number of sperm retained several days after mating. Finally, we investigated the evolutionary histories of *atlas* and *kj* using a combination of BLAST- and synteny-based techniques. Both genes are found throughout the *melanogaster* group of species, but also in *D. virilis*, implying an origin at the base of the *Drosophila* genus. Both genes have also been lost in certain lineages, such as the *obscura* species group, suggesting that the genes' essential functions in *D. melanogaster* might have evolved well after their *de novo* origins.

557B Rapid diversification and duplication of protamine (sperm chromatin) genes in *Drosophila*

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Many animals have independently acquired and deployed short, positively-charged proteins, called protamines, to tightly package sperm genomic DNA. Unlike highly-conserved histones, protamines evolve rapidly and show recurrent duplications in various lineages. However, the causes and consequences of this diversification remain poorly understood. The prevailing hypothesis posits that sexual selection, particularly sperm competition, drives protamine diversification. However, this model lacks experimental support and cannot explain the rapid evolution of protamines in species lacking sperm competition, including humans. To understand the biological forces driving protamine evolution, we first phylogenetically cataloged protamine diversity and innovation across *Drosophila* species. Protamine genes in *Drosophila* are not homologous to mammalian protamines; they independently arose from proteins containing a high mobility group (HMG) DNA-binding domain, which is commonly found in transcription factors. We found that, of 13 testis-specific genes with HMG domains, 11 have higher protein evolution rates (ω) than 95% of *Drosophila* genes. Using polarized McDonald–Kreitman tests, we showed that 4 of them are under positive selection in the *D. melanogaster* lineage. We found extensive duplication of protamine genes

across *Drosophila* species; these duplications are enriched on sex chromosomes. Several protamine genes that are essential for fertility in *D. melanogaster* have been lost in other *Drosophila* species. Moreover, two recently-evolved protamine genes are essential for fertility in *D. melanogaster*, whereas some ancestral protamine genes are not. Our analyses demonstrate recurrent selection and evolutionary turnover of protamine genes in *Drosophila*. We propose that young sex chromosome-linked protamine duplicates might be involved in the genetic conflicts between sex chromosomes by either inducing or suppressing meiotic drive.

558C Eya is a master regulator of egg chamber morphogenesis by coordinating epithelial and germline interactions

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In the developing *Drosophila* egg chamber, the follicle epithelium has to closely cooperate with nurse cells and the growing oocyte to produce fertile eggs. We found that the transcriptional co-factor Eya controls affinity of the apical epithelial surface for nurse cells and oocytes. This is necessary to drive a majority of morphogenetic changes throughout egg chamber development. Specifically, Eya expression dynamics guide segregation of anterior, main body and posterior cells over nurse cell and oocyte surfaces by stage 10A and, therefore, facilitate oocyte growth. Loss of Eya function gives rise to stage-specific phenotypes, ranging from a catastrophic loss of epithelial germline coverage to disruption of anterior cell flattening. Finally, manipulating Eya expression patterns in the epithelium interferes with the ability of the oocyte surface to expand its contact with the follicle epithelium, thereby disrupting the development of a fertile oocyte of proper size and shape. Our work sheds light onto how the close interaction between two tissues during organ development can be coordinated by a simple model of affinity regulation through one transcriptional regulator, and emphasizes the plasticity of epithelial behaviors during interactions with their surrounding tissues.

559A Investigating a role for septate junction proteins in planar polarity, cell shape changes and rearrangements during dorsal closure

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Septate junctions (SJs) are occluding barriers in invertebrate epithelia. In *Drosophila*, more than 20 genes are required for the formation or maintenance of SJs. Loss-of-function mutations in core SJ components are embryonic lethal, with defects in developmental events such as head involution and dorsal closure (DC) that occur prior to the formation of a mature SJ. This indicates a role for SJ genes in mid-embryogenesis, independent of their occluding function. However, the mechanistic role of SJ genes during morphogenesis is unknown. To address this, we are studying the function of SJ genes during dorsal closure (DC). DC occurs during mid-embryogenesis to seal a dorsal gap in the epidermis that results from germ band retraction. DC is initiated by Jun-N-terminal kinase (JNK) signaling cascade and is driven by contraction of the extraembryonic amnioserosa cells that temporarily cover the dorsal surface, and by elongation of the epidermal cells surrounding the gap. This epidermal elongation requires planar polarized expression of various molecular components including actin and tubulin. Also, local cell intercalations at the leading-edge relax the tension across the tissue. Here we hypothesize that SJ genes may be required for aspects of planar polarity during DC, failure of which may contribute to abnormalities in cell shape changes and rearrangements and ultimately defective DC. To test this, we analyzed fixed tissue of SJ mutant embryos for defects in epidermal elongation, cell rearrangements and planar polarity during DC. We observe that JNK signaling occurs normally in SJ mutant embryos, but the smooth organization of the leading-edge cells is disrupted, particularly during advanced stages of DC. Epidermal cells in SJ mutant embryos show significant defects in cell shape compared to wild type embryos, including a lower aspect ratio, reduced area and increased circularity of their apical surfaces. In addition, SJ mutants fail to maintain robust organization of microtubules and actomyosin in the epidermis towards the end of DC. However, SJ mutants show no significant defects in cell intercalations at the leading-edge during DC. We are investigating defects in cell shape changes and planar polarity in embryos with region-specific knockdown of SJ proteins. Also, we will use live-image analysis to examine SJ mutants for defects in microtubule and filopodial dynamics during DC.

560B Investigating the role of two conserved morphogens, Hh and Dpp, in the regulation of synchronized epithelial growth

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Animal development frequently requires multiple epithelia to grow in coordination with each other, producing proportional and functional multilayered organs. The Drosophila wing disc, the precursor to the adult wing and thorax, consists of two closely associated epithelia: the disc proper and the peripodial epithelium (PE). These grow synchronously during larval

development, even when growth of the wing disc as a whole is markedly perturbed. Studies of the disc proper in isolation have yielded valuable insights into growth regulation in epithelia. However, due to the scarcity of research on the PE, it is unknown how the two layers of the disc coordinate their growth.

In the disc proper, two conserved morphogens, Hedgehog (Hh) and Decapentaplegic (Dpp/TGF-B), have long been known as crucial growth regulators. As secreted morphogens involved in disc proper growth, these two morphogens are candidate regulators of PE growth, potentially acting to coordinate growth between the layers. We investigated the role of Hh and Dpp in PE growth, using newly characterized genetic drivers that make the PE more genetically accessible. By performing timed, PE-specific knockdowns of the Hh pathway component Smo, we found that Hh is required only for very early growth of the PE, and that later PE growth can occur in the absence of wild-type Hh signaling. We used a temperature-sensitive Hh allele to determine that PE Dpp expression is Hh-dependent, as is the case in the disc proper. However, PE-specific disruptions of Dpp reception show that, in sharp and surprising contrast with the disc proper, the PE is able to grow and develop almost completely normally in the absence of detectable Dpp signaling. Our work shows that Hh and Dpp are not major growth regulators in the PE, suggesting the existence of other signals which regulate PE growth and coordinate it with growth of the disc proper.

561C Ion channel mediated signaling in wing development

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Cells must communicate precisely for correct tissue patterning. Recent evidence has indicated that in addition to using molecular pathways to instruct patterning, cells communicate via electrical signaling mediated by ion channels. Using the *Drosophila melanogaster* wing as a model for morphogenesis, our lab has identified 46 ion channels that are important for wing development. In addition, we have found that spontaneous calcium waves and transients occur in both the *Drosophila* larval wing disc and the pupal wing. Of the ion channels that we identified as required for proper wing development, five of them, Best2, SK, Stim, Orai, and SERCA, regulate or respond to intracellular calcium in at least in some cellular contexts, suggesting that regulation of intracellular calcium may be important for wing development. Using immunohistochemistry staining of downstream pathway components in the known canonical developmental signaling pathways, we found that Best2, a calcium activated chloride channel, is required for proper BMP and Notch signaling at the larval stage and correct BMP signaling at the pupal stage. Our results suggest that ion channels play an important role in the tissue patterning of the *Drosophila melanogaster* wing, and we identify a number of ion channel candidates for future study to further understand mechanisms by which ion channels influence morphogenesis.

562A Malvolio, the *Drosophila* ortholog of the mammalian NRAMPs, is a fork head target required for embryonic salivary gland morphogenesis

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Malvolio (Mvl) is a member of the SLC11 family of metal ion transporters, and is the Drosophila ortholog of the mammalian natural resistance-associated macrophage proteins (NRAMPs). The family members (NRAMP1 and NRAMP2) function as general metal ion transporters and use proton motive force to transport Fe^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, and Co^{2+}. The key roles for NRAMPs are in maintaining ion homeostasis essential to support the antimicrobial activities of phagocytic cells. Here we report a likely role for Mvl in the morphogenesis of embryonic salivary gland (SG). Mvl expression in the SG is fork head-dependent. Examination of multiple Mvl mutant alleles reveals cell invagination defects leading to morphological abnormalities of the SG tube. Mvl-GFP localizes to the luminal membrane of SG epithelium, and is enriched in basal cytoplasm. The localization of adherens junction marker E-cadherin and polarity marker Bazooka in *Mvl* mutants is comparable with the wild-type SGs. The localization of apical polarity determinant Crumbs in *Mvl* mutants, however, showed decreased enrichment compared to the wild-type SGs. The *Mvl* mutants, furthermore, show severe cuticle defects and also feature a loose assemblage of a yet unidentified population of cells in the anterior region of the embryo. That the Fe^{2+} transport activity of MVl is essential for SG morphogenesis was implied by the cell invagination defects—phenocopying the *Mvl* mutants—resulting from a deficiency that includes deletion of an iron-binding septate junction component (*Transferrin 2*). Thus, the role of Mvl in the embryonic SG presents an opportunity to investigate the link between cellular ion homeostasis and morphogenesis.

563B Scaling between cell area and stress fiber number adjust cell elongation and mechanosensitivity during epithelial tissue dynamics.

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The scaling of the properties and dynamics of biological systems with their size is central to organismal growth, morphogenesis, physiology and metabolism. However, such scaling remains a poorly explored question in the field of cell mechanics and mechanosensitivity. Here, by examining how *Drosophila* epithelial tissue responds to morphogenetic forces, we identified a class of apical stress fibers (aSF) that anchor to the adherens junctions. Combining experiments and modeling, we found that the number of aSF scales with cell apical area and that this scaling is critical to prevent the elongation of larger cells in response to morphogenetic tissue stress. Furthermore, we uncovered that aSF confer a scaling between Hippo/YAP activity and apical area to regulate epithelial cell proliferation. The scaling between aSF number and cell size is mainly driven by the number and distribution of tricellular junctions, which leads in larger cells to an increase in both aSF nucleation rate and life-time. Tissue morphogenesis and proliferation entails major changes in epithelial cell area driven by mechanical forces; our work highlights how in turn cell mechano-sensitivity scales with cell area to control tissue dynamics.

564C Spatially patterned cell death affects wing local growth and morphogenesis

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Programmed cell death, or apoptosis, is an ubiquitous process during morphogenesis. It has been suggested that apoptosis was required to buffer developmental noise leading to Drosophila wing *size* variation [1]. Regarding tissue *shape*, the mechanisms underlying the instructive and/or buffering roles of apoptosis in the regulation of wing *shape* are unclear, especially since apoptosis has been described as unpatterned and scarce in this tissue [2]. To address the question of the contribution of apoptosis to wing shape morphogenesis, we first characterised the effects of know-down of proapoptotic gene *hid* and of induction of ectopic death by optogenetically overexpressing Dronc. Using a precise morphometric approach, we found consistent effects on tissue shape: inhibiting apoptosis leads to rounder wings whereas ectopic apoptosis leads to more elongated shapes.

We next characterized the spatial map of cell death in the larval wing pouch with an unprecedented spatial resolution. First, we monitored the activity of apoptotic caspases and found spacially reproducible patterns of caspase activity. Second, we used a clonal approach to quantify the spatial pattern of cell death and local growth heterogeneities during larval development, and found a consistent pattern between local caspase activity and cell death. Our data show that, contrary to previous findings, there is patterned cell death during larval wing morphogenesis. In addition we show that cell death contributes to govern the shape of the tissue in a quantitative way.

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[2] Milan et al. 1997. *PNAS*.

565A Characterizing cell adhesion and cytoskeletal networks during development of the amnioserosa, a squamous epithelium

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Epithelia can be shaped through cytoskeletal rearrangements and remodeling of cell adhesion complexes. There are many well-studied actin- and myosin-based mechanisms of epithelial morphogenesis, but far fewer examples of microtubule (MT)-based mechanisms. *Drosophila* embryogenesis requires the support of the amnioserosa, an extraembryonic tissue that undergoes dynamic developmental changes. The amnioserosa is initially made up of hexagonal columnar cells with MT arrays running apicobasally. These MT arrays then rotate 90° to become aligned with the tissue plane as the cells to become squamous and spindle-shaped. We are investigating subsequent steps of amnioserosa development and how the cells maintain cell-cell adhesion. After their rotation, the MT networks re-organize. At stage 8, each network displays an organization individualized for each cell with MTs running along the apical, lateral and basal surface of the cell. At stage 11, MT organization becomes more coupled between cells and MTs display greater enrichment at the level of adherens junctions (AJs). Individual MT bundles also seem to rearrange, as evident from the appearance of a streaks of Patronin-GFP (a MT (-)end binding protein). Although actin networks localize at AJs, junctional actin and myosin levels appear lower in the amnioserosa than in the neighbouring epidermis. RNAi of α-catenin, the linker protein between AJs and actin, strongly fragmented DE-Cadherin-positive AJs in the epidermis, whereas DE-Cadherin in the amnioserosa remained continuous. In contrast, RNAi of Bazooka (Baz), a scaffold protein and junctional organizer, resulted in amnioserosa AJ fragmentation, whereas AJs in the epidermis remained continuous. With mild fragmentation of amnioserosa AJs following Baz RNAi, MT networks remained

largely intact, but with greater AJ fragmentation, MTs became focused at residual AJs. To examine how Baz acts at amnioserosa AJs, its localization was examined. Using multiple probes, Baz was found to form large puncta along amnioserosa AJs by stage 11. These puncta were much larger than Baz puncta in the epidermis, and were more sparsely distributed along AJs. Overall, compared with the epidermis, the amnioserosa gains a distinctive association between MTs and AJs, and relies on Bazooka more than α-catenin for the continuity of AJs across the tissue.

566B Irregular oscillations in apical cell area during sex comb rotation in *D. melanogaster*

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Epithelia are studied intensively not only because they are considered the primary building block of animal complexity but also because in humans, about 90% of cancers originate in epithelial cells. In recent years, live imaging of fruit fly epithelia has shown that tumour growth outcompetes the surrounding wild type epithelium, thus leading to tissue rearrangements such as changes in cell size. Here, we studied the morphogenesis of an epithelial tissue during sex comb rotation, a normal process which mimics in some respects the cell competition observed during tumour progression. The sex comb is a row of bristles present on the first leg of *Drosophila melanogaster* males. During development, these bristles rotate from a perpendicular to a parallel position on the leg axis. As this row of bristles moves, the epithelia below the sex comb increases in area, while the epithelia above the comb dramatically decreases in cell area. While previous work focussed on the cellular processes involved in comb rotation by studying the average changes in apical cell area, here we focus on the dynamics of cells in four regions cells surrounding the developing comb, and examine the change in apical area of these cells as the comb rotates. Using a fly line containing Ubi*DE*cadGFP which outlines cell boundaries we live imaged and recorded 3 time lapses videos taken at 23 and 36 hours after pupation.
We quantified the apical area of multiple 5-cell clusters per region by tracing the cells in ImageJ. We found three different types of changes in apical cell area in the regions studied: 1) expansion, 2) compression, and 3) little to no change in size. Surprisingly, independent of the regions studied or behaviour observed, all cells exhibited a high degree of oscillation in apical cell area. In other words, while this tissue displays gradual and directional changes in size, epithelial cells show irregular pulses, increasing and reducing size during morphogenesis. Although these finding resemble the “ratcheting” mechanism described during fruit fly embryonic stages, the oscillations found here seem to be more stochastic in time and space. Altogether, our observations raise the question of how the cells surrounding the comb display irregular oscillations, but the tissue overall is changing in a gradual manner. In addition, future work needs to evaluate whether other epithelial systems display a similar behavior and whether cancer progression could modify the cellular oscillations described in this work. Our next goal is to reexamine tumour cell behavior to see if tumour cell competition exhibits similar oscillations and if differences in the rate of these oscillations may be associated with tumour competition outcomes.

567C Degenerating *Drosophila* larval epidermal cell layer drives epithelial tissue closure during thorax development

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Epithelial tissue gap closure ensues when the development of an organ requires the fusion of its two contralateral halves. Thorax closure in *Drosophila* represents one such tissue closure event wherein contralateral progenitor halves of the adult thorax, the heminotal epithelia (HEs), migrate and zip, while the larval epidermal cell (LEC) layer gives way. LECs have been considered as a ‘passive substrate’ so far and its role in thorax closure has not been understood so far. Here we show that the LEC layer acts as the dynamic structure that generates pull forces which is relayed to the HEs underlies thorax closure. During thorax closure, the LEC layer displays active cell contractions—via non-muscle myosin II and actin—besides cell delamination and death, culminating in overall tissue shrinkage. Pull force thus generated by the shrinking LEC layer, is then relayed to the HEs by their mutual adhesions via βPS and αPS3 integrins. Suppression of cell death in the LEC layer by a gain of p35 slows down HEs migration, while the surviving LECs overcrowd and impede HEs zipping. Further, abrogation of non-muscle myosin II activities in the LECs, or knockdown of integrins in either LECs or HEs arrest thorax closure. Mathematical modeling of thorax closure further confirms these biophysical underpinnings of thorax closure wherein a degenerating LEC layer mediates its own succession. The mechanism of tissue closure revealed here appears ancient in origin and appears to underlie wound healing as well.

568A Dynamic analysis of the *DPP* dorsal open phenotype

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Dorsal closure is one of the last morphogenetic events of *Drosophila* embryogenesis. The closure not only generates a continuous epidermis but also shapes the embryo as a maggot. At the cellular level, the epidermis migrates dorsally to enclose a hole covered by a squamous tissue, the amnioserosa. Often used as a model to study wound-healing, dorsal closure differs from classic healing as it is concomitant with the movement of other organs within the embryo. The mesoderm also migrates toward the dorsal midline as the future heart forms, and the head of the embryo involutes within the thorax, a process known as head-involution. Here we investigate the interaction between several organs that move synchronously during closure. The mutant for the *DPP* receptor *thickveins* displays a dorsal closure phenotype thus providing us with an excellent paradigm to study the coordination of tissue morphogenesis in the late embryo. Surprisingly, the precise caracterisation of the violent evisceration of *thickveins* embryos reveals that the overal migration speed of the dorsal epidermis is not affected. Rather, our dynamic analysis suggests that a number of defects at the interfaces between migrating organs is at the origin of the lethal phenotype. We will propose a dynamic model to explain DPP function during late embryonic development.

569B Hedgehog on the move: Control of epithelial spreading and fold formation during late *Drosophila* embryogenesis

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Morphogens are not only important for the patterning of cell identities in early development but also regulate the spatial distribution of cytoskeletal protein activity during morphogenesis. As a key example, Hedgehog signaling regulates the patterning of cell tension during the late stages of *Drosophila* embryogenesis. However, it is still unclear how Hedgehog regulates these processes at different stages of epidermal spreading and subsequent fold formation. To address this knowledge gap, we performed a quantitative analysis to investigate the spatiotemporal dynamics of Hedgehog signaling and cell shape changes during head involution. We identified two major phases of epidermal spreading characterized by the velocity of leading-edge movement toward the anterior head region. In addition to the previously reported defects in tissue spreading, mispositioning of segment widths, we observed inhibition of fold formation with ectopic activation of Hh signaling. Depending on the level of ectopic Hedgehog signaling, the outcome of head involution varies from the absence of spreading and fold formation to the formation of new actomyosin boundaries, which were not reported previously. Also, we found that there exists a choreography of Hh expression dynamics that correlates with specific steps in tissue spreading and fold formation. Canceling this spatiotemporal patterning of Hedgehog activity by uniform activation resulted in a nonuniform pattern of repressed E-cadherin levels accompanied by increased, nonuniform levels of Myosin. Overall, this study defines the functional relationship between patterned Hh signaling and cell mechanical processes that occur during tissue spreading and epidermal fold formation.

570C Interplay between patterning and morphogenesis of the cephalic furrow

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The cephalic furrow is a deep epithelial fold that demarcates the head-trunk boundary of *Drosophila* during gastrulation. It is formed by a unique series of morphogenetic events combining cell shape changes and tissue invagination. The process begins with a single row of blastoderm cells shortening in their apicobasal axis. These initiators and their neighbouring cells then progressively invaginate deep into the yolk sac. The resulting furrow is symmetric and displays stereotypic cell morphologies at the infolding point and outer invagination edges, suggesting the entire structure is under precise genetic control. While *buttonhead* and *even skipped* are required to specify the initiator cells, it is unclear if other genes contribute to the patterning of the cephalic furrow sub-regions or to the process of invagination. To better understand the interplay between molecular patterning and morphogenesis, we investigate known and newly identified genes involved in cephalic furrow formation using multiplexed *in situ* hybridization and lightsheet microscopy. By analyzing the expression of relevant genes at single-cell resolution, we generate a molecular map of the developing cephalic furrow during wild type embryogenesis. We then describe how these molecular arrangements are disrupted in mutant embryos, and investigate the impact of mis-specification to the morphogenesis of the cephalic furrow by imaging at high-temporal resolution. We find that perturbing the molecular arrangements at the head-trunk interface often disrupts the normal process of cephalic furrow invagination, but that most mutants still exhibit reminiscent initiator cell behaviors. This suggests the patterning of cells around the infolding point and furrow edges can also affect the invagination process independently of the specification of initiator cells. Interestingly, late transient epithelial folds of variable shapes commonly occur in the cephalic region of mutant embryos, suggesting that mechanical forces of adjacent tissues might contribute to the process of cephalic furrow invagination. Altogether, these observations highlight the tight coordination between molecular patterning and morphogenetic events that control the cephalic furrow formation, and provide clues about the role of the cephalic furrow in *Drosophila* gastrulation.

571A Characterization of novel *Drosophila* Egf receptor signaling targets with roles in eggshell structure and morphology

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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 were observed to play roles in normal eggshell structure and morphogenesis. Gene mutant/knockdown phenotypes include decreased chorionic integrity, shortened eggs, and various dorsal appendage malformations, as well as decreased fertility. We have used the CRISPR-Cas9 system to create mutations in some of these "morphogenesis genes." These mutants have so far recapitulated the previously observed phenotypes, and in at least one case resulted in the observation of an additional phenotype in our null mutant, not seen in the original P-element insertion. We are currently using these CRISPR mutants for further study and characterization of the genes.

572B The muscle founder cell identity gene *apterous* regulates muscle attachment

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Skeletal muscles come in a variety of shapes and sizes important for functions like running or eye blinking; however, we do not yet understand the mechanisms that generate muscle fibers with discrete morphologies. In the *Drosophila* embryo, muscle properties like shape, attachment and orientation are specified by the expression of transcription factors known as founder cell identity genes. Over a dozen founder cell identity genes are known to be expressed in incompletely overlapping subsets of muscles to regulate final muscle characteristics. To determine how morphological information is translated from identity gene transcription factors to cellular processes controlling muscle size and shape, we focused on the identity gene *apterous*, which is expressed in six muscles in each abdominal hemisegment. We found that embryos with gain or loss of *apterous* function displayed attachment defects, including missing or incorrect attachments. Using time-lapse confocal microscopy we have found that misexpression of *apterous* in the somatic musculature leads to loss of muscle attachment upon the onset of contractions, resulting in embryonic death. We have shown that overexpression of *apterous* leads to loss of both beta-PS Integrin and alpha-PS2 Integrin from myotendinous junctions. Our work establishes a clear function for *apterous* in the regulation of muscle attachment, linking changes in gene expression to alterations in muscle morphology.

573C Environmental mercury toxicity perturbs indirect flight muscle development: Effects on neuroligin 1 implicates neuromuscular junction-specific targets

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Methylmercury (MeHg) is an environmental, organic mercurial compound that is ubiquitously found in seafood. MeHg is a developmental neurotoxicant capable of causing cognitive and motor deficits in children. The presentation of motor deficits suggests that MeHg may act on muscle-derived targets in the developing neuromuscular system, and potentially the neuromuscular junction (NMJ). We previously conducted a genome-wide association study (GWAS) using *Drosophila melanogaster*, which revealed many neuromuscular-associated genes that accompanied a muscle phenotype of myospheres in the indirect flight muscles (IFM). By assessing morphological and functional phenotypes of adult structures formed during pupal metamorphosis, following larval exposure to MeHg, we explored the existence of muscle-derived MeHg targets that might act in conjunction with neural targets at the NMJ. The IFM neuromuscular morphology was visualized using fluorescent reporter fly strains and immunostaining and neuromuscular function was assessed via eclosion and flight behavior. Through transgenic modulation of the Nrf2 pathway activity in either muscle or neural lineages during development, we first demonstrate that protecting either muscle or neuron development moderates MeHg toxicity and protects IFM morphogenesis, seen by a reduction in myosphere number. This rescue in IFM development parallels an improvement in eclosion and flight ability, indicating that both the developing muscle and motor neurons contribute to the MeHg-induced

phenotypes and prompting us to explore a role for the NMJ. Neuroligin 1 (nlg1), an identified GWAS candidate, is a muscle-restricted NMJ-associated factor and the heterodimeric synaptic partner of neurexin-1 (nrx1). Through RT-qPCR, we show that *nlg1* expression, relative to other nlg and nrx members, is selectivity repressed early in pupal metamorphosis by MeHg. Muscle-specific overexpression of *nlg1* partially rescues MeHg-mediated deficits in both eclosion and flight. These findings indicate that both muscle and neurons are targets of MeHg, the forming NMJ is a MeHg-sensitive component of the neuromuscular system, and highlights *nlg1* as a candidate that mediates MeHg's disruption of NMJ development. Future research will extend investigations of Nlg1 and its heterotypic protein interactions at the NMJ as targets of MeHg, which may underlie the etiology of MeHg induced motor deficits. Supported by R01ES025721, R01 ES010219, and T32ES007026

574A Secondary structure of the novel myosin binding domain WYR and implications within myosin structure

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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). Fln has a three domain structure that includes WYR, a novel 52 aa domain conserved throughout Pancrustacea. In this study we (i) test the hypothesis that WYR binds the LMM, (ii) characterize the secondary structure of WYR, and (iii) examine the structural impact WYR has on the LMM. Circular dichroism at 260-190 nm reveals a structural profile for WYR and supports an interaction between WYR and LMM, further supported by cosedimentation with a stoichiometry of ~2.4:1. The WYR-LMM interaction results in an overall increased coiled-coil content while curtailing alpha helical content. WYR is found to be composed of 15% turns, 31% antiparallel beta, and 48% 'other' content. A hypothetical structure of WYR including an antiparallel beta hairpin between Q92-K114 centered on an ASX or beta turn around N102, with a G1 bulge at G117, is proposed. The Drosophila LMM segment used, V1346-I1941, encompasses conserved skip residues 2-4 and is found to possess a traditional helical profile but is interpreted as having <30% helical content by multiple methods of deconvolution. This is the largest segment of the Drosophila LMM characterized by CD and this low helicity may be affiliated with dynamic behavior of the structure in solution or inclusion of a known non-helical region in the C-terminus. Our results support the hypothesis that WYR binds the LMM and that this interaction brings about structural changes in the coiled-coil. These studies implicate fln, via the WYR domain, for distinct shifts in LMM secondary structure that could influence structural properties and stabilization of the thick filament, scaling to modulation of whole muscle function.

575B Cellular basis of coupling between the central clock and the peripheral prothoracic gland clock in *Drosophila melanogaster*

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In *Drosophila melanogaster*, the emergence of adult fly is controlled by the circadian clock, which restricts the time of 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. We have recently shown that central brain clock neurons communicate with the PG clock by transmitting time information to neurons that produce the neuropeptide, PTTH, and that these neurons in turn communicate with the PG via the PTTH neuropeptide. However, the cellular mechanism by which PTTH neurons transmits the time information to the PG has not been described. Similarly, it is not known whether other PTTH-independent signal transduction mechanisms in the PG are relevant to the rhythm of adult emergence. Here, using genetic manipulations in the PG, we evaluated whether Gq-signaling pathways independent of PTTH transduction could regulate the rhythm of adult emergence. Using immunofluorescence, we observed daily changes in the levels of PTTH present in the terminals of the PTTH neurons in the PG. Finally, using a genetically encoded calcium sensor, we show that calcium levels in PTTH neurons are under circadian control. Establishing how PTTH signaling affects the PG clock during adult emergence could serve as a paradigm for understanding how central and peripheral clocks are coordinated.

576C A sex-specific switch between visual and olfactory inputs underlies adaptive sex differences in behavior

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While males and females largely share the same genome and nervous system, they differ profoundly in reproductive investments and require distinct behavioral, morphological and physiological adaptations. How can the nervous system, while bound by both developmental and biophysical constraints, produce these sex differences in behavior? Here we uncover a

novel dimorphism in *Drosophila* that allows deployment of completely different behavioral repertoires in males and females with minimum changes to circuit architecture. Sexual differentiation of only a small number of higher-order neurons in the brain leads to a change in connectivity related to the primary reproductive needs of both sexes - courtship pursuit in males and communal oviposition in females. This study explains how an apparently similar brain generates distinct behavioral repertoires in the two sexes and presents a fundamental principle of neural circuit organization that may be extended to other species.

577A Loci of individuality in the *Drosophila* olfactory circuit

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Individuality is a fundamental aspect of behavior that is observed even in isogenic flies reared in the same environment. Broad individual-to-individual behavioral distributions are observed in odor preference, exploratory handedness bias, and other sensory and motor modalities. Behavioral variation may confer evolutionary benefit as a "bet hedging" strategy against fluctuating environments. While some neural circuit elements have been identified as controlling the extent of variability in some behaviors, the exact circuit mechanisms underlying individual differences in sensorimotor behaviors are largely unknown.

Olfaction is a powerful model for studying neural loci of individuality, as the relevant circuit elements are well characterized and generally stereotyped across individuals. By combining behavioral and neural activity recordings within the same individuals, we have recently identified loci of individuality within the antennal lobe. To measure behavior, we tracked individual flies in linear chambers in which each half was perfused with one of two odors, MCH or OCT. Individual odor preference scores were calculated as the fraction of time the fly spent in one half of the chamber. To measure neural activity, we performed two-photon imaging of ORN (Orco-gal4) or PN (gh146-gal4) calcium dynamics across glomeruli of individual flies in response to a panel of odors. A linear model of glomerular activations on odor preferences revealed that idiosyncratic PN, but not ORN, dynamics from a subset of glomeruli were predictive of OCT-MCH odor preference.

While we have found that idiosyncratic activity in a subset of PNs constitutes a locus of behavioral individuality, how these idiosyncratic dynamics arise remains unknown. Local inhibitory neurons (LNs) are one likely source of neural variation, as LN wiring patterns differ widely across individuals (Chou et al, 2010), and silencing LNs decreases behavioral variation (Honegger, Smith, et al, 2019). Here we test the hypothesis that morphological variation in LNs across individuals drives individual variation observed in PN activity using an *in silico* circuit model of the AL that resolves ORNs, PNs, and LNs.

We utilize behavioral and neural activity recordings and mathematical modeling to identify loci of individuality in the fly olfactory circuit. This work contributes to our understanding of the mechanistic underpinnings of variation in odor preference and decision-making even within seemingly stereotyped neural circuits.

578B Identification of neural circuitry underlying *prickle*-mediated seizures in *Drosophila.*

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Epilepsy is a neurological disorder predicted to affect 65 million individuals worldwide. Our laboratory has previously shown that mutations in the Planar Cell Polarity (PCP) gene *prickle* cause myoclonic-like seizures in flies similar to the seizures observed in human patients with mutations in orthologous *PRICKLE* genes, identifying a genetically tractable model system for studying this atypical epilepsy. Using this model, we thus aim to understand the mechanisms by which disruptions in a PCP gene known to broadly affect developmental processes can result in a highly conserved seizure phenotype. To do so, we have leveraged the Gal4/UAS system to drive expression of *prickle* in specific neuronal circuits in order to help identify candidate neurons whose dysfunction underlies the seizure phenotype. As a first step, we have shown that pan-neuronal expression of *prickle* in homozygous null *prickle* mutant flies suppresses the seizure phenotype, thereby demonstrating that the phenotype is neuronally derived. To further define whether a specific neurotransmitter-based neuronal subset is associated with the seizures (similar to what has been observed for channelopathy epilepsies such as Dravet Syndrome), we have driven *prickle* expression in either glutamatergic, GABAergic, serotonergic, dopaminergic, or cholinergic neurons. Somewhat surprisingly, none of these experiments resulted in suppression of the seizures, suggesting that the seizure phenotype likely results from the dysfunction of complex neuronal circuits rather than specific neuron types. We are now targeting expression of *prickle* in broader brain regions of the *prickle* mutants, including those associated with motor and posture control such as the central complex, lateral accessory lobe, gnathal ganglion, and saddle. Preliminary evidence suggests that driving prickle

expression in the gnathal ganglion partially suppresses the seizure phenotype, which would identify a portion of the *prickle*-mediated seizure circuitry in the brain. As a reciprocal experiment, expression of *prickle* in all neurons outside the brain fails to rescue the seizure phenotype, thus providing the first evidence that, similar to humans, fly seizures may be brain-derived. This work is supported by NIH/NINDS R01NS098590.

579C Dopamine-based mechanism for transient forgetting

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Active forgetting is an essential component of the brain's memory management system. Forgetting can be permanent, in which prior memory is lost completely; or transient, in which memory exists in a temporary state of impaired retrieval. Such temporary blocks on memory seem universal, and can disrupt an individual's plans, social interactions, and ability to make rapid, flexible and appropriate choices. However, the neurobiological mechanisms that cause transient forgetting are unknown. Here we identify a single dopamine neuron in *Drosophila* that mediates memory suppression resulting in transient forgetting. Artificially activating this neuron failed to abolish the expression of long-term memory. Rather, it briefly suppressed memory retrieval, with memory becoming accessible with time. The dopamine neuron modulates memory retrieval by stimulating a unique dopamine receptor expressed in a restricted physical compartment of the axons of mushroom body neurons. This mechanism for transient forgetting is triggered by interfering stimuli presented just prior to retrieval.

580A Fungi hitch-hike via the Toll pathway into the brain to induce neurodegeneration and thrive on the immobile host

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Parasites have evolved behaviour manipulation strategies to exploit their host for nutrition and reproduction. In Drosophila, the fungus *Enthomophthora muscae* affects the nervous system, altering behaviour. Furthermore, volatile compounds produced by fungi can cause loss of dopaminergic neurons and locomotion deficits, reminiscent of Parkinsonism. The molecular mechanisms by which pathogens may affect the brain and behaviour are unknown, and here we explore whether Toll signalling may be involved. Toll receptors in Drosophila, like Toll-like-Receptors in mammals, are responsible for innate immunity. Toll receptors also regulate embryonic dorso-ventral patterning and nervous system development. In the central nervous system, Tolls normally bind ligands of the neurotrophin family to regulate the neuronal number, connectivity, structural synaptic plasticity, and behaviour. We are investigating how the fungus *Beauveria bassiana* alters the *Drosophila* brain and will present evidence on the underlying signalling mechanism, cellular outcomes and behaviour. Altogether, our findings will provide insight into the evolutionary race between organisms and how to protect and maintain brain health in humans.

581B Inhibitory circuits in grooming

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Nervous systems control the assembly of multiple flexible actions in order to produce complex behaviors. Grooming behavior in *Drosophila* is an excellent model to study neural circuits that regulate coordinated, sequential execution of movements. Serial grooming actions are observed in flies coated with dust. These flies choose to clean only one body part at a time, demonstrating mutual exclusivity among competing choices, and they prioritize cleaning anterior body parts over posterior ones, demonstrating hierarchical suppression (Seeds et al., 2014; Hampel et al., 2015, 2017). We investigate the role of specific inhibitory interneurons in regulating the sequential choice of grooming actions.

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the fly and vertebrate brain (Hosie et al., 1997; Ennel et al., 2007; Fei et al, 2010). Twelve GABAergic lineages are present in each segment of the ventral nerve cord (VNC) in *Drosophila* (Lacin et al., 2019; Harris et al., 2015). We performed a targeted behavioral screen to identify specific inhibitory neurons that disrupt normal grooming. We identified subsets of GABAergic lineage 13 neurons that regulate two types of coordination: intra-segmental (between the left and right limbs) and inter-segmental (foreleg-hind leg). Two hemilineages, 13A and 13B, project to the ipsilateral and contralateral regions respectively in a given segment of VNC (Harris et al., 2015). Activation of some neurons results in disruption of left-right leg coordination, and flies also prioritize posterior body cleaning over anterior.. Preliminary analysis of their connectivity using an electron-microscopy dataset (Maniates-Selvin et al., 2020) revealed presynaptic commissural interneurons, descending neurons, and intersegmental neurons. These inhibitory neurons or others may connect directly to sensory neurons, since RNAi knockdown of Rdl receptors in mechanosensory neurons also results in a similar phenotype.

The combination of targeted behavioral screens and neural circuit mapping using EM data provides a way to determine how neural circuit motifs contribute to limb coordination and action selection.

582C The targets of the transcription factor DATILÓGRAFO reveal the blueprint of a neuronal unit that generates decisions

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The ability to make decisions in a changing environment, such as seeking shelter, protecting territories, and selecting mates, is essential for animal survival and species perpetuation. Despite the significance of decisions, the molecular and cellular mechanisms behind decision-making are still poorly understood. Previously, we showed that the transcription factor DATILÓGRAFO (DATI) is required for the decision of accepting or rejecting courting males in *Drosophila* and identified brain regions involved in this processing. To understand the mechanisms of neuronal processing of decision-making regulated by DATI, we searched the *Drosophila* genome for genes containing DATI binding sites. Almost 90% of the genes identified have homologues in humans that also contain DATI binding sites and nearly 10% of them interact physically with APP, the protein involved in familial Alzheimer's disease. These results reveal a conserved gene network likely required for essential cognitive functions. To functionally validate the requirement of these genes in logical processing, we knocked down 39 of them in *Drosophila* and identified 9 genes that are required for decisions. This is the largest number of genes involved in female-decision making identified in a single screening. This group of genes constitutes the blueprint of a fundamental neuronal unit that generates decisions, which we refer to as the DATI neuroprocessor. This neuroprocessor deploys two excitatory receptors, nAChRα6 and nAChRα7, and two inhibitory receptors, Dop2R and GluClα. In addition to these receptors, we also identified the receptor anchor protein CORA; the regulator of translation BRU3; the negative chromatin regulator HDAC4; a novel gene we here name *dati switch* (CG1677/*ditch*), which is predicted to regulate mRNA production and decay; and the calcium channel Ca-α1T, which has been implicated in burst firing. By using spatial statistics, we show that CORA regulates the localization of the receptors identified and creates a parallel circuit that performs two functions. The first function is the transduction of excitation to a center where acceptance is generated, and the second is the suppression of excitation in other paths that elicit alternative behaviors. In addition to the parallel circuit we have also identified a serial component in the circuit whose potential function is to create temporality in the process of acceptance.

583A Aversive Training Induces Both Pre- and Postsynaptic Suppression in *Drosophila*

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The α'β' subtype of *Drosophila* mushroom body neurons (MBn) is required for memory acquisition, consolidation and early memory retrieval after aversive olfactory conditioning. However, *in vivo* functional imaging studies have failed to detect an early forming memory trace in these neurons as reflected by an enhanced G-CaMP signal in response to presentation of the learned odor. Moreover, whether cellular memory traces form early after conditioning in the mushroom body output neurons (MBOn) downstream of the α'β' MBn remains unknown. Here, we show that aversive olfactory conditioning suppresses the calcium responses to the learned odor in both α'3 and α'2 axon segments of α'β' MBn and in the dendrites of α'3 MBOn immediately after conditioning using female flies. Notably, the cellular memory traces in both α'3 MBn and α'3 MBOn are short-lived and persist for less than 30 min. The suppressed response in α'3 MBn is accompanied by a reduction of acetylcholine (ACh) release, suggesting that the memory trace in postsynaptic α'3 MBOn may simply reflect the suppression in presynaptic α'3 MBn. Furthermore, we show that the α'3 MBn memory trace does not occur from the inhibition of GABAergic neurons via $GABA_A$ receptor activation. Since activation of the α'3 MBOn drives approach behavior of adult flies, our results demonstrate that aversive conditioning promotes avoidance behavior through suppression of the α'3 MBn-MBOn circuit.

584B Altering neural activity during pupal stages affects the bang-sensitivity of adult *Drosophila*

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Epilepsy is a common neural disorder hallmarked by recurrent, unprovoked seizures. The bang-sensitive (BS) phenotype in *Drosophila* offers a useful model for studying the development of neuronal excitability and epilepsy. One recently identified bang-sensitive locus is *julius seizure* (*jus*), which encodes a novel transmembrane protein and is responsible for seizure

sensitivity. Additionally, the bang-sensitive allele *sda*$^{iso7.8}$, attributed previously to the *aminopeptidase N* gene, was determined to be an allele of the *jus* gene and is now known as *jus*$^{iso7.8}$. Our prior work suggested that *jus* expression during the early pupal development was critical to prevent bang-sensitivity in adult flies. Here we refined the critical period of *jus* expression to pupal stages P6-8. We hypothesized that, in *jus* mutant animals, altered activity of pupal neurons during the critical period may contribute to bang-sensitivity in adults. Testing this hypothesis would require a GAL4 line that expresses GAL4 in *jus*-expressing neurons. We tested the ability of different GAL4 lines with putative enhancers from the *jus* locus for their ability to rescue bang sensitivity with a UAS-*jus* transgene and for their efficacy in causing bang sensitivity when expressing a UAS-*jus*RNAi construct. 90B09, a construct containing the *jus* promoter, was the most effective in genetic rescue and in driving bang sensitivity with RNAi. Using the 90B09 enhancer GAL4 line, we expressed multiple copies of UAS-*shibire*^{ts} in *jus*$^{iso7.8}$ heterozygous animals to see if reducing neurotransmitter release from *jus*-expressing neurons during the mid-pupal alters bang-sensitivity in the adult. Our preliminary data suggest that the bang-sensitive phenotype is exacerbated by the expression of *shibire*^{ts} when animals are heated to the non-permissive temperature during the critical phase as compared to controls. We plan to further investigate the role of activity in jus-expressing neurons in the development of epileptogenesis.

585C The molecular mechanism of the Dscam1 phosphorylation in dendritogenesis of aCC motoneurons

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Proper formations of axons and dendrites are essential for the functional nervous system. It has been known that several extrinsic and intrinsic factors cooperate to determine the initiation of axonal development. However, the understanding of the molecular mechanism underlying dendritic formation is limited. To study it, we selected aCC (anterior Corner Cell) motoneurons as a model system. It has been known that aCC motoneurons have stereotypical and simple dendritic development, which the contra-lateral dendrites are generated from the stem of the axon at 13 µm from the cell body at 13 hours after egg laying. Recently, we have reported that the loss of *Dscam1* in aCC motoneurons causes defective phenotype on their dendritic formations. Interestingly, this evidence suggests that Dscam1 serves as an essential cue in dendritic formation; thus we provide a novel role of Dscam1 in CNS development. We also identified that adaptor proteins, Dock and Pak are involved in the Dscam1 signaling pathway to generate dendrites at their proper positions. However, there are some missing links to fully understand this pivotal signaling pathway. For instance, how does Dscam1 interact with its downstream effector Dock? One of the well-known modifications on Dscam1 is the tyrosine phosphorylation. Additionally, Dock is an adaptor protein that recognizes phosphorylated tyrosine residues via its binding domain. Consequently, we predict that the phosphorylation of Dscam1 is the key event to trigger the downstream signaling cascade. Thus, to elucidate the role of Dscam1 phosphorylation in aCC dendritogenesis, we expressed non-phosphorylatable Dscam1 in Dscam1 knock-down embryos. Compared to overexpression of wild type Dscam1, the mutated Dscam1 fails to rescue the defective dendritic phenotype. In addition, we developed the detection techniques based on the proximity ligation assay (PLA) or the bimolecular fluorescent complementation (BiFC) to investigate the Dscam1 phosphorylation event in cellular level. In the PLA, we use two antibodies for detecting tyrosine phosphorylation of Dscam1. PLA signals can be visualized only if Dscam1 gets phosphorylated. In the BiFC, we apply split GFP fragments into Dscam1 and its adaptor protein Dock. The reconstituted GFP signals can be obtained only if Dscam1 gets phosphorylated and Dock recognizes the phosphorylated Dscam1. We will present the recent progress of the phosphorylation reporter techniques and discuss the results and the phosphorylation mechanism of Dscam1.

586A Temporal regulation of neuronal maturation by a chromatin anti-looping factor

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The nervous system undergoes dramatic reorganization to become fully mature, but chromatin-based mechanisms underlying neuronal maturation remain poorly defined. The tissue-specific chromatin insulator antagonist Shep promotes *Drosophila* neuronal maturation by repressing expression of master regulator genes specifically in maturing neurons. To understand the mechanism of Shep repression of a key target gene, *brat*, we performed 4C-seq on CNS-derived BG3 cells. We found that Shep depletion leads to increased *brat* promoter looping with proximal regions enriched for H3K4me1. Subsequent luciferase reporter assays verified enhancer activity of one candidate region when transfected into BG3 but not non-neural S2 cells. Furthermore, depletion of Shep from BG3 cells does not affect either enhancer or promoter activities in this artificial context, suggesting that Shep does not simply inactivate either module. *In vivo* 3C in dissected tissue validated Shep inhibition of looping in pupal but not larval brains. Finally, we performed ATAC-seq on FACS-sorted pupal neurons and found that Shep depletion increases chromatin accessibility in the immediate vicinities of the *brat* enhancer and promoter, indicating correspondence between increased chromatin accessibility and increased looping frequency in Shep-depleted neurons. Genome-wide, regions with increased accessibility resulting from Shep depletion are enriched for H3K4me1 and

contain genes that also increase in expression, suggesting extensive Shep-mediated enhancer closure during neuronal maturation. Our results provide the first evidence for a chromatin anti-looping factor that regulates temporal gene expression during organismal development.

587B Homeodomain protein Six4 prevents the generation of supernumerary *Drosophila* type II neuroblasts and premature differentiation of intermediate neural progenitors

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In order to boost the number and diversity of neurons generated from neural stem cells, intermediate neural progenitors (INPs) need to maintain their homeostasis by avoiding both dedifferentiation and premature differentiation. Elucidating how INPs maintain homeostasis is critical for understanding the generation of brain complexity and various neurological diseases resulting from defects in INP development. Here we report that Six4 expressed in *Drosophila* type II neuroblast (NB) lineages prevents the generation of supernumerary type II NBs and premature differentiation of INPs. Loss of Six4 leads to supernumerary type II NBs likely due to dedifferentiation of immature INPs (imINPs). We further demonstrate that Six4 inhibits the expression and activity of PntP1 in imINPs in part by forming a trimeric complex with Earmuff (Erm) and Pointed P1 (PntP1). Furthermore, knockdown of Six4 exacerbates the loss of INPs resulting from the loss of PntP1 by enhancing ectopic Prospero (Pros) expression in imINPs, suggesting that Six4 is also required for preventing premature differentiation of INPs. Taken together, our work identified a novel transcription factor that that plays important roles in maintaining INP homeostasis.

588C Investigating the localization and function of laminin and dystroglycan in *Drosophila* wrapping glia development

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The health of the peripheral nervous system (PNS) is largely dependent on proper glial cell functioning during development. Myelinating and non-myelinating Schwann cells (MSCs and NMSCs, respectively) are glial cells in the PNS that ensheathe and protect axons. Communication between Schwann cells and the extracellular matrix (ECM) is essential for PNS development. The ECM protein laminin is important for MSC and NMSC development. The laminin receptor dystroglycan (Dg), part of the dystrophin-glycoprotein complex (DGC), is also important for MSC development, however very little is known about the mechanisms underlying the role of laminins in NMSC development, including the involvement of Dg and the DGC in this process. We use developing *Drosophila* wrapping glia (WG), which ensheathe axons similarly to NMSCs, as a model to study the role of laminin/Dg in NMSC development. Laminins are heterotrimers, composed of an alpha, beta, and gamma subunit. We found strong expression of LanA, one of two laminin alpha subunits in *Drosophila*, around WG. Knockdown of LanA in WG eliminated LanA expression around WG and caused WG swellings similar to those seen from laminin gamma subunit knockdown in a previous study. These data suggest that LanA is expressed by WG. Preliminary data suggests that LanA is most often found at the adaxonal WG membrane (between WG and axons), rather than the abaxonal WG membrane (between WG and its adjacent glial layer, subperineurial glia). Given that laminin is a cue for polarity in other cell types, this adaxonal-biased expression pattern may indicate a form of WG polarization, a feature that has not been well understood in WG thus far. Dg is also expressed on WG membranes, and knockdown of Dg and dystrophin (a component of the DGC), leads to WG ensheathment failure. These results indicate that Dg and dystrophin are important for WG development. Future directions of the project include investigating whether other known components of the DGC also play a role in WG development, what are the downstream signalling pathways mediating this process, and whether other ligands besides laminins bind Dg in the WG (due to the different WG phenotypes from laminin and Dg knockdown). Due to the highly conserved nature of laminins and the DGC to their vertebrate counterparts, our results may have implications for NMSC development—thus improving our understanding of the factors underlying PNS health and development.

589A Novel regulation of asymmetric cell division in neural stem cells by Kin17

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Drosophila neuroblasts divide asymmetrically to generate a self-renewing neuroblast and a differentiating daughter cell. In order to drive fate in these daughter cells, fate determinants, such as Brat, Numb, and Prospero, are segregated to the differentiating daughter cell. To segregate these factors properly, cell polarity must be established during mitosis. Miranda is a scaffold protein required for the localization and segregation of Brat and Prospero, which is restricted to the basal pole of the cell through the activity of the kinase aPKC at the apical pole. We have identified a novel regulator of the basal localization of Miranda, Kin17, whose loss leads to localization of Miranda and Prospero to the centrosome, Prospero localization to the nucleus, a reduction in the mitotic rate, and a reduction in brain size. While localization of Miranda to the centrosome has

been previously reported, the mechanism of this localization has not been studied. We have determined that in wild-type cells, localization to the centrosome is cell-cycle dependent, with a peak of localization in prophase, while Kin17 mutants maintain localization of Miranda to the centrosome during the entire cell cycle. Using genetic methods, we have determined that the phosphorylation state of Miranda mediates its localization to the centrosome, Miranda phosphorylated at Serine-96 localizing to the centrosome. aPKC phosphorylates this residue, however, reduction of aPKC activity does not rescue the Kin17 phenotype. We have identified that Kin17 regulates Miranda localization through the activity of Protein Phosphatase 4, which localizes to the centrosome during mitosis, as knockdown of PP4 subunits leads to an accumulation of Miranda at the centrosome. Knockdown of Kin17 leads to a reduction in the protein and mRNA levels of the PP4 targeting subunit, Falafel, and expression of Falafel in the Kin17 RNAi background leads to a partial rescue of the Kin17 phenotypes. We propose that Kin17 regulates the localization of Miranda during neuroblast asymmetric division through ensuring the proper levels of Falafel, and that loss of Kin17 and the subsequent reduction in Falafel levels, leads to mislocalization of Miranda and defects in asymmetric cell division.

590B Investigating the role of condensins in brain development using condensin deficient *Drosophila* models of microcephaly

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1 in 800-5000 babies in the United States are born with microcephaly. Mutations in specific proteins, including condensin proteins, have been shown to cause microcephaly in humans and in mouse models. However, the mechanisms by which condensin mutations lead to the development of microcephaly are not well understood. Condensins are conserved complexes that are important for regulating chromosome organization.

Previously, our lab discovered that condensins also repress the expression and movement of retrotransposons in both flies and human cells. Retrotransposons are DNA elements that, upon expression, can copy themselves through the action of an encoded reverse transcriptase, and mobilize through the process of retrotransposition. Their uncontrolled activity can cause genetic instability and is linked to the development of cancers and neurological diseases.

Our lab has developed a model in the fruitfly, *Drosophila melanogaster,* to study the mechanisms by which condensin depletion leads to microcephaly. Our data shows that knockdown of condensin subunits SMC2, SMC4, CAP-D2, CAP-D3 and CAP-H in the developing fly result in decreased brain volumes. Additionally, we found that microcephaly begins after the third-instar larval stage of development in condensin deficient flies. Immunostaining for the cell death marker, *Drosophila* caspase 1, revealed increased cell death in the optic lobes of the pupal brain, which contain stem cells that give rise to a significant portion of the adult *Drosophila* brain. Interestingly, qRT-PCR analyses demonstrate that retrotransposon expression is increased in developing condensin-deficient brains. Excitingly, condensin-mediated microcephaly is partially rescued by allowing the flies to develop on food containing Nucleoside Reverse Transcriptase Inhibitors (NRTIs). Together, these findings suggest that condensins may repress retrotransposon expression and activity in the developing *Drosophila* brain to prevent microcephaly.

591C tRNA methyltransferase TRMT9B regulates synaptic growth and function

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Regulation of neuronal gene expression is crucial for proper neuronal development and function. Transfer RNAs (tRNAs), which recognize codons and add the appropriate amino acid to growing polypeptides, dynamically regulate mRNA translation, and their disruption is associated with neurological disorders. tRNAs are heavily post-transcriptionally modified to regulate their structure, stability, and fidelity.
In a candidate genetic screen, we identified the uncharacterized CG42261 gene as a novel regulator of synaptic growth and function. CG42261, also known as fire dancer (fid) for its proposed role in pain sensation, is the Drosophila homolog of human TRMT9B. TRMT9B is one of two homologs of the yeast tRNA methyltransferase TRM9 found in all animals. In yeast, TRM9 methylates wobble uridine at position 34 of the anticodon loop to reinforce cognate codon-anticodon pairings, resulting in increased translation of specific mRNAs. In animals, this role appears to be primarily carried out by the second TRM9 homolog, ALKBH8. Notably, ALKBH8 is ubiquitously expressed, whereas TRMT9B is enriched in the nervous system. TRMT9B has been

studied for its role as a tumor suppressor gene, yet its biochemical function and role in the nervous system remain largely unknown. To further characterize the role of TRMT9B in the nervous system, we have generated null and endogenously tagged alleles. We observe TRMT9B localization in both neuronal cell bodies and at synapses. Loss of TRMT9B results in significant ectopic synapse formation at the larval neuromuscular junction, demonstrating a role for TRMT9B in negatively regulating synapse formation. TRMT9B mutants also exhibit reduced neurotransmitter release despite the presence of ectopic synapses, indicating aberrant synapse function. Analysis of tRNA methylation in TRMT9B mutants indicates a role in promoting specific wobble uridine modifications in the brain and points to potential mRNA targets that we are investigating for roles in synaptic growth. Together, our findings demonstrate a key role for tRNA modification in nervous system development and function.

592A The central role of the R7 photoreceptor in insect eye development and evolution

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One of the most heavily studied cell fate specification events in biology is recruitment of R7 photoreceptors in the developingDrosophila retina. New evidence suggests that R7 specification is also a target of evolutionary change and that modification of R7 has been used to enable a wide range of visual adaptations, such as to change the distribution of ommatidial types across the retina in several species of Diptera, for improved target detection in house flies, and for expanded color vision in butterflies. We first present evidence that the genes and signaling pathways involved in initial R7 specification are highly conserved across the insects. Here, we use antibody stains and CRISPR/Cas9 tests of gene function in butterflies and wasps. Despite deep conservation in how retina cell types are specified, considerable diversity exists in insect eyes across species which live in a wide range of environments and which have varying natural history requirements. We characterize Rhodopsin diversity across 25 families of Diptera and compare expression patterns in five species to what is known from Drosophila. In one example, even flies which distribute their receptors similarly to Drosophila sometimes make different color comparisons, such as Olive Flies, which compare one UV wavelengths (Rh3) to another UV (Rh4) instead of comparing UV to blue wavelengths in the “pale” ommatidial type. In another example, male Musca domestica house flies have instead sacrificed color vision entirely in favor of motion detection in a region of their eye dedicated to pursuing females, known as the “Love Spot”. We used single cell sequencing to identify male-specific cell types and evaluate changes in gene expression in Love Spot R7s during development. We have identified genes that specify Love Spot fate and have used genetic tools inMusca and Drosophila to test regulatory relationships between candidate factors. We argue that Love Spot R7s are a novel neural type and uncover the genetic modifications used to produce this example of increased neural complexity. Despite deep conservation of a retina “ground plan” across the insects, specific changes in a highly conserved developmental program have allowed an impressive diversity of specialized features and functions to evolve.

593B How do muscles and motoneurons meet for life?

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Every species possesses its stereotypical mode of locomotion that is under the control of the neuromuscular system. Locomotion results in movements that are essential for animal survival. Diseases of this system are at the origin of handicaps with severe social and economic consequences. Its study has become a key challenge for the scientific community in order to develop efficient therapies. Our long-term project is to understand the coordinated development and maintenance of the three main components of Drosophila melanogaster neuromuscular system: motoneurons (MNs), muscles and glia. This system is stereotyped and conserved between individuals. During development, the nerve terminals are in front of several choice points of muscular connection. For that, a mechanism has to be at the heart of the specific recognition between axon and muscle, and this, in a short window of time. Despite a lot of knowledge on the initial steps controlling the axon guidance, the identities of molecules involved in the axon-muscle recognition remain unknown. Our hypothesis is that each muscle and MN will express differentially molecules that are at the origin of the specific recognition.
Until now, studies in the Drosophila muscle-axon recognition have been made in the larval neuromuscular system, a 2D system, where each muscle is composed of one fiber and innervation happens after the muscle formation. For our project, we use the leg muscles model, a 3D system, which gives the possibility to work on a model where each muscle is made of several fibers and where myogenesis and innervation happen at the same time. Our strategy is first to understand the mechanism controlling the specific muscle-axon recognition during development. We will perform live imaging and fixed tissues experiments to visualize and understand the development of myoblasts and motoneurons at the same time. Second, we will do RNA profiling during development in order to find proteins expressed in MNs and muscles that mediate specific axon-muscle recognition. Then, the candidate molecules will be analysed in order to define their function in this system by RNAi knockdown and overexpression.

This project will lead to novel biological concepts that will increase our fundamental knowledge on developmental biology. Understanding the mechanisms that specify the muscle innervation will allow finding efficient ways to face neuromuscular diseases.

594C A KDM5-Prospero transcriptional axis functions during early neurodevelopment to regulate mushroom body formation

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Intellectual disability (ID) disorders affect up to 2% of the population and are characterized by an IQ score less than 70 with deficits in adaptive functioning. Our research focuses on the KDM5 family of transcriptional regulators, mutations in which account for 1-3% of inherited ID ranging from mild to severe. Although recent advances in comparative genomic hybridization and whole exome sequencing have revealed over 75 KDM5 mutations segregating in families with inherited ID, the molecular mechanisms by which KDM5 proteins impact neuronal function remain largely unknown, leaving patients without effective treatment strategies.

Here, we utilize the *Drosophila* Mushroom body (MB), a major learning and memory center within the fly brain, to demonstrate that KDM5 is specifically required within ganglion mother cells (GMCs) and immature neurons for proper neurodevelopment. Utilizing Targeted DamID (TaDa), we identify a core network of KDM5-regulated genes within GMCs and immature neurons that are critical modulators of neurodevelopment. Significantly, we find that a majority of these genes are direct targets of Prospero (Pros), a transcription factor with well-established roles in neuronal growth and guidance. We further demonstrate that Pros can physically and genetically interact with KDM5 to orchestrate a transcriptional program critical for proper MB development.

To better understand the molecular mechanism(s) through which KDM5 functions to regulate MB development, we generated a library of fly strains each bearing a conserved ID patient-derived KDM5 missense mutation. We demonstrate that fly strains bearing patient mutations in an A/T Rich Interacting Domain (ARID) and a C_5HC_2 zinc finger domain of unknown function present with profound MB defects. Interestingly, fly strains bearing patient mutations that disrupt KDM5's histone demethylase activity do not present with MB morphological defects, suggesting that KDM5 may function through demethylase-independent mechanisms to regulate MB development. This is significant, as the prevailing model linking KDM5 dysfunction to ID assumes that altered demethylase activity of KDM5 is largely responsible for such deficits. We are currently investigating how KDM5 may utilize these domains in coordination with Pros to guide neuronal development.

595A SWI/SNF complex subunit Snr1 regulates proliferation and differentiation in the Drosophila optic lobe

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Mutation in the human gene SMARCB1, which is a core member of the SWI/SNF complex, has been linked to nerve sheath tumours in Schwannomatosis. The SWI/SNF complexes are a conserved family of ATP-dependent chromatin remodeling complexes. During nervous system development, the SWI/SNF complexes play an important role in maintaining pluripotency and governing cell fate decisions. Although the loss of SMARCB1 has been shown to alter SWI/SNF complex activity, how this results in over proliferation and tumor development remains unclear. To investigate the role of SMARCB1 in regulating proliferation and differentiation in the nervous system, we study the orthologous gene in *Drosophila melanogaster*, *Snr1*. We hypothesized that knocking down Snr1 would promote proliferation at the expense of differentiation. We used immunofluorescence and confocal microscopy to characterize the role of Snr1 in the different cell types of the developing optic lobes of Drosophila larvae. We found that Snr1 is expressed in neuroepithelial cells and a subset of optic lobe neuroblasts. To investigate the effects of the loss of Snr1 in specific cell types, we knocked down Snr1 by RNA interference. Knockdown of Snr1 specifically in neuroepithelial cells resulted in reduced proliferation and disorganization of the optic lobe. In contrast, knockdown of Snr1 in neuroblasts resulted in the optic lobe being overgrown. Clonal analysis indicated that this over-proliferation was due to a defect in differentiation of neural stem cells into neurons. In glial cells, knockdown of Snr1 resulted in reduced cell numbers and altered organization. Our results indicate that Snr1 regulates proliferation and differentiation in a cell-type and stage specific way during optic lobe development.

596B The RNA binding protein Nab2 regulates gene expression during brain development

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During nervous system development, neurons must form connections with specific pre- and post- synaptic cells in order to form functional neuronal circuits. Functional circuits are essential for the nervous system to receive sensory information from the surrounding environment and then respond in an appropriate manner. Circuit formation typically relies upon diffusible and contact-dependent ligands that are released from target cells. These ligands interact with receptors on the growth cones of developing axons and guide the growth cone towards or away from synaptic targets. Guidance of an axon towards a specific synaptic target also relies on precisely defined spatial and temporal patterns of gene expression. In order to function correctly, axon guidance ligands and receptors must be expressed by specific cells and during specific developmental time points. Therefore, identification of the mechanisms that regulate gene expression during nervous system development will enhance our understanding of circuit formation.

In the current study, we have investigated the mechanisms by which the evolutionarily conserved RNA binding protein, Nab2, regulates *Drosophila* brain development and neuronal circuit formation. We previously demonstrated that Nab2 is necessary for both memory formation and correct development of the fly mushroom bodies, a region of the brain required for olfactory learning and memory. Since Nab2 is an RNA binding protein, we hypothesized that problems in mushroom body circuit formation observed in flies lacking Nab2 were the result of changes in gene expression during brain development. To test this hypothesis, we identified the developmental stage when defects in mushroom body development are first observed in flies lacking Nab2. RNA sequencing was then used to identify mis-regulated genes in Nab2 null brains from this developmental stage. Preliminary evidence suggests that Nab2 loss may alter expression of genes involved in synaptic communication and the unfolded protein response during nervous system development. Interestingly, comparison of changes in gene expression during brain development to those observed in adults suggest a consistent pattern of Nab2 target transcripts across developmental time points. In summary, these studies contribute to our understanding of the cellular mechanisms controlling gene expression during axonal guidance and nervous system development.

597C

The *Drosophila* Fragile X Mental Retardation Protein controls dendrite arborization by regulating cytoskeletal remodeling *Hui Li*[1], Elizabeth Gavis[1] 1) Department of Molecular Biology, Princeton University, Princeton, NJ, United States.

Dendritic arbor development is a complex and highly regulated process. Post-transcriptional regulation mediated by RNA-binding proteins (RBPs) can play a particularly important role in dendrite morphogenesis by targeting on-site, on-demand protein synthesis. Here, using *Drosophila* class IV dendritic arborization (C4da) neurons as a model system, we show that an RBP, *Drosophila* Fragile Mental Retardation Protein (dFMRP), acts as a negative regulator of dendrite morphogenesis. In the absence of dFMRP, dendrites of C4da neurons display an over-branching defect with increased terminal branch dynamics compared to wild-type, suggesting that dFMRP might affect branching by regulating cytoskeletal dynamics. Indeed, we found that loss of dFMRP alters microtubule organization by increasing both stable microtubule levels along main branches and distal dendritic microtubule dynamics. Furthermore, we showed that *dfmr1* genetically interacts with *chickadee* (*chic*), which encodes the *Drosophila* profilin homolog, and dFMRP negatively regulates Chic expression likely through post-transcriptional regulation. Together, these results support a model in which dFMRP regulates dendrite morphogenesis by regulating cytoskeleton remodeling. Our studies provide insight into the regulation of dendritic patterning by RBPs, and also shed light on the neurodevelopmental pathology of Fragile X Syndrome.

598A Secretion of neuropeptides regulates Distal medulla (Dm) neuron mosaic formation in the *Drosophila* Visual System

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How neurons target their arbors to ensure they receive the proper number of inputs is a critical question in neurobiology. The highly ordered visual centers and powerful genetic tools available in *Drosophila* make it an ideal model to study this question. The medulla is the most complex brain region in the fly visual system and consists of 40,000 neurons of about 100 different types. Distal medulla (Dm) neurons are a class of neurons that resemble the amacrine cells of the mammalian retina. Like amacrine cells, the various classes of distal medulla neurons distribute themselves across the retina in an ordered, regularly spaced array known as a retinal mosaic. To understand how Dm neurons establish their territories and orientations to cover the entire topographic map, we selectively killed off large groups of Dm4 neurons by driving the apoptotic genes *grim* and *rpr* under the control of a Dm4-specific driver. Depleting large numbers of Dm4 neurons does not cause compensatory expansion of the surviving neurons' arbors, indicating that contact-mediated self-avoidance is not

likely to dictate neurite scaling. In contrast, ectopic expression of a temperature-sensitive dynamin (*shibire*^{ts}) to prevent secretion was sufficient to mistarget Dm4s, indicating that these cells use secreted information from neighboring cells to space themselves. To identify what molecules are involved in Dm neuron targeting, we searched through our existing adult scRNAseq dataset to identify candidate genes specifically expressed in Dm neurons. We performed an RNAi screen on these candidates and identified a neuropeptide, Nplp1, that is required for proper Dm neuron mosaic formation within the medulla. Cell-specific *nplp1* RNAi causes Dm4 neurons to mistarget, causing gaps within the adult medulla, indicating that Nplp1 is required cell-autonomously. scRNAseq data also suggests that these same Dm4 neurons are glutamatergic. Dm4-specific RNAi against *vglut* causes a similar Dm targeting defect as *nplp1*, and Dm4-specific *nplp1* RNAi prevents *vglut* expression in Dm4 neurons, indicating that Nplp1 is required for proper *vglut* expression. Our work suggests that these visual system neurons do not use contact-mediated repulsion to target, but rather, use secreted signals to instruct neuron targeting; this allows neurons to scale their arbors to ensure that the environment is uniformly sampled, a problem common to many neurons in sensory and central nervous systems.

599B Ionotropic Receptor-dependent cool cells control the transition of temperature preference in *Drosophila* larvae

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Temperature sensation guides animals to avoid temperature extremes and to seek their optimal temperatures. The larval stage of *Drosophila* development has a dramatic effect on temperature preference. While early-stage Drosophila larvae pursue a warm temperature, late-stage larvae seek a significantly lower temperature. Previous studies suggest that this transition depends on multiple rhodopsins at the late larval stage. Here, we show that early-stage larvae, in which dorsal organ cool cells (DOCCs) are functionally blocked, exhibit similar cool preference to that of wild type late-stage larvae. The molecular thermoreceptors in DOCCs are formed by three members of the Ionotropic Receptor (IR) family, IR21a, IR93a, and IR25a. Early-stage larvae of each Ir mutant pursue a cool temperature, similar to that of wild type late-stage larvae. At the late larval stage, DOCCs express decreased IR proteins and exhibit reduced cool sensitivity. Importantly, late-stage larvae that overexpress IR21a, IR93a, and IR25a in DOCCs exhibit similar warm preference to that of wild type early-stage larvae. These data suggest that IR21a, IR93a, and IR25a in DOCCs navigate early-stage larvae to avoid cool temperatures and the reduction of these IR proteins in DOCCs results in animals remaining in cool regions during the late larval stage. Together with previous studies, we conclude that multiple temperature-sensing systems are regulated for the transition of temperature preference in fruit fly larvae.

600C Terminal selector genes link neuronal fate with wiring specificity in the *Drosophila* visual system

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Cascades of transcription factors (TF) in neural stem cells are responsible for generating the enormous diversity of cells types in nervous systems, from flies to humans. However, the gene-regulatory mechanisms that establish and maintain these cell fates in postmitotic neurons and instruct their specific morphology, connectivity and physiology remain unclear. The *Drosophila* optic lobes provide an excellent model system to address these questions with around 200 morphologically distinct neuronal types whose connectome has been almost completely characterized. In order to understand the cell-type specific transcriptional programs employed at different stages of neuronal differentiation, we have generated a very large single-cell RNA sequencing atlas spanning all stages of optic lobe development (Ozel, Simon et al. 2020, *Nature*). We found that unique combinations of 113 TFs, which are strongly enriched in homeobox proteins, are sufficient to define each of the 175 optic lobe neuronal types in our dataset (median: 8 TFs per cell type) throughout development as well as in adults. We hypothesized that these TFs represent terminal selectors that are activated in each neuron immediately after their birth and function as top-level regulators of their cell-type specific gene expression throughout their life. Accordingly, we show that modification of these TF 'codes' with knock-down and ectopic expression experiments in postmitotic neurons is sufficient to induce complete *in vivo* transdifferentiation between neuronal types. We identified a bistable loop formed by the mutually repressing TFs Mef2 and Aop in closely related transmedullary neurons. This ensures that transformations between them are all or none, with no intermediate phenotypes. We also performed computational network inference analysis using the *Inferelator* pipeline to understand the downstream effectors of these terminal selectors. This revealed that terminal selectors interact with other TFs that are transiently activated in response to extrinsic signals at specific time points to activate the cell-type specific cell-surface molecules and other effectors that instruct distinct morphological features and the synaptic partners of each neuron. Our results provide a unified framework of how specific fates are maintained in postmitotic neurons, and reveal the regulatory mechanisms that ensure the robustness of these cell fate choices. They open up new avenues to understand synaptic specificity through gene regulatory networks.

601A Bisphenol A differentially impacts neurodevelopment in *Drosophila melanogaster* from distinct genetic backgrounds

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Evidence indicates that the interaction between environmental chemicals with specific genetic variants are linked to neurodevelopmental disorders (NDDs). Thousands of genes have been linked to NDDs; by comparison, the role of environmental chemicals in conjunction with genetic risk factors to confer risk of NDDs has fallen behind. Bisphenol A (BPA) is an abundant chemical used in the synthesis of plastics and epoxy resins. Well known for its endocrine disrupting abilities, BPA has also recently been implicated in NDD etiology. However, the mechanism by which BPA disrupts neurodevelopment and its impacts when combined with NDD-associated genes are unclear. In this study, we investigated the neurodevelopmental impacts of BPA within two genetic strains of *Drosophila melanogaster—w1118* and the Fragile X syndrome (FXS) model. FXS flies have a loss-of-function mutation in *fragile X mental retardation 1 (dFmr1),* the ortholog of an NDD-associated gene in humans. We demonstrate that in wild-type flies BPA causes increases in larval locomotion (both reorientation events and peristaltic contractions), repetitive grooming behavior in adults, and axon guidance defects in the mushroom bodies adult brains. Remarkably, BPA has either the complete opposite or insignificant impacts for these same phenotypes in the FXS flies—upon BPA exposure, reorientation is reduced, peristalsis is unaffected, grooming is reduced, and axon guidance defects are reduced. This study is the first to demonstrate that BPA may elicit a gene-environment interaction with an NDD-associated gene.

602B Uncovering the mechanism of slit function in PNS development

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Slit is a secreted ligand expressed in the ectoderm and plays an important role during neurogenesis. In the peripheral nervous system (PNS), removing slit results in defects in the morphology and the migration of the lch5 chordotonal neurons. In order to gain a deeper understanding of how slit functions in this developmental process, we have over-expressed slit in the neurons, glial cells, or ectoderm of Drosophila embryos, and examined PNS neurons in each situation. Preliminary results show that over-expression of slit in glial cells results in a few defects, including an axonal branch from one section that connects to another, loss of certain dorsal neurons, and neurons that are completely out of place. Additionally, over-expression of slit in the ectoderm shows that the cluster tails from the dorsal section are shorter than those found in wild type embryos. Currently, we are in the process of over-expressing slit in neurons, over-expressing robo2 in these same cell types, as well as over-expressing slit and robo2 in these different cell types in a slit, robo and robo2 mutant backgrounds. This data demonstrates the importance of slit in PNS development, and we hope to provide insight into the mechanism of slit function in the PNS.

603C The role of the cell cycle regulator dacapo (dap) on embryonic PNS development

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The embryonic peripheral nervous system (PNS) is a wonderful system to examine a myriad of cellular processes, including cell migration, cell-cell, and cell-environment interactions, and cell cycle regulation. However, many of the details of these processes remain a mystery. In this study, we seek to examine the role of the gene *dacapo (dap)* PNS development, specifically, in cell cycle regulation. Dap is a known cyclin-dependent kinase inhibitor and has been shown to be upregulated after the last mitosis in arresting cells in G1/G0 before terminal cell differentiation. *dap* is suspected to be necessary for entry into the S phase and to arrest cell proliferation. Preliminary results show defects in PNS neuronal development in *dap* loss-of-function mutants. Currently, we are quantifying these defects while also characterizing the effect of *dap* over-expression on PNS development. Through these studies, we seek to answer if/how Dap inhibits specific actions within the cell cycle or if other genes influence it.

604A Neural stem cell quiescence is controlled by daughter cell-mediated Notch activation in *Drosophila*

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Stem cells enter and exit quiescence as part of normal developmental programs and to maintain tissue homeostasis during adulthood. We report that *Drosophila* neural stem cells (neuroblasts) utilize the evolutionarily conserved Notch cell-cell signaling pathway for quiescence entry. When Notch activity is reduced, quiescence is delayed or altogether bypassed, with some neuroblasts dividing continuously during the embryonic to larval transition. We find that neuroblasts express both Notch

and the Notch ligand Delta. During mitosis, Delta is partitioned symmetrically between NBs and their GMC daughters and after division, GMC-localized Delta trans-activates Notch in neuroblasts. During embryogenesis, neuroblast Notch activity increases due to continued GMC production and Notch transactivation. Increasing Notch inhibits neuroblast cell cycle progression and reduces Delta levels, consequently promoting quiescence while attenuating Notch signaling. Thus, a Notch-dependent feedback mechanism induces neuroblast quiescence, which gives developing animals time to acquire dietary nutrients to restart divisions and continue developmental growth programs.

605B Measuring the Impact of Bisphenol A on Nonassociative Learning and Memory in *Drosophila melanogaster* Using the Endoparasitoid Wasp Predator-Response Paradigm

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Neurodevelopmental disorders (NDDs) are a heterogeneous group of disorders caused by both genetic and environmental factors. Cognitive impairments—like diminished learning and memory—is a common feature of many NDDs, often caused by changes in synaptogenesis and/or synaptic plasticity. One environmental factor that is increasingly being shown to impact neurodevelopment is bisphenol A (BPA). BPA is a high-volume chemical that is produced worldwide and used in the synthesis of polycarbonate plastics and epoxy resins. The lipophilic structure of BPA allows it to cross placental and blood-brain barriers, making it particularly concerning as a putative risk factor for NDDs in humans. The purpose of this study is to use the common fruit fly, *Drosophila melanogaster*, to determine if developmental exposure to BPA impacts learning and memory in adults. Studies have shown that wild-type female fruit flies can learn that the presence of an endoparasitoid wasp poses an imminent threat to her offspring and respond by reducing the rate of oviposition (ie. egg-laying). The females can also remember the threat, which is exhibited through an extended depression of oviposition rate. We exposed fruit flies to BPA (1 millimolar) during embryonic and larval development and then used this experimental paradigm to assess learning and memory. We found that BPA exposure did not affect learning, but did significantly reduce memory in *w1118* flies. To extend this work, we are now examining how BPA impacts learning and memory in Canton S flies, as well as flies carrying a null mutation in *fragile X mental retardation 1 (fmr1)*—a gene associated with NDDs in humans.

606C Regulators of retrograde BMP signaling during neuromuscular junction development in *Drosophila*

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The canonical Bone Morphogenic Protein (BMP) pathway helps coordinate the growth and development of synapses in both vertebrates and invertebrates. The *Drosophila* neuromuscular junction (NMJ) is a glutamatergic synapse used as a model for the AMPA-type excitatory synapses of the mammalian central nervous system, which develop in a structurally similar way. Retrograde BMP signaling is critical for the scaling growth of the synaptic termini, or boutons, proportional to larval muscle growth. Autocrine BMP signaling, however, is critical for embryonic NMJ morphogenesis and later organization of presynaptic active zone structures. In the absence of any BMP signaling, NMJs severely undergrow and do not release neurotransmitter normally. Though nearly twenty years have passed since the identification of the core BMP signaling components regulating synaptic growth and function, we still have an incomplete understanding of how the core pathway itself is regulated. We identified Tao, a conserved serine/threonine kinase implicated in autism, as an inhibitor of retrograde BMP signaling during the scaling growth of the larval NMJ. Loss of neuronal *Tao* resulted in supernumerary boutons, suggesting that Tao normally inhibits NMJ growth. Previous studies showed that Tao activates the conserved Hippo pathway by initiating a kinase signaling cascade. In NMJ development, however, Tao functions independently of the Hippo pathway. We are currently investigating whether Tao similarly behaves as an upstream kinase in a signaling cascade to inhibit BMP signaling. Finally, in follicle cell morphogenesis, Tao regulates cell-cell adhesion through modulating endocytosis. We will present recent progress made in understanding the mechanism by which Tao might be inhibiting the BMP pathway by regulating endocytic processes.

607A Probing the role of *Drosophila* thrombospondin in larval NMJ formation

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Thrombospondin (TSP) is an extracellular matrix glycoprotein that plays a role in synaptogenesis in the mammalian brain. In mammals, TSP is released by astrocytes at glutamatergic synapses. The TSP family is composed of 5 members in humans; there is a single homologous gene in *Drosophila melanogaster* (D-TSP) and there is conservation in the protein domains involved in TSP's function in synaptogenesis. It has not been investigated if D-TSP plays a role in synaptogenesis in *D. melanogaster*. Here

we determined if D-TSP modulates synaptogenesis in the *D. melanogaster* larval NMJ and if TSP modulates locomotor behavior associated with NMJ function. We hypothesized that D-TSP would be necessary for normal NMJ formation and locomotor behavior. We used the GAL4-UAS system to knock down D-TSP in specific tissues. We quantified features of the NMJ structure and locomotor behavior in larvae with either normal D-TSP expression or decreased expression in either muscles, neurons, or both. We used immunohistochemistry to visualize larval NMJ structure at muscle 4 in segments A3 and A4, using phalloidin for muscles, HRP for presynaptic axons, and DLG for the postsynaptic density as markers. Locomotor behavior was assessed by analyzing videos capturing the 45-second trajectories of larvae moving freely on a gridded surface. Our preliminary results suggest a change in NMJ morphology and locomotor behavior 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 control larvae to a majority of NMJs having 3 or more branches when D-TSP is knocked down. 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 movement. Larvae with decreased TSP in muscles may have trajectories that reach further away from the point of origin and have lower curvature, but we need more data to confirm this result. We are in the process of validating the knockdown of D-TSP by RT-qPCR and finishing analysis of the full data set for locomotor activity. Next we will quantify other behaviors, including head-turning of the larvae. 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.

608B Developmental exposure to the neurotoxicant polychlorinated biphenyl-95 elicits a synergistic gene by environment response in *fmr1* mutant *Drosophila melanogaster*

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The interaction of environmental chemicals with specific genetic susceptibilities is linked to neurodevelopmental disorders (NDDs). However, identification of specific environmental chemicals that interact with genes to confer risk to NDDs like autism spectrum disorder (ASD) remains a critical gap in our understanding of disease etiology. Given that there are over 80,000 chemicals in use that have undergone little to no toxicological testing, the field is in dire need of an efficient method for chemical analysis. This project involves the development of assays using *Drosophila melanogaster* for identification of chemicals that molecularly converge with *fragile x mental retardation 1 (fmr1)*. Mutations in *FMR1* cause Fragile X syndrome (FXS) and are the most common, monogenic cause of ASD. The role *FMR1* plays during neurodevelopment is functionally conserved from flies to vertebrates. Data from vertebrate model organisms suggests that gestational exposure to the environmental neurotoxicant, polychlorinated biphenyl-95 (PCB-95), can increase risk of ASD. Loss of *fmr1* in *Drosophila* causes a decreased courtship index (CI; a quantitative measure of genetically programmed behaviors) and impaired axon trajectory in the adult brain. We exposed developing fruit flies to PCB-95 and used the courtship assay to determine that exposure to nanomolar concentrations of PCB-95 significantly decreases the CI in *wild-type (wt)* flies in a dose-dependent manner, and exhibits a synergistic reduction in the CI of *fmr1* mutant flies. We used immunohistochemistry and confocal microscopy to examine adult brains and found a dose-dependent increase in axon pathfinding defects in the mushroom body (MB) of *wt* flies, which is the same phenotype caused by loss of *fmr1*. This finding suggests that PCB-95 may confer increased risk of ASD in individuals with *FMR1* mutations; they affect similar neurodevelopmental processes and thus indicate potential molecular convergence in developing neurons.

609C Netrins and receptors control Drosophila optic lobe organization and transmedullary neuron axon targeting

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The precise guidance of developing axons to their targets is essential for neural circuits formation. In the Drosophila visual systems, Transmedullary neurons (Tms) connect medulla neuropil with lobula neuropil, relaying visual information between them. The developing Tms first leave medulla neuropil, then join the inner chiasm and finally target to specific lobula layers. How Tms axon target to the correct lobula layer is not well-known.We found that Netrins (NetA and NetB), and their receptors, Unc-5 and Frazzled, are expressed in the developing visual system, with netB specifically enriched in the lobula neuropil. In *netAB* double mutants, the organized structure of the lobula neuropil is lost, the inner chiasm is impaired as reflected by various Tm neurons choosing the wrong routes, and Tm3 neurons target at the wrong layer. Consistently, with knocking down fra in all Ap+ neurons, Tms choose wrong routes and Tm3 neurons target at the wrong layer. By knocking down fra specifically in various Tms, including Tm1, Tm3 and Tm9, we found only Tm3 showed targeting defects of their axon terminals, and the inner chiasm is normal. These data indicate that netrins/fra signaling acts both cell-autonomously and non-cell-autonomously in Tm targeting. What's more, we found netA and netB act redundantly in forming intact inner chiasm and organized lobula neuropil, whereas only netB is necessary for Tm3 layer-specific targeting. Diffusibility of netB is also required for Tm3 layer-

specific targeting. We found redistribution of netB was regulated by fra and unc-5. In summary, we found that by expressing in different cells types, netrins and receptors orchestrate the organization of the Drosophila optic lobe.

610A Parkinson's disease genes interact with ATP7 to regulate copper distribution and availability in *Drosophila melanogaster*

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Copper is an essential element for enzymes that catalyze oxygen-dependent reactions. When an organism is exposed to either excess copper or deprived of copper, this micronutrient becomes detrimental. A mechanism used to control copper distribution and availability involves the ATPase transporter, ATP7. This X-linked transmembrane protein is responsible for delivering copper into the lumen of the cell by utilizing both endocytic and exocytic mechanisms. Mutations in ATP7 have been shown to cause Menkes disease and Wilson's disease, which both share the phenotype of neurodegeneration. These genetic disorders with ATP7 defects both lead to mechanisms of neurodegeneration that is likely shared with other, more common neurodegenerative diseases, such as Parkinson's disease. A screening of possible candidate genes that interact with ATP7 was conducted by inhibiting a Parkinson's disease gene in a ATP7 loss of function background. We find that five of the Parkinson's disease genes showed a genetic interaction with ATP7, indicating that the mechanisms of neurodegeneration caused by ATP7 mutations may be conserved in Parkinson's disease. These interactions and their link to neurological disorders will further discussed.

611B Neuromuscular Defects in a Drosophila Model of Muscular Dystrophy

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Recent evidence has demonstrated that synaptic defects are among the earliest hallmarks of neurodegenerative diseases, including neuromuscular defects seen in Amyotrophic Lateral Sclerosis (ALS). However, much less is known about synaptic defects in diseases of the muscle, including Muscular Dystrophies. Here we describe the neuromuscular defects that precede muscle cell loss in a Drosophila model of Duchenne Muscular Dystrophy, which is caused by a mutation in the *dystrophin* gene. Through analysis of *dystrophin* mutants as well as tissue-specific knockdown of *dystrophin*, we show the progressive loss of functional and structural integrity at adult Neuromuscular Junctions (NMJs) that precede cell death. We also characterize defects in synaptic proteins in both pre-synaptic motor neurons as well as post-synaptic muscle cells. Together, our results describe the early pre- and post-synaptic deficits present in a Drosophila model of Duchenne Muscular Dystrophy and provide a better understanding of the mechanisms that ultimately lead to muscle cell death.

612C The Role of Copper in Parkinson's Disease

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Parkinson's Disease (PD) is a neurodegenerative disease caused by the death of dopaminergic neurons in the substantia nigra region of the brain. PD is characterized by the presence of dysfunctional mitochondria and increased levels of oxidative stress. Though a handful of genes, such as parkin and PINK1, have been identified in familial forms of PD, most cases are sporadic. Therefore, it is thought that environmental factors may act on genetic risk factors to promote disease onset. Therefore, we are exploring the relationship between copper toxicity, which has been linked to other neurological disorders, and parkin and PINK1. We are testing the effect of environmental exposure to copper as well as altering copper levels genetically by manipulating the copper transporter ATP7, which is mutated in the neurodegenerative disorder, Menkes disease.

613A mir-277 targets *hid* to ameliorate Aβ42-mediated neurodegeneration in *Drosophila* eye model of Alzheimer's Disease

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Alzheimer's disease (AD), an age-related progressive neurodegenerative disorder, exhibits reduced cognitive functions with no cure to date. One of the reasons for AD is the extracellular accumulation of Amyloid-beta 42 (Aβ42) plaques. Misexpression of human Aβ42 in the developing retina of *Drosophila* exhibits AD-like neuropathology. Accumulation of Aβ42 plaque(s) triggers aberrant signaling resulting in neuronal cell death by an unknown mechanism(s). We screened for microRNA which

post-transcriptionally regulates the expression of genes by degrading mRNA of the target genes. In a forward genetic screen using miRNAs, we identified mir-277 as a genetic modifier of Aβ42-mediated neurodegeneration. Gain-of-function of mir-277 rescues Aβ42 mediated neurodegeneration whereas loss-of-function of mir-277 enhances Aβ42 mediated neurodegeneration. Moreover, misexpression of higher levels of mir-277 in the GMR>Aβ42 background restores the retinal axonal targeting indicating functional rescue. Furthermore, we have identified head involution defective (*hid*) as one of the targets of mir-277 by Fly TargetScan and validated by luciferase assay and qPCR. The *hid* transcript levels are decreased by one third when mir-277 is misexpressed in the GMR>Aβ42 background in comparison to the GMR>Aβ42 fly model. Here we provide a mechanism of how mir-277 modulates Aβ42 mediated neurodegeneration by regulating *hid* transcript levels and demonstrate its neuroprotective role in Aβ42-mediated neuropathology.

614B Trans-synaptic transmission of mutant huntingtin aggregates is mediated by phagocytic glia in *Drosophila* brains.

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Huntington's Disease (HD) is an inherited neurodegenerative disorder caused by expansion of a CAG nucleotide repeat in exon 1 of the huntingtin (Htt) gene. Expansion of the trinucleotide repeat domain beyond a pathogenic threshold of 37, results in Htt protein that cannot fold properly and causes it to self-assemble into protein aggregates. These aggregates are visible as dense, proteinaceous inclusions within neurons and glia of HD patient brains. Emerging evidence supports the hypothesis that mutant Htt (mHtt) aggregates and other pathogenic aggregates associated with neurodegenerative diseases (e.g. Alzheimer's disease, frontotemporal dementia, Parkinson's disease, and amyotrophic lateral sclerosis) spread between cells similar to infectious prions—aggregates transfer from cell to cell and nucleate the aggregation of normally-soluble, cognate wild-type proteins. We have previously demonstrated that mHtt protein aggregates transfer from *Drosophila* presynaptic olfactory receptor neurons (ORNs) to the cytoplasm of postsynaptic projection neurons (PNs) and there effect prion-like conversion of wild-type Htt (wtHtt) proteins. Remarkably, mHtt transmission from presynaptic to postsynaptic neurons is inversely correlated with neuronal activity and requires Draper, a glial scavenger receptor homologous to the mammalian astrocytic receptor MEGF10. We also found that mHtt aggregates originating in presynaptic ORNs make an obligatory visit to the cytoplasm of Draper-expressing glia before reaching the cytoplasm postsynaptic PNs, suggesting that phagocytic glia drive mHtt aggregate spreading. Current efforts to investigate the mechanism underlying prion-like transfer of mHtt aggregates suggest that certain Rab GTPases play a critical role in aggregate spreading between these different cell types in the fly CNS. Evidence gathered propose that vesicular trafficking events in both presynaptic and postsynaptic neuronal populations effect trans-synaptic transfer of mHtt aggregates. This is parallel to our proposal that Draper-dependent phagocytosis is necessary for prion-like movement as both mechanisms involve highly regulated vesicular machinery.

615C Expression of P3 ($A\beta_{17-42}$) in Drosophila produces Alzheimer's-like pathology and exacerbates the effects of $A\beta_{1-42}$

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Alzheimer's Disease (AD) is a heritable neurodegenerative disorder that progressively decreases an individual's cognitive abilities and is the most common underlying cause of dementia. More than 50 million people are living with AD worldwide; the rate of diagnosis and death from AD has increased over the years and is projected to continue to increase. The amyloid precursor protein (APP) is a transmembrane protein expressed in neurons that undergoes proteolytic cleavage by β-secretase and ɣ-secretase to produce the 42 amino acid amyloid-beta (Aβ) peptide, which is thought to be a primary factor in AD pathology. Research on Aβ has shown that excess Aβ forms aggregates, including amyloid plaques and that aggregation of Aβ is associated with neurodegeneration, leading to memory loss, behavioral changes, and cell death. APP is alternatively cleaved by α-secretase and ɣ-secretase to produce p3, which is composed of amino acids 17-42 of Aβ. Earlier work had suggested p3 might be non-amyloidogenic, but recent research suggests it also forms aggregates. Since p3 is made up of a portion of Aβ, we hypothesized that p3 has similar neurodegenerative effects to Aβ. To test this hypothesis, we generated transgenic Drosophila expressing p3 and compared them with Aβ expressing flies, Aβ/p3 co-expressing flies, and non-expressing controls, to test and compare the peptides' neurodegenerative effects. Expression of each transgene was confirmed through RTPCR. Longevity analysis was performed to monitor lifespan and the Rapid Iterative Negative Geotaxis (RING) assay was conducted to test motor skills of flies expressing the peptides pan-neuronally. To further assess effects of these peptides, they were expressed in fly eyes using the GMR-Gal 4 driver and electron microscopic images were taken to assess the levels of physical degradation in the ommatidia of protein-expressing flies. Our data indicate that p3 has similar, though somewhat less severe effects on lifespan, behavior and eye morphology as compared to Aβ when expressed independently. The p3 peptide also appears to increase the severity of Aβ's effects when the two peptides are co-expressed.

616A Investigating metabolic reprogramming in Frontotemporal Dementia

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Metabolic reprogramming is a common hallmark of many diseases. In recent years the focus on metabolic change in cancerous tissues has increased. However, fewer studies have investigated the metabolic shifts in neurodegenerative diseases. Metabolic reprogramming in neurodegenerative disease has been well documented and glucose uptake is even used as a key diagnostic indicator for some of these diseases. We are utilizing established Drosophila models the neurodegenerative disease, Frontotemporal Dementia (FTD), to investigate metabolic changes using whole brain energy utilization assays. This assay allows us to assess whether the changes observed are similar or distinct between these diseases. We are also studying the underlying molecular mechanism leading to the overall metabolic change. The goal of this project is to determine if metabolism could be a potential target for treatment of FTD.

617B Asymmetric loss of apical domains during light induced retinal degeneration in *Drosophila* photoreceptor cells.

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The plasma membrane of photoreceptors (PRCs) of the retina is highly differentiated into distinct domains namely the apical, sub-apical, basolateral domains and the adherens junctions. The apical domain, outer segments in vertebrates and rhabdomeres in invertebrates, houses the components of the phototransduction machinery. Maintenance of the apical membrane, is crucial for the proper function of PRCs. Improper maintenance of the apical domain results in a decline in function, leading to retinal degeneration. To get a deeper insight into the molecular and cellular events leading to degeneration, we compared the integrity of plasma membrane domains of healthy PRCs with those of light stress-induced degenerating PRCs. We utilized established degenerative disease models involving mutations in the evolutionary conserved polarity genes (*crumbs* and *D-Lin7)*. Degeneration is associated with the loss of rhabdomeric surface area and with an altered turnover of Rhodopsin from the rhabdomeres. Here, we focused our observations on the other principal component of the rhabdomere, filamentous Actin (F-Actin).
Our results corroborated with previous reports that a primary hallmark of light-induced degeneration is an early loss of rhabdomere integrity. We noted a loss of F-actin levels in these rhabdomeres, suggesting a gradual decline in rhabdomere maintenance. Whilst rhabdomeric integrity is lost, we did not observe a concomitant loss in integrity of other plasma membrane domains such as the sub-apical domain (stalk membrane), or the adherens junctions. Finally, we observed that loss of rhabdomeric integrity, as revealed by F-actin and Rhodopsin localization, is asymmetrical, starting at the proximal side of the retina (next to the synapse) and progressing towards the distal side. Moving forward, we are interested to understand the underlying basis of the asymmetric susceptibility of the rhabdomere to degeneration.

618C Cyclin-dependent kinase 8 regulates mitochondrial morphology and modulates a Parkinson's disease model in Drosophila

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Cyclin-dependent kinase 8 (Cdk8) is a serine/ threonine kinase, which functions in regulating RNA polymerase II mediated transcription. Cdk8 forms a complex with Cyclin C (Cyc C), Mediator 12 (Med 12), and Mediator 13 (Med 13) that interacts with the core mediator complex in a reversible fashion. While the regulatory role of Cdk8 in transcription is well-studied, we identified novel functions of Cdk8 in regulating the mitochondrial morphology in Drosophila. When Cdk8 is knocked down ubiquitously, progeny with desired genotype have phenotypic effects including held-up and droopy wing postures, reduced life span, and defects in both flight and climbing abilities. Surprisingly, the observed phenotypic effects are characteristics of flies with either PTEN-induced putative kinase 1 (Pink1) or Parkin mutations, which are two well-known players associated with Parkinson's disease (PD). Pink1 and Parkin normally function in regulating the homeostasis of mitochondria in a process known as mitophagy. Impaired or dysfunctional mitochondria will be recognized by Pink1 and Parkin and targeted for degradation by autophagy. Since tissue specific knocked down of Cdk8 in either muscles or neurons also resulted in impaired climbing ability, we hypothesized that Cdk8 functions in a common pathway with Pink1 and Parkin in mitophagy. Ectopic expression of Cdk8 significantly suppressed defects caused by the loss of function *pink1* allele, $pink1^{B9}$, including rescue of the thorax indentation phenotype which is due to muscle degeneration, and rescued climbing activity relative to $pink1^{B9}$ mutants. In addition, mitochondrial and muscle fiber morphologies were restored when Cdk8 was overexpressed in the $pink1^{B9}$ mutant background. Finally, we examined the effect of Cdk8 on mitochondria under physiological conditions. Expression of Cdk8 is tightly associated with mitochondrial dynamics, as fission-like morphology occurred when Cdk8 expression was reduced and

fusion-like morphology was found after overexpression of Cdk8. Together, we demonstrate that Cdk8 may exert mediator-independent functions in regulating the homeostasis of mitochondria, and it may serve as a potential therapeutic target for patients with PD.

619A Structure-function relationships of the axonal endoplasmic reticulum tubular network

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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, help establish and maintain this network, and mutations affecting them cause an axon degenerative disease, hereditary spastic paraplegia (HSP).

Two striking features of axonal ER are its continuity over distances that are massive on a subcellular scale, and its narrow diameter. This continuity potentially makes it a channel for long-range communication, independent of action potentials or microtubule transport, like a “neuron within a neuron”. Unlike ER tubules elsewhere, tubule diameter in axons is often so small as to make the the lumen invisible. This poses a paradox - that the neuron goes to a lot of effort to generate a continuous tubular network, yet also apparently to limit continuity of its lumen.

We aim to understand how axonal ER architecture is formed, and its functional roles. We identified markers for axonal ER in *Drosophila*, which show continuity through axons and synapses. Despite the apparent stability of ER network in axons, it is highly dynamic. Its amount, continuity, and tubule diameter, depend on ER-shaping HSP proteins including reticulons and REEPs.

We hypothesize that (1) occasional ER gaps in HSP mutants can explain degeneration of longer but not shorter axons; (2) additional unknown proteins are required to form axonal ER tubules; (3) homeostatic mechanisms maintain sufficient dynamic ER tubules in axons to avoid gaps in the network or accumulation of excess ER; (4) ER architecture is important for its roles in axon physiology and maintenance; (5) its minuscule lumen has functional consequences for ER continuity.

To test these models, we have developed ways to monitor the physiological consequences of altered axonal ER architecture in *Drosophila*. Loss of the ER-shaping protein Rtnl1 subtly alters presynaptic ER organization, but has substantial effects on Ca^{2+} handling in different organelle compartments. Using mutations in ER-shaping proteins that increase ER tubule diameter, we have tested whether the narrow lumen diameter constrains diffusion of its contents. Finally, we are developing forward and reverse genetic strategies to identify more of the processes of ER organizational homeostasis in axons. Our model is that ER organization is important for spatial distribution of the physiological processes that it regulates.

620B A new concept explaining the cell biology of axons and axon pathology

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To develop remedial strategies for neurodegeneration, we need to improve our understanding of the cell biology of neurons. Here we demonstrate how *Drosophila* can be used as a powerful tool to achieve this goal.

We focus on axons, the cable-like neuronal processes that wire our nervous system. These delicate axons must survive for an organism’s lifetime, making them key lesion sites in neurodegeneration. Our prime aim is not to explore mechanisms of decay, but we ask the fundamental question of how an axon can be maintained long-term. For this, we focus on the maintenance of the continuous microtubule (MT) bundles in axons that provide the highways for life-sustaining axonal transport. Understanding their maintenance will provide new conceptual frameworks to explain also gene-linked axonopathies.

Addressing our question in fly has enabled us to functionally study and classify >50 actin- and MT-binding and -regulating proteins (Neural Dev 10.1186/s13064-019-0134-0). From this, we could extract novel mechanisms and concepts explaining axon pathology and long-term maintenance. Newly extracted concept is illustrated by our “dependency cycle of local axon homeostasis” (animated summary: tinyurl.com/y4bd42yd): (1) axonal transport is indispensable for axon function/physiology; (2) transport requires MT bundles as essential highways; (3) however, transport mechanically damages MT bundles; (4) therefore, MT bundles have to be actively maintained by MT-binding proteins; (5) this maintenance absolutely requires cargoes and physiology delivered via axonal transport - thus closing the dependency cycle. Breaking this cycle at any point leads to

bundle/axon decay, explaining why trauma, intoxication, genetic defects, or ageing can cause comparable pathologies.

Here I will explain genes and mechanisms underpinning this model. Long-term maintenance of MT bundles includes roles (a) of EB1/spectraplakins in guiding polymerising MTs into bundles, (b) of cortical collapse factors eliminating off-track MTs that leave the bundles, (c) of MT polymerisation machinery in maintaining MT volume and bundle organisation, and (d) of the actin cortex in upholding MT polymerisation. To illustrate how MT maintenance depends on axon physiology, I will explain our findings that loss of kinesin-1 and -3 cause severe MT bundle decay through patho-mechanisms that involve harmful ROS production.

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621C Unravelling the molecular and cellular mechanisms underlying altered lipid metabolism in *Drosophila* model of Huntington's disease

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Huntington's disease (HD) is a dominantly inherited, progressive disorder marked by prominent atrophy of striatal and cortical brain regions. HD is caused by $(CAG)_n$ repeat expansion in *huntingtin* (*HTT*) gene which translates into a mutant form of the ubiquitously present Huntingtin (HTT) protein. Disease phenotype sets in once the number of glutamines in the HTT protein crosses 35, wherein, extensive peripheral metabolic dysfunction coexists with overt neuropathy. Loss of body weight despite normal to high caloric intake remains a critical determinant of the disease progression and a challenge for therapeutic interventions. Earlier in HD fly model, we have reported amendment in level of major macromolecules with disease progression along with significantly dysregulated lipid levels. Furthermore, it was observed that the fatbody of diseased flies undergoes immense structural and functional modification and therefore, we intended to monitor cellular and molecular perturbations, if any, contributing to metabolic changes caused by pan-neuronal expression of mHTT (mutant Huntingtin; 93 glutamine repeats) protein. Interestingly, we found aberrant transcription profile of the key lipolytic and lipogenic effector genes, *lipin* and *brummer*, in HD flies with disease progression. Moreover, we found that fat body undergoes extensive alteration in vital cellular processes and eventually surrenders to elevated apoptotic cell death at terminal stage of the disease. Extensive mitochondrial dysfunction and calcium dyshomeostasis were observed in fat body with disease progression, which might be contributing to the increased apoptosis. Present study provides an insight into the mechanisms through which neuronal expression of mHTT might be inflicting profound systemic effects, specifically on lipid metabolism, and may further open new therapeutic avenues for alleviation of this multidimensional and devastating disease.

622A Age-dependent changes at the neuromuscular junction for an identified adult leg motor neuron in a knock-in *sod1* model of ALS

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease affecting motor neurons (MNs) and leading to paralysis and death in affected individuals. Previously, we used a gene-replacement strategy to introduce disease-causing mutations at conserved positions in the orthologous *Drosophila sod1* (*dsod1*) gene. Flies homozygous for $dsod1^{G85R}$ die as pharate adults while $dsod^{H71Y/H71Y}$ and $dsod1^{null/null}$ eclose but are short-lived. Here, we characterize changes at the neuromuscular junction for $dsod1^{H71Y}$ and $dsod1^{null}$ alleles in an identified MN. Homozygotes for both alleles display progressive leg-dragging phenotypes for the 3rd metathoracic leg. In dissected legs, we assessed age-dependent changes in a single identified motor neuron (MN-I2) innervating the tibia levitator muscle. At adult eclosion, MN-I2 of $dsod1^{H71Y/H71Y}$ or $sod1^{null/null}$ flies is patterned similar to wild type flies indicating no readily apparent developmental defects. At 10 days post-eclosion, MN-I2 shows an overall reduction in arborization with bouton swelling and loss of the post-synaptic marker *discs-large* (*dlg*) in mutant *dsod1* adults. In addition, increases in polyubiquitinated proteins correlate with the timing and extent of MN-I2 changes. Because similar phenotypes are observed between flies homozygous for either $dsod1^{H71Y}$ or $dsod1^{null}$ alleles, we conclude these NMJ changes are mainly associated with *dsod1* loss of function. Together these studies characterize age-related morphological and molecular changes at an identified adult MN and similar techniques can be applied to other motor neuron diseases.

623B Expression of the Human Antimicrobial Peptide, LL-37, Attenuates the Deleterious Effect of $A\beta_{42}$ in a *Drosophila* Model of Alzheimer›s Disease

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Alzheimer's disease (AD) is characterized by amyloid plaques, composed largely of aggregates of the peptide (Aβ), in affected areas of the brain. Aggregates of Aβ, especially soluble oligomers, have been shown to be neurotoxic and evidence indicates these aggregates play an important role in AD pathology. While the function of Aβ is not well understood, recent studies show that the Aβ peptide has antimicrobial properties (Kumar et al., 2016) suggesting it functions as an antimicrobial peptide. In addition, Aβ has been shown, in binding assays *in vitro,* to interact with the known human antimicrobial peptide, LL-37, (De Lorenzi et al., 2017), though it is unknown whether LL-37 and Aβ interact *in vivo.* If LL-37 and Aβ interact directly *in vivo*, LL-37 might have an effect of Aβ aggregation and, as a result, on its neurotoxicity.
To determine whether LL-37 has an effect on Aβ neurotoxicity *in vivo*, we first generated transgenic *Drosophila* that carry human LL-37 under UAS regulation. We used this transgenic fly, along with the AD model fly we previously generated, which carries a UAS-$A\beta_{42}$ transgene, to generate the following four groups of flies expressing these transgenes pan-neuronally: 1) flies expressing $A\beta_{42}$ only; 2) flies expressing LL-37 only; 3) flies co-expressing $A\beta_{42}$ and LL-37; and 4) control flies expressing neither $A\beta_{42}$ nor LL-37. Results showed that while $A\beta_{42}$-expressing flies had significantly shorter lifespans than controls, adults co-expressing $A\beta_{42}$ and LL-37 flies lived longer than flies expressing only Aβ. The LL-37/$A\beta_{42}$ co-expressing flies had lifespans that were shorter than controls, indicating that co-expression partially ameliorated the detrimental effects of $A\beta_{42}$ on lifespan. Additionally, in developmental studies, dual expressing flies had an increased survivorship to adult eclosion compared to flies only expressing $A\beta_{42.}$
In addition to these longevity assays, we conducted RING climbing assays, at different ages, to assess whether there are age- and genotype-related differences in behavior among these same four *Drosophila* genotypes. Our previous experiments indicated that $A\beta_{42}$-expressing flies have impaired locomotor function when compared to the wildtype controls. This allowed us to assess behavioral differences in flies expressing LL-37 individually and in flies co-expressing LL-37 and Aβ to determine if LL-37's modulation of $A\beta_{42}$ effects on lifespan is mirrored in its effects on behavior, reflecting a possible modulation of $A\beta_{42}$'s deleterious effects on neuron function. Together our data indicate that LL-37 partially attenuates the deleterious effects of Aβ, so might play a role in modulating progression of AD pathology.

624C Functional Screen of Lysosomal Storage Disorder Gene Interaction with Alpha-Synuclein Toxicity in *Drosophila*

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Parkinson's disease (PD) is a common and incurable neurodegenerative disorder with strong evidence for heritability. *GBA* is one of the most common genetic risk factors for PD, with carriers of loss-of-function variants having a 5- to 10-fold increased risk of PD. Complete loss of *GBA* causes a metabolic disorder called Gaucher's disease, one in a group of over 50 rare diseases called lysosomal storage disorders (LSDs). In an exome-wide association study including 1156 subjects with PD, we discovered a significant variant burden among 53 LSD gene loci in addition to *GBA*, suggesting that other LSD genes also contribute to PD risk. To investigate further, I have utilized a *Drosophila* transgenic model to experimentally confirm which LSD genes likely modify PD pathogenesis. In flies, pan-neuronal over-expression of human α-synuclein induces Lewy body-like PD pathology along with progressive, age-dependent neurodegeneration and locomotor impairment. Using more than 300 independent RNA-interference strains, I knocked down 94 conserved LSD genes and screened for enhancers or suppressors of α-synuclein-mediated neurodegeneration. My screen has identified 19 genetic modifiers whose knockdown strongly enhances the α-synuclein-associated locomotor phenotype, including homologs of well-established PD risk genes (e.g. *GBA, SCARB2*, and *SMPD1*), as well as many novel gene candidates (*ARSB, IDS, IDUA, LIPA,* and *NPC1)*. Altogether, I have identified three metabolic pathways of interest: regulation of ceramides and sphingolipids, breakdown of glycosaminoglycans, and trafficking/metabolism of cholesterol. Interestingly, the metabolites involved in these pathways constitute major components of lipid rafts, which are particularly important as organizing structures at the synaptic membrane. My results support a model in which LSD gene loss-of-function increases PD risk by promoting α-synuclein toxicity in neurons and further highlight a central role for the lysosome in PD pathogenesis.

625A HAP40 is a conserved central regulator of Huntingtin

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Perturbation of Huntingtin (HTT)'s physiological function is one postulated pathogenic factor in Huntington's disease (HD). However, little is known how HTT is regulated *in vivo*. In a proteomic study, we isolated a novel ~40kDa protein as a strong binding partner of *Drosophila* HTT and demonstrated it was the functional ortholog of HAP40, an HTT associated protein shown recently to modulate HTT's conformation but with unclear physiological and pathologic roles. We showed that in both flies and human cells, HAP40 maintained conserved physical and functional interactions with HTT, loss of HAP40 resulted in similar phenotypes as HTT knockout, including animal viability and autophagy, and more strikingly, HAP40 depletion significantly reduced the levels of endogenous HTT, while HAP40 was mostly degraded via the proteasome in the absence of HTT. Interestingly, polyglutamine expansion in HTT did not affect its affinity for HAP40. However, HAP40 modulated HD pathogenesis in *Drosophila* model by regulating the overall protein levels and potentially also the toxicity of full-length mutant HTT. Together, our study uncovers a conserved mechanism governing the stability and *in vivo* functions of HTT, and demonstrates that HAP40 is a central and positive regulator of HTT and a potential modulator of HD pathogenesis

626B The genetic and neuronal bases of a coevolved reproductive trait in female *Drosophila*

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Sensory inputs during mating, and an individual's response to variation in these inputs, has the potential to affect reproductive success. Although newly evolved sexual traits have often been studied in males, the coevolution of newly evolved sexual traits in females with which these male traits interact during mating has received far less attention. The epandarial posterior lobes (PLs) are reproductive structures that are found only among males of the four species that belong to the *Drosophila melanogaster* species complex. The PLs do not come into contact with female internal reproductive structures, but instead insert between two abdominal segments during copulation. The PLs are essential for the male to grasp the female during mating, which makes them a candidate for a tactile mating signal. PL size and shape vary dramatically among the four sister species, and we have identified several genomic regions between *D. mauritiana* and *D. sechellia* that specify species-specific variation in PL morphology using a large collection of genetic introgressions between these two species. Our data show that when pure species *D. sechellia* females mate with introgression males that have interspecific PL morphology, they reduce the number of eggs they lay. However, when females and males from the same introgression genotype mate, female egg-lay amounts are similar to those of pure species matings. To further analyze this behavioral phenotype, we studied the PL insertion site on female abdomen. We found a series of *fruitless*- and *Piezo*-expressing mechanosensory neurons at the PL insertion sites on the female abdomen, which very in number among the four species. Our experiments in *D. melanogaster* show that although *Piezo* null females display a normal mating position and duration when mated with wild-type males, they lay fewer eggs compared to females from the control wildtype crosses. We used the GAL4-UAS system to manipulate the expression levels of two genes that specify PL development and generated males with increased/decreased PL size. We used these males to test the role of *Piezo*-expressing neurons in receiving sensory clues from the PL, and our preliminary data show that *Piezo* knock-out females lay even fewer eggs when they mate with males that have smaller PLs. These *Piezo*-expressing neurons appear to extend their axons to the abdominal ganglion, and we are currently working to identify their postsynaptic connections.

627C Alternative splicing of *Drosophila* Dopamine 2 Receptor in Memory Circuits

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Alcohol activates and disrupts the reward memory circuitry in the brain. This results in neuroadaptations that affect the ability to respond to reinforcers, and increases susceptibility to addiction. The molecular changes that alter plasticity within these circuits are not well understood. Using a *Drosophila* model, we have recently discovered that alternative splicing occurs in mushroom body neurons after alcohol exposure. The mushroom body is a central brain structure that plays a key role in encoding memories for sensory cues. We hypothesize that alcohol changes memory circuit function and behavior by changing the molecular landscape in these neurons through alternative splicing. In support of this, reducing expression of spliceosome genes in adult mushroom body neurons blocks formation of alcohol associated memories, demonstrating the necessity of RNA processing in memory formation. Furthermore, reducing expression of genes that are alternatively spliced, like the *Dopamine-2-Receptor* (*Dop2R*), in adult MB neurons reduces ethanol memory formation. However, the functional consequences of splicing of these genes is unknown. To test the thus, I am focusing on one alternatively spliced gene, *Dop2R*.

We generated mutants that have forced expression of the naïve *Dop2R* isoform expressed in control flies, and the alternatively spliced *Dop2R* isoform expressed in alcohol treated animals. My goal is to test how these mutations affect the cell biology and physiology of mushroom body circuits, and how this ultimately affects memory and behavior. This will provide insight to how alcohol induces lasting changes in the brain to affect reward response and confer vulnerability to addiction.

628A Temporal Clustering of Alcohol-Responsive genes in *Drosophila*

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Alcohol abuse disorders are highly disabling conditions that provoke life-long struggles to over 30% of the US population. As a neural depressant, alcohol has the ability to cause long-lasting changes in the molecular identity of neurons within the brain through compensatory neuroadaptations of the nervous system. The link between how exactly casual consumption disrupts homeostatic function in the brain to perpetuate an addictive state, is still unknown. Our hypothesis suggests that alcohol exposure leads to long-term dynamic changes in gene expression in the brain that ultimately results in dysfunctional homeostatic neuroadaptations. To further understand how alcohol abuse perpetuates changes in neuroadaptation in the brain, analysis of the temporal transcriptional dynamics that occur in response to alcohol exposure will lead great insight into how gene expression is ultimately affected during alcohol abuse disorders. In order to observe how gene expression is progressively altered in response to alcohol exposure, flies were subjected to a single acute sedating dose of alcohol vapor for 30 minutes. The brains of *D. melanogaster* were then dissected at days 1, 2, 3, 4, 6 and 7 after alcohol exposure, including the brains of control flies that did not receive alcohol, and sequenced using RNA-seq technology. Clustering analysis of genes that showed significant changes in gene expression across time was performed to elucidate the different temporal transcriptional responses to ethanol exposure. The results demonstrate, that with 18 clusters comprising 4,191 genes total, there exists a wide variety of transcriptional responses to alcohol exposure. Further investigation of the alcohol-responsive genes contained in each cluster and their genetic pathways will lead further insight into alcohol's molecular targets and the human disease conditions that arise from alcohol abuse disorders.

629B Temperature-dependent synaptic growth of the *Drosophila* NMJ affects 1s boutons and requires autophagy, the ubiquitin-proteasome system, and MAP Kinases.

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The *Drosophila* Neuromuscular Junction (NMJ) is used to study molecules and molecular mechanisms regulating synapse formation and function. The muscle 6/7 is innervated by two motoneurons (MNs); MN-1s and MN-1b. The synapse formed by MN-1s, has a small synaptic area and reduced subsynaptic reticulum (SSR) while the MN-1b synapse has a larger synaptic area and increased SSR. Interestingly, they are functionally diverse, differing in both basal transmission and plasticity.
It has been reported that the rearing temperature can affect synaptic growth at the NMJ. We reared animals at 15°C, 25°C and 29°C. We observed that animals reared at 29°C had a 186% increase in 1s synaptic boutons when compared to animals reared at 15°C, while the 1b boutons remained constant at all temperatures. This result indicates that motor neurons might be differentially sensitive to changes in temperature.
Next, we identified autophagy as a key regulator of temperature-dependent synaptic growth. Autophagy is a positive regulator of synaptic growth. *atg1* loss of function mutants induced a temperature-independent undergrowth with a decrease of 1s boutons. Then, we accessed Ref (2)P, a component of protein aggregates that diminishes with autophagy activity. Animals reared at 29°C had a decrease of Ref (2)P staining, indicating activation, and protein degradation by autophagy. In addition, using lysotracker we tested if there was a difference of acidic compartments that could indicate change in autophagy activity. Animals reared at 29°C had more lysotracker staining than animals reared at 15°C, indicating higher autophagic activity in animals reared at 29°C. We then tested a known target of Atg; Highwire (Hiw) an E3 ubiquitin ligase. Hiw is a negative regulator of synaptic growth and *hiw* loss of function mutants induced a temperature-independent overgrowth. We hypothesize that, at 29°C, there is little suppression of synaptic growth due to the high activity of autophagy and the ensuing low activity of Hiw. In contrast, at 15°C, reduced autophagic activity provokes increased Hiw activity and suppresses synaptic growth. Finally, we identified the Map Kinases Wallenda (Wnd) and P38b, known targets of Hiw, as key regulators of the synaptic growth of animals reared at 29°C. Indeed, at 29°C, *wnd* and *p38b* loss of function mutants had reduced synaptic growth when compared to control animals. We also found evidence of a genetic interaction between *wnd* and *p38b* suggesting that they function in the same pathway to regulate synaptic growth at 29°C. In this study we identified the requirement of *atg1* and *hiw* genes for the regulation of temperature-dependent synaptic growth. In addition, we identified a novel role for Wnd and P38b in regulating the synaptic growth of animals reared at higher temperature.

630C The *Drosophila* voltage-gated ERG potassium channel *seizure* affects developmental time and oxidative stress resistance

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The highly conserved ERG potassium channels play crucial roles identified in both neural and cardiac systems. In *Drosophila*, nervous system function of the ERG homolog *seizure* (*sei)* has been shown to provide protection from heat-induced seizures, and to impact cardiac function in flies. The human homolog (hERG) contributes to both genetic and pharmacological causes of the cardiac Long QT Syndrome (LQTS), and affects seizure susceptibility. Thus, *sei* loss of function leads to conserved detrimental phenotypes in neural and cardiac systems. Here, we show that *Drosophila sei* mutants exhibit additional phenotypes that may be beneficial. *sei* mutants display decreased developmental time and increased resistance to oxidative stress, due to function of the channel within the nervous system. Neuronal overexpression of *sei* leads to opposite phenotypes: delayed development and reduced resistance to oxidative stress. Along with seizure and cardiac phenotypes, these findings suggest that *sei* expression levels may be finely tuned based on detrimental effects of too little or too much expression with respect to different phenotypes. Additionally, these findings suggest possible impacts of hERG mutations beyond previously known seizure and cardiac phenotypes in humans.

631A The effect of male and female cellular identity on regulation of expression by male-specific isoforms of *fruitless*

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Fruitless is one of two well characterized transcription factors that are directly sex-specifically spliced downstream of the sex determination hierarchy. Proteins encoded by *fruitless* are known to be involved in both early development and in male mating behaviors, such as courtship. The fruitless locus encodes many protein variants and has a complex pattern of alternative transcript isoforms. In total, there are fifteen annotated transcript isoforms that share a common protein-protein binding domain and differ for other regions, for example in the first exon and in the DNA binding domain. These include both transcripts common to males and females expressed from P2-P4 promoters and sex-specifically spliced transcripts expressed from the P1 promoter. Three types of male-specific transcript isoforms are expressed from the P1 promoter in the Drosophila brain, each has a different DNA-binding domain (A, B and C). Most *fru* neurons express all three of these isoforms. However, each isoform has a unique expression profile with some neurons showing isoform specific expression. Interestingly, while Fru-MB and Fru-MC have distinct large effect courtship phenotypes, knock out mutations of Fru-MA have only a modest impact on male mating behavior. Here we use expression profiling of a series of over-expression and sex transformed genotypes to isolate the effects of Fru-MA on gene expression in *fru* expressing neurons in both male and female cellular environments. Incorporating previously published surveys of binding sites and occupancy, we examine the regulatory role of co-expression of male-specific isoforms and the importance of male/female cellular identity in the function of Fruitless isoforms in regulating gene expression.

632B A *Drosophila*-Based Method of Assaying the Effect of Autism-Associated Missense Variants

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Autism spectrum disorders (ASD) encompass neurodevelopmental conditions which impair social interaction, restrict interests, and cause repetitive behaviors. Advances in the field found individuals with ASD have a higher incidence of *de novo* mutations as compared to control cohorts. Though this work has implicated hundreds of genes in ASD pathogenicity, the way these genes contribute to ASD remains elusive. Variants of unknown significance introduce additional challenges, as many genes have no established functional assays. This necessitates the development of novel assays to determine if the variant has an effect on protein function.
We present findings from both a rescue- and over expression-based screen performed in *Drosophila* designed to assess the function of missense, *de novo* variants in 73 genes from the Simons Simplex Collection ASD cohort. Our rescue-based screen

utilized T2A-GAL4 lines, which replace fly genes orthologous to human genes of interest with a GAL4. This knockout was lethal in 47 (43%) of cases. Expressing the human ortholog rescued 17 (46%) of 37 tested non-viable lines. 5 (29%) of the instances where the human reference could rescue, the variant could not, suggesting loss-of-function.
Our overexpression screen used humanized flies that express either the reference or variant human allele under the control of a UAS element. We found 21 (30%) cases where the phenotype from expressing the human variant or reference allele was different, 17 (23%) cases where expressing either the reference or variant had comparable phenotypes, and 35 (48%) cases where expressing neither allele caused a phenotype. Finally, we utilized humanized flies to determine if the expression of human alleles altered one or more stereotyped patterns of behavior. We screened the knockout, reference, and variant-expressing flies for changes in the amount of time spent courting, moving, grooming or copulating. 8 (53%) human allele-expressing lines displayed one or more altered behaviors. In 5 (63%) of these cases the variant-expressing and reference-expressing lines had different behavioral phenotypes.
This screen shows the power of *Drosophila* to perform high-throughput, *in vivo* assays to determine the pathogenicity of missense variants of unknown significance relevant to neurodevelopmental disorders such as ASD. This method also helps determine if an allele is loss- or gain-of-function by comparing the phenotypes between lines expressing the reference or variant allele.

633C Diverse spiking signatures of excitability mutations emerge from a stereotypic seizure-flight sequence in *Drosophila*

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High-frequency electrical stimulation across the brain triggers seizures in humans, mice and flies. In *Drosophila* such stimulation induces stereotypic electroconvulsive seizure (ECS) discharges which manifest across the nervous system and can be monitored through spiking in the large indirect flight muscles (DLMs) in conjunction with microphone recordings of wing beats. During flight, the DLM motor neuron spikes rhythmically (~5 Hz) providing Ca^{2+}influx for stretch-activated myogenic contractions which powers wing beats (~200 Hz). In contrast, during ECS discharges, a distinctive sequence of firing modes have been reported: an initial discharge (ID, ~50 Hz), a quiescent period, and a delayed seizure discharge (~30 Hz peak). Although widely in studies of circuit excitability, most studies employing ECS in *Drosophila* examine the induction threshold, with considerably less attention on alterations in firing patterns during the discharges.
Using an isolated stimulation configuration, we discovered that wild-type (WT) flies would reliably display sustained flight activity following DD (DD-flight). Compared to air-puff triggered flight, DLM spiking during DD-flight was higher (~10 – 20 Hz) while the wing-beat frequency was lower (~170 Hz). We observed a similar motor sequence in other WT strains and in other *Drosophila* species. Furthermore, we observed DD-flight after the surgical removal of sensory structures required for air-puff triggered flight, including the halteres, antennae and wings.
Across broad ranges of excitability mutants, we found that spiking activity during the DD can serve as a 'signature' of the specific manifestation hyperexcitability. Non-linear dynamical systems analyses revealed clear distinctions between two classes of hyperexcitable mutants: 'leg-shakers'(e.g. *Sh*, *qvr*) that twitch under ether anesthesia, and 'bang-sensitives' (e.g. *eas*, *sda*) which display mechanical shock-induced seizures. In leg-shaker mutants, with disrupted I_A K^+currents, the DD-evoked flight sequence was largely intact, but with a declining wing beat frequency, presumably reflecting alterations direct flight muscle biophysical properties. In contrast, bang-sensitive mutants displayed mutant-specific DLM ECS discharge patterns, with varying duration, firing frequency and regularity. However, a common feature across the bang-sensitive mutants was the complete absence of DD-evoked flight.
This work demonstrates that our quantitative treatments of spike patterning can provide succinct signatures for a variety of mutations-specific vulnerabilities in motor circuits.

634A Leptin and insulin signaling interact to modulate olfactory sensory neuron function in *D. melanogaster* larva.

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An animal's satiety state modulates olfactory sensory neuron (OSN) sensitivity and function. Appropriate starvation-dependent modulation of olfactory sensitivity underlies key health-related behaviors, such as foraging and eating, while inappropriate modulation leads to eating and metabolic disorders. Therefore, it is important to understand the molecular mechanisms underlying OSN modulation. Recent work from our lab showed that insulin signaling plays a crucial role in mediating starvation-dependent modulation of olfactory behavior in *D. melanogaster* larvae. Like insulin, leptin is another anorectic peptide implicated in the modulation of olfactory neurons and eating disorders in humans. Therefore, we asked whether the leptin receptor also expresses in larval OSNs. We showed that domeless, a receptor for Upd1 (leptin homolog in fly), expresses in OSNs. Next, we found that altering Upd1/dome signaling in OSNs significantly affected larval foraging behavior. Based on

these initial results, we hypothesized that leptin and insulin signaling interact within OSNs to mediate starvation-dependent modulation of olfactory function. To address this hypothesis, we decreased Upd1/dome signaling in OSNs and monitored its effects on insulin receptor (InR) levels using immunocytochemistry. We found that decreasing Upd1/dome signaling in OSNs increased InR levels. This result suggested that leptin and insulin signaling interact in OSNs, with insulin signaling potentially downstream of leptin signaling. It also suggested that an inverse relationship between the two signaling pathways helps mediate starvation-dependent OSN function changes. In support of this, we showed that decreasing InR expression in OSNs increased OSN spiking in response to odors while decreasing dome-receptor expression in OSNs resulted in the opposite result—decreased OSN spiking in response to odors. Together, these results expand our understanding of the molecular mechanisms underlying OSN modulation and offer new hypotheses to test. Ultimately, these neuromodulatory mechanisms are fundamental to our understanding of how neural circuits support animal cognition and behavior.

635B Steroid hormone signaling activates thermal nociception during *Drosophila* peripheral nervous system development

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Sensory neurons enable animals to respond to environmental changes and avoid harm. An intriguing open question concerns how the neuronal properties which enable sensation are activated during development. *Drosophila melanogaster* larvae undergo a behavioral transition by robustly activating a thermal nociceptive escape behavior during the second half of larval development (3rd instar) (Sulkowski et al. *Biological Bulletin* 2011), but the sensory changes and mechanisms which accompany this transition were previously unknown. Here, we report that heat-induced Ca^{2+} activity in the primary nociceptors, the multimodal Class IV dendritic arbor (C4da) neurons, increases from 2nd to 3rd instar; however, the ultraviolet light-induced Ca^{2+} activity in C4da neurons decreases during the same period of larval development. We find that activation of ecdysone signaling is sufficient to promote precocious nociceptive responses in 2nd instar larvae and suppress *subdued* (encoding a TMEM16 channel) expression. We find that Subdued functions not only to suppress heat-induced nociceptor activity in the 2nd instar but also to promote ultraviolet light-induced activity in the C4da neurons. Thus, steroid hormone signaling suppression of *subdued* expression facilitates the sensory transition of C4da neurons. These roles are in addition to the requirement of *subdued* for thermal nociception in 3rd instar larvae (Jang et al. *Journal of Biological Chemistry,* 2015) and highlights the importance of developmental context in determining the encoding of sensory cues. There is considerable interest in understanding the function of the transcriptionally unique identities which sensory neurons undergo during development (Sharma et al. *Nature*, 2020). Our study provides mechanistic insight into the developmental signals and sensory regulators important for generating a sensory transition in nociceptive neurons.

636C The Role of *Tricornered* in the Development of Glia in the Nervous System

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Glial cells are vital components in the nervous system and are involved in the processes of axon ensheathment, axon targeting, and organ shape. These processes are crucial to a properly functioning nervous system. In previous research, the protein, Raw, was shown to reduce glial proliferation and migration in the developing eye disc of Drosophila. While Raw has been demonstrated to negatively regulate JNK signaling, its mechanism of action remains unclear. Studies in the context of dendrite adhesion and patterning in sensory neurons, have demonstrated that Raw interacts with Tricornered (Trc), an NDR family kinase. Trc is a component of the Hippo signaling pathway that regulates progression through the cell cycle. Even though Trc is placed in this pathway, its function is not well understood. We hypothesize that Trc functions cooperatively with Raw to regulate glial development. In order to explore the role of Trc in glia, knockdown experiments were performed. Trc knockdown in glia of the eye imaginal disc results in reduced numbers of glia. This reduction in glia could be due to glial cell death, reduced proliferation, or defects in glial migration. Preliminary data reveals low levels of cell death upon trc knockdown, suggesting the reduction in glial number is due to defects in proliferation or migration. Experiments are underway to test these possibilities. Future experiments will focus on the relationship of Raw, Trc, and Hippo signaling in the context of glial development.

637A A LOV-based light-gated expression system for intersectional genetics.

Inês M.A. Ribeiro[1], Wolfgang Essbauer[1], Romina Kutlesa[1], Alexander Borst[1] 1) Max Planck Institute of Neurobiology.

The ability to drive exogenous expression of genes of interest in tissues and cell types, under control of specific enhancers has revolutionized biological discovery. In *Drosophila melanogaster*, the UAS-GAL4, Split-GAL4 and LexA-LexAop expression systems are widely relied upon for genetic access to single neuron types. In cases where existing enhancers are not specific

enough for the target neuron type, methods of expression in subsets of cells relying on the combination of heat shock promoter with recombinases, such as MARCM, Flybow or MCFO, are used. In particular, many neuron types in the visual system are composed of several dozens of cells topographically organized, each portraying a small part of the visual field. The topography in the visual system has been implicated in several computations underlying processing of visual information, such as detection of looming or motion, as well as in supplying positional information of visual landmarks. To test roles for topography in visual neural circuits it is necessary to genetically access the same subsets of cells in a consistent manner. We created the LOV-LexA genetic tool that enables light-gated expression in subsets of neuronal cells in a given expression pattern with spatiotemporal resolution. The light oxygen voltage (LOV) domain from *Atena sativa* phototropin I (1) is able to cage short peptides in the dark, and expose them under blue light. We inserted a short nuclear localization signal (NLS) into the evolved LOV (eLOV)(2) and fused it to a modified form (3) of the LexA bacterial transcription factor. Expression of reporter genes under control of LexAop is absent when driving LOV-LexA expression in fat body and several neurons in flies raised at 18ºC, but not in multi-nucleated muscle. Subjecting whole larvae, pupae or flies to light pulses 458nm leads to an increase in the expression of the LexAop reporter gene in fat body and neurons. Induction of expression in subsets of neuronal cells is achieved by restricting exposition to light with an external pinhole. The LOV-LexA tool, together with the recently described Photo-GAL4 (4), thus forms another layer of intersectional genetics that will allow for direct hypothesis testing regarding the role of topography in processing of visual cues. These tools will also enable restriction of expression to subsets of cells in several tissues using broadly expressed GAL4 lines, to compare cells differing solely in expression of a gene of interest within the same organism.

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638B Single-cell transcriptional responses to cocaine exposure in the Drosophila brain.

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Previous studies identified polymorphisms in candidate genes associated with variation in consumption of cocaine among lines of the Drosophila Genetic Reference Panel, and RNAi-mediated targeted gene disruption implicated dopaminergic projections to the mushroom bodies. To identify specific cell populations that respond to acute cocaine exposure, we analyzed single-cell transcriptional responses in duplicate samples of flies that consumed fixed amounts of sucrose or sucrose supplemented with cocaine, sexes separately. After exposure, 20 brains for each sample were dissected, pooled, and dissociated. Cells were separated and lysed, and cDNA was synthesized using Chromium 10x microfluidics followed by sequencing on an Illumina Novaseq. Integration of all eight samples distributed across sexes, conditions and replicates resulted in a dataset of 86,224 cells. Unsupervised clustering of this population yielded 36 distinct clusters. Annotation of clusters based on their gene markers revealed that all major cell types (neuronal and glial), as well as neurotransmitter types from most brain regions, were represented (including the optic lobe and the mushroom body). Differential expression analysis within individual clusters indicated cluster-specific responses to cocaine. Specifically, clusters corresponding to glia, T1 and T4/T5 neurons of the optic lobe, Kenyon cells, and photoreceptor cells showed dramatic transcriptional responses following cocaine exposure. Some clusters also showed significantly divergent responses across the sexes. Additionally, transcriptional responses to cocaine in most clusters were considerably more pronounced in male than in female brains. Thus, cocaine exposure elicits sexually dimorphic transcriptional responses in both glia and neurons in multiple compartments of the Drosophila brain. Supported by1U01-DA041613.

639C Subcellular localization of 5-HT receptors in the nervous system of Drosophila melanogaster

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Serotonin (5-HT) receptors are widely expressed in the nervous system of Drosophila and participate in neuronal modulation of many aspects such as sleep, circadian behaviors, aggregation, courtship and mating. The MiMIC (Minos Mediated Integration Cassette) lines, which couple the expression Gal4 with specific genes, together with the upstream activation sequence (UAS)-expression system have told us some information such as cell type specific expression of 5-HT receptors. However, due to a lack of antibodies targeting the five types of receptors, the endogenous expression and subcellular expression pattern of the receptors are still unclear. In this project, we label the endogenous 5-HT receptors with seven tandem repeats of superfold GFP fragment 11 (GFP11) and the HA tag via CRISPR/Cas9 editing of the fly genome. In this methodology, the HA tag labels all the receptors in the animal body, while GFP11 fragment will specifically reveal the receptor in cells and locations where its

complementary fragment, GFP1-10, is introduced in a cell type specific manner. In immunostaining targeting the HA tag, we found that the endogenous 5-HT1A and 5-HT7 receptors were highly expressed in the mushroom body and the ellipsoid body, respectively. These localization patterns are consistent with the previous studies with the MiMIC lines. In future study, we will use GFP1-10/11 reconstitution to check the cell type specific distribution of 5-HT receptors.

640A Chromatin based reprogramming of courtship behaviors with social experience

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Many behaviors consist of innate and experience-dependent components. While developmental gene expression programs establish structure and function of neural circuits, a lot less is known about how sensory experience modifies these programs to execute innate or learned behaviors. We use fly olfaction and courtship as a model to understand how changes in genes regulation with social experience drive innate and experience-dependent behaviors. Courtship provides a great system to study this question because it has both innate and experience-dependent components driven by two transcription factors, Fruitless (Fru) and Doublesex (Dsx), respectively. Fruitless is necessary and sufficient to drive male innate courtship behaviors and is expressed in approximately 2000 interconnected neurons. Socially isolated *fru* mutant males do not court. However, if grouped, they use olfaction – through pheromone sensing- to learn to court with flies around them, regardless of their sex. This learning also requires the gene *dsx,* which is co-expressed with *fru* in a subset of courtship decisions neurons. *Fru* mutants that are smell blind or lack *dsx* fail to learn courtship when group housed. We hypothesize that social experience-dependent changes in gene expression through epigenetic mechanisms drive such behavioral adaptations. Previously, we showed that chromatin around *fru* gene is modified with sensory experience and pheromone receptor signaling in the olfactory receptor neurons (ORNs). We recently found that changes in *fru* regulation leads to differential expression of ion channels and neuromodulation, which alters the pheromone sensitivity ORNs and competitive courtship advantage of males. We predicted that social experience and ORN activity can also regulate chromatin around *fru* and *dsx* in the central circuits to regulate innate and learned courtship behaviors. To identify social experience dependent changes in gene regulation that modify courtship circuit structure and function, we analyzed chromatin around *fru* and *dsx* in the central circuits of the brain. We found that group housing and social experience modified active chromatin marks around *fru* and *dsx*. Interestingly, signaling from different pheromone receptors elicited differential effects on chromatin around both genes in the central brain. In addition, we found that open chromatin marks around *dsx* increase in the brain when *fru* mutant are grouped. This suggests that social experience and ORN circuit activity can modify *dsx* regulation in the brain and mediate courtship learning in *fru* mutants. Our results provide insights into the fundamental mechanisms by which sensory experience drive behavioral plasticity and learning, via chromatin-mediated changes in the expression of genes critical for neural circuit structure and function.

641B Cold Sensation in Drosophila Larvae

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Thermosensation is crucial to the survival of all animals. Temperature-sensitive neurons and receptors located in the nervous system sense temperature cues in an evolutionarily conserved manner. Unlike heat sensation, the mechanisms of cold sensation are less understood. Using custom-designed electronic devices that can cool rapidly and precisely, we observed several cold-induced behavioral responses in fly larvae. Interestingly, DOCC neurons, which are known for sensing cooling, are not required for these larval cold avoidance responses, suggesting that other unknown sensory organs may mediate cold sensation in fly larvae. We tested some genetic mutants lacking known cold sensors and found that they are not necessary for larvae to sense cold. Our results show that Drosophila larvae sense and respond robustly to cold stimuli through novel neural and genetic pathways.

642C Determination of curcuminoid targets mediating sex-specific neurological differences in *Drosophila*

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Oxidative stress, which occurs from an imbalance of reactive oxygen and nitrogen species 28 (RONS) and both endogenous and exogenous antioxidants, promotes aging and underlies sex-29 specific differences in longevity and susceptibility to age-related neurodegeneration. Curcumin, a yellow pigment derived from turmeric and shown to exhibit antioxidant properties

as an ROS scavenger, influences the regulation of genetic elements in endogenous antioxidant pathways. To investigate the role of curcumin in sex-specific in vivo responses to oxidative stress, Drosophila were reared on media supplemented with 0.25 mM, 2.5 mM, or 25 mM curcuminoids (consisting of curcumin, demethoxycurcumin, and bisdemethoxycurcumin) and neuroanatomical and behavioral parameters were assessed. Inducible ELAV-GeneSwitch constructs were used to interrogate potential molecular targets of curcumin in the Drosophila nervous system that underlie sex-dependent alterations in neural function. High levels of curcuminoids results in sex-specific changes to larval brain size and turning rate in an open field. Taken together, these results suggest that the influence of curcuminoids in the nervous system likely relies on changes in gene expression, and that sexual dimorphism exists in the in vivo response to curcuminoids.

643A Synaptic Ultrastructure at the Drosophila Neuromuscular Junction

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Fast and efficient intercellular communication in the brain is orchestrated through the release of neurotransmitter from presynaptic nerve terminals. The “active zone”, a specific presynaptic specialization, is the primary mediator for transmitter release during cell-to-cell communication. Transmitter-filled vesicles dock, prime, and ultimately fuse with the presynaptic membrane at active zones to release transmitter. While the functional roles of several active zone proteins have been determined, the resolution limitations of standard electron microscopy (~50 nm) have limited the identification of spatial relationships. Electron Tomography permits the 3D analysis of 0.5 nm virtual slices through a single 50-70 nm sample. Recently, the 3D active zone ultrastructure at the mouse and frog neuromuscular junction have been determined at a sufficient level of resolution to begin assessing functional spatial relationships. One spatial relationship that has newly been studied, using this high-resolution 3D tomography, is that of the transmitter-filled synaptic vesicles and their associated pre-synaptic membrane at the neuromuscular junctions of frog. This study, conducted by Dr. McMahan’s lab at Texas A&M, revealed that the physical contact area between the vesicle membrane and the plasma membrane (VM-PM) shows a normal distribution. Moreover, the VM-PM contact area distribution shifts to the left when the neuromuscular junctions are fixed during stimulation. These results demonstrate that not only is the VM-PM contact area measurable using electron tomography but that we can also use it as a correlate of synaptic vesicle priming. This proposal aims to exploit the fast, cost-effective, genetic system of Drosophila to test a mutation in synaptotagmin, the Ca^{2+} sensor required for vesicle fusion, hypothesized to play a role in synaptic vesicle priming. We will also use the 3D data to begin identifying the presynaptic ultrastructure at the active zone and start determining the molecular mechanisms mediating neurotransmitter release.

644B Copia, of many copies and the Drosophila NMJ

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Copia is an abundant retrotransposon found in *Drosophila melanogaster*. It is found as a full length or spliced form and we hypothesize that copia may play a significant role at the Drosophila neuromuscular junction. Copia was found enriched together with Arc1 in extra-cellular vesicles derived from S2 cells. From RNA-sequencing data coupled with digital PCR, we determined that copia has several full-length forms that are found in differing abundancy in the Drosophila larval brain and body wall muscles. We also show that, in the Drosophila larval brain, the short-spliced form (copia Gag) is highly abundant compared to the full-length version. We are performing RIP-seq, and digital RT-qPCR to establish the interactome of copia and perform electrophysiological recordings in copia RNAi flies to establish the physiological role of this retrotransposon.

645C Understanding How a Human Short Sleep Mutation offsets the Negative Effect of Sleep Deprivation

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Sleep is an essential physiological process important for many biological functions. Inadequate sleep leads to many health issues including cognitive impairment and neurodegenerative diseases. However, natural short sleepers exist in the human population who sleep less than the average individual with no negative impacts on health. For example, a proline-to-arginine mutation in the human *dec2* gene *(dec2^{P384R})* is associated with a short sleep phenotype and individuals with this mutation do not seem to exhibit the usual effects of sleep deprivation. Mechanistically, the *dec2^{P384R}* mutation induces overexpression of the wake-promoting gene, *prepro-orexin,* also known as *hypocretin (Hcrt)* to promote prolonged wakefulness. We have found that over-expressing mammalian *dec2^{P384R}* in *Drosophila* sleep-controlling neurons results in significantly less sleep time, mimicking the human short sleep phenotype. *dec2^{P384R}* mutant flies also show signs of improved health. Notably, *dec2^{P384R}* mutant flies live longer and are more stress-resistant compared to control flies. Thus, our fly model provides an opportunity to understand the pro-health mechanisms of the *dec2^{P384R}* mutant. Currently, we are examining the molecular pathways by which *dec2^{P384R}* mutants

achieve stress resistance and longer lifespans. The results of our studies could uncover novel pro-health targets that could be manipulated to reduce the negative impacts of sleep deprivation and aging.

646A Roles of somatic and microdomain astrocyte Ca^{2+} signals in chronic pain sensitization following nerve injury

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Chronic pain following acute nerve injury is a debilitating condition associated with serious comorbidities and opioid dependence. Understanding the neurological mechanisms that underlie chronic pain is essential for prevention and treatment. The transition from initial injury to neuropathic pain is mediated by central sensitization, which involves reversible changes to nociceptive neurocircuitry in the central nervous system. Much of our understanding of central sensitization focuses on neurons, while the role of astrocytes is less defined. Astrocytes normally maintain and modulate synapses, but they become reactive and exhibit aberrant Ca^{2+} signals during central sensitization. How these Ca^{2+} signals are generated and how they modulate astrocyte function in central sensitization are unknown. Astrocytes generate both somatic Ca^{2+} transients and microdomain Ca^{2+} signals at fine processes. Somatic Ca^{2+} transients are activity dependent and occur downstream of metabotropic neurotransmitter receptors, and they can induce gliotransmitter and cytokine release from astrocytes. Ca^{2+} microdomains, on the other hand, are spontaneous and asynchronous and occur close to synapses, where they may regulate localized control of synaptic activity. Importantly, while we cannot experimentally separate somatic and microdomain Ca^{2+} signals in mammalian astrocytes, *Drosophila melanogaster* astrocyte somatic and microdomain Ca^{2+} channels are clearly defined: somatic Ca^{2+} transients are generated by the TrpA channel Waterwitch (Wtrw), and Ca^{2+} microdomains are generated by TrpML. Our objective is to define the roles of astrocyte somatic and microdomain Ca^{2+} signals in central pain sensitization in *Drosophila* by combining genetic manipulation of Wtrw and TrpML expression with astrocyte Ca^{2+} imaging and a behavioral assay of pain sensitization. We show that acute injury due to single leg amputation in adult flies results in enhanced Ca^{2+} signals in astrocytes using the Transcriptional Reporter of Intracellular Ca^{2+} (TRIC). We also show that acute injury results in increased pain responsiveness after seven days, indicative of chronic pain sensitization. We are now testing the roles of Wtrw and TrpML in these responses. Our results will bring new understanding to the role of astrocyte signaling in pain sensitization and may offer novel therapeutic targets for the prevention or treatment of chronic pain.

647B Sensory organs of *Drosophila* larvae: morphology and ultrastructure

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The *Drosophila melanogaster* larva allows to study various aspects of behavior including taxis, kinesis and even learning and memory. All behavior that the animal exhibits is based on processing of sensory information from environmental stimuli, the internal state and acquired experience. But how do larvae actually perceive their environment? What sensory organs are they equipped with? Does the ultrastructure of these sensory organs allow for conclusions about their function? Drosophila melanogaster larvae possess external sense organs on their head, thoracic, and abdominal segments specialized to receive diverse environmental information. As in humans, most of the sensory organs are concentrated at the head of the *Drosophila* larva. There are four major external organs, the terminal organ (TO) the dorsal organ (DO), the ventral organ (VO) and the labial organ (LBO). The 3D ultrastructure of the latter 3 is described in this study using focus-ion-beam scanning electron microscopy (FIB-SEM) and serial section transmission electron microscopy (ssTEM). In addition, all external sensillum types of the trunk are described. We provide data on the subcellular configuration and morphology of all individual sensilla and link them to putative sensory function. The ultrastructural description of the main larval sense organs of *Drosophila* will serve as a basis for further molecular and functional examination.

648C Ammonia detection in *Drosophila melanogaster* is mediated by a non-canonical receptor in previously unidentified olfactory neurons

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Many insect vectors of disease are attracted to ammonia, a kairomone secreted by humans in sweat and breath. Other insects, including the model organism *Drosophila melanogaster*, are also attracted to low levels of ammonia. In Drosophila, antennal ac1 olfactory sensilla are highly sensitive to ammonia, and this response is thought to be mediated by the IR92a receptor found in IR92a+ olfactory receptor neurons (ORNs). However, such receptors are poorly conserved across insect species. In Drosophila, ac1 ammonia responses also rely on an ammonium transporter, Amt, found in the same sensilla. Here, we demonstrate that ac1 sensilla contain a previously unidentified 4th ORN that expresses both Amt and a related ammonium transporter, Rh50. Surprisingly, neuronal ablation experiments suggest that the 4th ORN mediates ammonia responses in ac1 sensilla. This was confirmed by analysis of ammonia responses in a newly generated IR92a mutant fly line. GCaMP imaging and electrophysiological experiments suggest that ammonia responses in the 4th ORN do not rely on canonical odor receptor co-receptors and that these ORNs respond exclusively to ammonia. In other organisms, members of the evolutionarily conserved ammonium transporter family are highly selective for ammonium, and some are electrogenic. We propose that charged ammonium molecules are transported into the ORN by these transporters, and that ammonium ions directly depolarize the cells. This is in contrast to all other odors, which act on ligand-gated receptors. Our data indicate that misexpression of Amt in an ammonia-insensitive neuron confers ammonia responses to this cell. Thus, members of the ammonium transporter family may act as conserved, noncanonical odor receptors for this ecologically important odor.

649A Employing TurboID and Ribosomal Profiling to examine the molecular defects caused by Intellectual Disability-associated mutations in KDM5

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Mutations in *kdm5*, a lysine demethylase, across multiple domains have been shown to cause intellectual disability (ID) in humans as well as in multiple model organisms. To understand the link between mutations in *kdm5* and ID, we have generated a genetic toolkit that includes 8 fly strains that harbor ID-associated point mutations. Our initial studies of an ID-associated point mutation that behaves indistinguishably from a demethylase dead (Dmd) form of KDM5 showed drastically reduced global protein translation in *Drosophila* heads, and not thoraces, compared to wildtype. Moreover, one third of ribosomal protein (Rp) transcripts are reduced, suggesting that KDM5 is required to maintain the expression of Rp genes. To discover how the dynamics of translation are changing *in vivo* in our ID mutant fly strains we are performing ribosomal profiling using RPL3-Flag as a target to precipitate in-tact ribosomes and the mRNA contained within them. These 20-40 base pair sequences can reveal if mutated KDM5 affects all translation or translation of a unique subset of genes that may be particularly important in neurons. Moreover, this method can uncover differential translation dynamics within the same gene such as premature termination, pause sites, and internal entry sites. We are also interested in understanding the molecular defects of KDM5-ID mutations, since these occur mostly in domains that are currently of unknown function. To do this, we are employing proximity labeling using TurboID, a promiscuous biotinylator. We are creating both N and C terminal KDM5 fusions to ascertain interactors in both wildtype and ID mutant brains. TurboID is advantageous to immunoprecipitation (IP) as it allows *in vivo* labeling of interacting proteins during a short time window, is more specific and reduces background. Biotinylated proteins can be precipitated using streptavidin and analyzed through mass spectrometry to form a library of Kdm5 interactors. Combined, employing TurboID and ribosomal profiling will allow a novel and highly specific window into how both canonical and non-canonical functions of KDM5 impact the fly brain.

650B An in vivo RNAi screen to identify genes involved in subcellular localization of the Golgi kinase Four-jointed

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Four-jointed (Fj) is a Golgi kinase that phosphorylates extracellular domains of the atypical cadherins Fat and its binding partner Dachsous (Ds). Phosphorylation of Fat enhances Fat-Ds interactions and phosphorylation of Ds suppresses its interactions. Fj regulates cell growth and planar cell polarity (PCP) by modulating Fat-Ds interactions in *Drosophila* development. Golgi complex is a stack of cis-, medial-, and trans-cisternae, and the cisternae are present as dispersed stacks (units) in *Drosophila* cells. We previously found that Fj was localized to a subset of Golgi units in the cells of the wing imaginal discs. To investigate molecular mechanisms by which Fj was localized to a subset of Golgi units, we decided to identify genes regulating subcellular localization of Fj. When Fj tagged with the V5 epitope (Fj:V5) was expressed in the cell lines derived from wing imaginal discs, some of cell lines had a similar property in Fj:V5 localization as the wing imaginal discs although the other cell lines did not. By using mRNA seq data analysis between these cell lines, we selected genes whose expression level correlates with tendency on the localization property of Fj. These genes were considered as candidate genes for regulating Fj localization. Therefore, we performed RNAi knock down the expression of the candidate genes in the wing imaginal discs of the third instar larvae, and investigated the subcellular localization of Fj. As a result, we identified a

novel gene *four-jointed localization factor 1* (*flf1*). The number of Golgi units which Fj was localized were decrease by knock down of *flf1* in the wing imaginal discs. Moreover, *flf1* RNAi resulted in a reduction in the distance between the anterior and posterior crossveins on the adult wings. Notably that wing abnormality is also found in the mutations of *fj*. Collectively, these results suggest that *flf1* is required for both proper subcellular localization of Fj and normal wing development.

651C Role of Intramembrane Spastic Paraplegia Proteins in Organization of Axonal ER and ER-mitochondria Contacts in Drosophila

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The Hereditary Spastic Paraplegias (HSPs) are a group of rare, clinically and genetically heterogeneous, inherited neurodegenerative and neurodevelopmental diseases characterised by spasticity and lower limb weaknesses. More than 80 causative genes are known, and some of them imply the importance of endoplasmic reticulum (ER)-**a neuron within a neuron**-function and morphogenesis in HSPs. These HSP proteins are Spastin (SPG4), Atlastin (SPG3A), Receptor Expression Enhancing Protein 1 (REEP1/SPG31) and Reticulon (SPG12), which share a common feature of one or two intramembrane hairpin domains that can recognise or drive curvature of ER membrane. Proteins of the REEP and reticulon families appear to be responsible for forming most peripheral ER tubules in yeast. In Drosophila, removing these families leads to fewer ER tubules in axons, of wider diameter, although there is no widespread absence of tubules. Therefore, other proteins must be involved in shaping the tubular ER network in Drosophila axons. Another HSP protein with predicted hairpin domains is C19orf12; this is therefore another candidate protein for contributing to shaping the axon ER network. Mutations in this gene are found in patients with autosomal recessive HSP and Neurodegeneration with Brain Iron Accumulation (NBIA). C19orf12 protein colocalises with mitochondria and ER, and with ERmitochondria contacts. Most C19orf12 mutations are found in predicted transmembrane (TM) regions. To investigate possible roles of C19orf12 in ER and mitochondria structure and function, we have generated loss-of-function mutants of the widely expressed Drosophila ortholog of C19orf12, CG3740, using P element excision and CRISPR/cas9. These mutants are homozygous viable, as are quadruple mutants lacking CG3740 and all the widely expressed reticulon and REEP proteins, suggesting that these 4 proteins together are not sufficient for tubular ER formation. Testing of ER and mitochondria morphology in these mutants is in progress. For this purpose, we have also generated flies carrying a split-GFP reporter for ER-mitochondrial contact sites. To identify additional candidate proteins for ER shaping, we have also performed bioinformatic analyses of proteins shown by proteomic analysis to be enriched on ER tubules. These analyses have identified most of the known proteins with roles in ER shaping, implying that it may also be a good way to identify additional proteins with similar roles.

652A Clu bliss particles function in the translation and import of nucleus-encoded mitochondrial proteins

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A common immediate mechanism to regulate gene expression under conditions of cell stress is localization of mRNA to ribonucleoprotein (RNP) granules for post-transcriptional regulation. Upregulation of RNP granules such as stress granules and P-bodies is well-characterized in cellular stress responses, where they affect transport, translation, and stability of mature mRNAs. We have shown that the nucleus-encoded gene *clueless (clu)* is necessary for properly functioning mitochondria. *Drosophila clueless* mutants are sterile and have direct and systemic mitochondrial dysfunctions. Clu exists in female germ cells as large cytoplasmic, mitochondria-associated particles under healthy cellular conditions. However, in contrast to other RNP granules, Clu particles disperse under mitochondrial, oxidative, or nutritional stress. Short periods of starvation cause Clu particle dispersion and refeeding flies causes them to reform, demonstrating the reversible nature of their dynamics. In addition, we have found insulin is both necessary and sufficient for their formation. We have also shown that Clu is a ribonucleoprotein. Clu physically interacts with mRNAs through its tetratricopeptide domain. Using co-immunoprecipitation and mass spectrometry analysis, we have shown that Clu physically interacts with many different ribosomal proteins present in the small and large ribosomal subunits, multiple translation initiation and translation elongation factors, and the mitochondrial transporters TOM20 and Porin in the outer mitochondrial membrane. Sucrose gradient analysis demonstrates that Clu sediments in the heaviest fractions with polysomes and that a shift to lighter fractions occurs upon ribosomal disassembly and in the absence of RNA. In addition, mitochondrial protein levels are also reduced in *Drosophila clu* mutants. Based on these findings, our current model is that Clu particles are RNP granules which may regulate the mRNAs of mitochondrial proteins bound for mitochondrial import in response to nutritional cues. Our current aims are to explore the role of Clu particles in the translation and import of mitochondrial proteins by elucidating whether the presence or absence of Clu particles affects translation and import of mitochondrial proteins and if the absence of global translation affects the stability of Clu particles using fixed and live imaging.

653B Orchestrated intragranular restructuring of distinct cargo during secretory granule maturation

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The mucous membrane present on the apical surface of vital tubular organs such as the gut, lungs, and urinogenital tract predominantly consists of large, high molecular weight glycoproteins called mucins. Mucins are synthesized and packaged in a condensed form into secretory granules within specialized cells known as goblet cells. Aberrations in mucin synthesis and biophysical properties are associated with many diseases, including inflammatory bowel disease, chronic bronchitis, and cystic fibrosis. Despite the importance of mucins in health, little is known regarding mucin synthesis, modification, and packaging into secretory granules. To understand the molecular basis of mucin packaging and secretion, we utilized *Drosophila melanogaster* salivary glands as a model system. Using a combination of *Drosophila*genetics and multimodality imaging (confocal and electron microscopy), we demonstrate that multiple mucins are packaged within the same secretory granule and display unique intragranular morphologies. Moreover, we found that these mucins undergo regulated restructuring over time to form mature granules. Furthermore, in a reverse genetics screen, we found important roles for Cl^-, Ca^{2+} transporters and the V-ATPase proton pump in the proper organization of the mucins and morphology of the secretory granules. We hypothesize that each mucin undergoes a regulated series of maturation events that results in proper packaging and spatial segregation. This represents a novel and efficient strategy to pack multiple, large proteins into one secretory granule yet maintain intragranular compartmentalization. These studies will ultimately advance our understanding of mucin secretory granule biogenesis and its impact on health and disease.

654C Structure function analysis of *defective proventriculus* (*dve*) in *Drosophila melanogaster* eye growth and development

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During development, axial patterning is required to establish Antero-posterior (AP), Dorso-Ventral (DV), and Proximo-Distal (PD) axes, which is crucial for the generation of a 3-dimensional organ from a monolayer organ primordium. Of the three axes, DV axis is the first lineage restriction event during eye development and any deviation results in developmental birth defects. In our study, we have used *Drosophila melanogaster* (Fruit fly) eye as a model system to understand the role of different domains of a new dorsal eye fate selector gene, *defective proventriculus* (*dve*, an ortholog of *SATB1*) in growth and development. In humans, *SATB1*, functions as a transcriptional regulator and chromatin organizer and requires tetramerization by the ULD domain. In *Drosophila* eye, *dve* regulates expression of *wingless (wg),* a negative regulator of eye. In the genetic hierarchy, *dve* acts downstream of GATA-1 transcription factor *pannier (pnr)* and upstream of *wg*. Loss-of-function of *dve* results in dorsal eye enlargement while gain-of-function results in eye suppression. We performed structure function analysis of Dve protein to elucidate the role of various domains in patterning, growth and development. We have developed several transgenic lines, which will allow us to induce expression of the specific domains of Dve protein and assay their effect on *Drosophila* eye growth and development. Dve has a ULD domain for tetramerization, HOX domains for DNA binding and PPP4R2 domain for H2AFX dephosphorylation. Here we present our results on ectopic induction of these domains and their effect on eye phenotype and wg expression in the developing eye.

655A Wingless and hedgehog pathway requirement for intrasegmental ring gland specification

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The *Drosophila* ring gland is formed by the fusion of three endocrine organs with different embryonic origin: the *corpora cardiaca*, the *corpora allata* (CA) and the prothoracic glands (PG). Previous work revealed that CA and PG primordia are specified in the maxillary and labial segments of the embryo. Key to the ring gland's formation is the activation of Snail expression in the CA and the PG primordia driven by a specific *snail* ring gland enhancer (*sna-rg*), which triggers epithelial to mesenchymal transition and migration of the endocrine primordia. STAT and Hox genes *Deformed* and *Sex combs reduced* are essential for CA and PG fate specification, but are expressed in a broad manner within the segment. Instead, endocrine primordia are specified only in the most anterior cells of the maxillary and labial segments. Here we show how *wingless* (*wg*) and *hedgehog* (*hh*) signaling pathways act through their intracellular effectors to restrict the precise localization of the CA and PG. First, we show that *wg*, but not *d-TCF* mutants, result in ectopic expression of *sna-rg* in a subset of cells of the anterior compartment. In addition, double *wg, d-TCF* mutants do not form ectopic primordia as does the *wg* mutant alone. This indicates that d-TCF acts as an indirect regulator of *sna-rg*, probably through modulation of a localized repressor. Second,

we show that *hh* mutants, but not *cubitus interruptus* (*ci*) mutants, completely downregulate *sna-rg* expression, suggesting that *ci* acts as a repressor of *sna-rg* only in the absence of *hh* signaling, and that *hh* signaling has no positive input over *sna-rg*. In summary, we propose that specific localization of the early ring gland primordia is achieved by a combination of repressive inputs controlled by segment polarity genes, which restricts its intrasegmental localization.

656B Epistatic analysis of phosphatidic acid metabolism genes reveals a regulatory role in Notch signaling by modifying receptor and Sapodo trafficking during *Drosophila* sensory organ development

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Notch pathway plays a crucial role during development and tissue homeostasis regulating binary cell-fate decisions. During asymmetric cell divisions of the *Drosophila's* sensory organ precursor cells, the regulated traffic of Notch modulates signaling pathway activity. Phosphatidic acid metabolic fates regulate signaling pathways by affecting receptor and ligand traffic. Nevertheless, its role in regulating binary cell-fate decisions during asymmetric cell divisions is not well known. Here we show that phospholipase D-derived phosphatidic acid (PLD-PA) activates Notch signaling by affecting Notch trafficking through the endosomal compartments in a mechanism that involves Sanpodo. Epistatic analyses indicate that PLD-PA and not phosphoinositides affect cell fate decisions during sensory organ development promoting ectopic activation of Notch in daughter cells where it is normally inactive. Removing a copy of Notch or Sanpodo suppresses this phenotype dominantly, while null mutants of Numb or the α-subunit of Adaptor Protein complex-2, enhance it. Removing a copy of Delta and Serrate enhances Notch activation, suggesting that cis-inhibition mediated by the ligand is affected and that ligand-independent activation of Notch might be induced. PLD-PA promotes Notch internalization and the enlargement of the early endosome compartment. Accordingly, in vivo experiments show decreased localization of Sanpodo at acidic late endosomal compartments, which is associated with increased internalization of the receptor. Together, these data suggest that PLD-PA activates Notch signaling by inhibiting Sanpodo trafficking towards degradation or by promoting ligand-independent activation at early endosomes. Understanding how genetic and environmental modulation of the membrane lipid composition affects Notch signaling during asymmetric cell divisions may help design therapies to Notch associated diseases.

657C Characterizing the Role of Doublesex in Creating Sexual Dimorphism in the Somatic Gonad

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The Doublesex (Dsx) and Mab-3 Related Transcription factor (DMRT) family proteins are paramount for sex determination in most animals, from planaria to flies, birds, mice, and humans. In *Drosophila*, the embryonic gonad is formed when a bipotent cluster of somatic gonadal precursors (SGPs) coalesces with the germ cells. Sex determination is regulated by X chromosome dosage, which activates an alternative splicing cascade that yields Dsx^F in females and Dsx^M in males. Both Dsx isoforms have the same DNA binding domain, but they regulate their targets differently to yield sexual dimorphism. Dsx, like mammalian Dmrt1, is first expressed in the somatic gonad during embryogenesis and is required for male vs. female gonad development. It is known that *dsx* is expressed in early SGPs during development, but the exact timing and role of Dsx in sex-specific cell fate specification during gonad development is unknown. We are taking a three-pronged approach to investigate the cell fate decisions that are regulated by Dsx, as well as the target genes it controls to make these decisions. First, we are using an endogenously tagged GFP-Dsx to characterize Dsx expression in the somatic gonad. We found that males continuously express Dsx, while female Dsx expression changes during development. Second, we are conducting a lineage analysis to characterize how SGPs contribute to ovary development – in particular, how do female SGPs achieve the greater diversity of ovarian somatic cell types compared to the testis? We are using G-TRACE, a *gal4-UAS* technique that permanently labels cells and allows for analysis at desired time points. Interestingly, using *tj-gal4*, which should label all embryonic SGPs, we found an unlabeled population of somatic cells, first visible in the larval apical cap, which migrate downwards between ovarioles during pupal development. Lastly, we are using small *dsx* LOF clones to investigate which cells of the gonad require autonomous sex information, and which cells may be regulated through cell-cell signaling. Ultimately, we aim to elucidate the mechanisms of Dsx in regulating the sex determination of *Drosophila* gonad somatic stem cell development.

658A Targeting glioblastoma cytoneme network: ODFs proteins

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Glioblastoma (GB) is the most common and aggressive malignant primary brain tumor of the central nervous system in humans, originated from neoplastic glial cells. It affects 1/100.000 persons and median survival is 14,6 months. Neoplastic glial cells are characterized by a high degree of proliferation, diffuse invasion in the cerebral parenchyma and resistance to conventional therapies (chemotherapy and radiation therapy). Patients suffer from progressive memory loss, defects in speech and language, epileptic seizures and vomiting, symptoms of neurodegenerative processes, compatible with synapse loss as an early event that occurs in neurodegeneration. All this suggests that GB is not only a tumor, but also a neurodegenerative disease, which could explain the failure of therapies so far. Thus, resistance to conventional cytotoxic therapies persists in the need to study the genetic and molecular basis of the disease, as it is now considered an incurable disease.
We propose to use Drosophila melanogaster model of GB which is one of the most reliable GB models available. Activating mutations of EGFR and PI3K pathways are the most common mutations in GB patients. The genetic activation of PI3K and EGFR in Drosophila glial cells faithfully reproduces the progression of a GB and associated neurodegeneration. Our group has extensively worked with this GB animal model and demonstrated that GB cells proliferate and invade the brain, which causes a reduction in the number of synapses in the neighboring neurons. In addition, tumor microtubes (TMs) are membrane protrusions that form a network among GB cells. TMs formation is also conserved in this Drosophila GB model, mediates the invasive and aggressive phenotype of the tumor and mediates neurodegeneration. Our aim is to identify the mechanisms that regulate the formation and growth of the connections network of the tumor. Based on that, we are focus on the role of ODFs, sperm cytoskeleton proteins, as cytoneme network modulators. Our results in the Drosophila tumor model show that ODF3B and ODF3L2 are upregulated during tumor progression. Moreover, the downregulation of these ODFs genes not only prevent tumor growth but also restore the glioma neurodegenerationassociated.

659B Early Role of Rif1 in the Repair of P-element Transposase-Induced DNA Double Strand Breaks

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Rap1 interacting factor (RIF1) is a structurally well-conserved protein in eukaryotes that plays an important role in pathway choice during the repair of DNA double strand breaks (DSBs). In human cells, Rif1 directly interacts with phosphorylated p53 binding protein (53BP1) to inhibit BRCA1 mediated resection of DSBs during G1, and hence promotes repair via non-homologous end joining. In contrast, *Saccharomyces cerevisiae* Rif1 was shown to promote resection and homology-directed repair of DSBs in both G1 and G2. Given the opposing repair functions of Rif1 in evolutionary distant mammalian and yeast cells, we have explored the DSB repair role of Rif1 in phylogenetic intermediate *Drosophila melanogaster.* Hatching assays and irradiation sensitivity assays were conducted to determine whether *rif1* mutant fly stocks exhibit any DSB repair defect during embryonic development or larval development. No such defect was observed. Next, in order to probe whether Rif1 influences repair pathway choice, as is seen in mammals and yeast, DSB repair assays were conducted. These assays allow for the determination of repair mechanism—end-joining or homology-directed repair—of site-specific DSBs in the male germ-line. *rif1* mutants exhibited no defects in the repair of I-*Sce*I endonuclease-induced breaks but, interestingly, a near-complete defect in the repair of *P*-element transposase-induced breaks. These results suggest that Drosophila RIF1 plays an early role in the repair of *P*-element transposase-induced breaks and may provide insight into the disparate roles of RIF1 in yeast and mammals.

660C Surveying the cell competition landscape by a *Drosophila* genetic screen

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Cell competition is a quality control process whereby cells with lower fitness (“loser cells”) are eliminated from the growing epithelium via the interaction with neighboring cells with higher fitness (“winner cells”). It has been shown that cells with heterozygous mutations in ribosomal protein genes (*Minute*/+ mutations), or homozygous mutations in apico-basal cell polarity, *Mahjong/VprBP*, or *Hel25E* genes are eliminated from *Drosophila* imaginal epithelium as losers of cell competition when surrounded by wild-type cells. While our understanding of the mechanisms of these cell competition phenomena has gradually progressed, it is still unclear whether the common mechanism exists among different cell competition phenomena

and what is the physiological trigger for cell competition. To address these, we conducted a large-scale EMS-based genetic screen in *Drosophila* to comprehensively isolate "loser" mutations over wild-type cells when introduced homozygously. As a result, we isolated 88 loser mutants from ~12,000 mutant lines. We next categorized the mechanisms of cell competition triggered by different mutations according to the dependence of cell competition on the previously identified cell competition regulators such as JNK, autophagy, caspase and transcription factor Xrp1. Intriguingly, more than half of the loser mutations depended on Xrp1 for their elimination, indicating that intracellular signaling activated by various loser mutations converge on the induction of Xrp1. We also sought to identify responsible genes for the loser mutations using the whole genome sequencing and found several interesting genes including Hel25E, which was previously reported. We are currently investigating the molecular mechanisms of cell competition triggered by different loser mutations, which will be presented.

661A Two distinct sources of calcium are required for recruitment of Annexins and their subsequent spatiotemporal regulation of RhoGEFs during cell wound repair

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Cells are constantly subjected to different types of damage leading to membrane tear and cytoskeletal disruption that must be rapidly repaired. Strikingly, cells can complete such repairs within a few tens of minutes using spatiotemporal regulated protein activity and recruitment. Rho family GTPases are indispensable for dynamic actin and myosin regulation and are spatiotemporally regulated by pre-patterning of three RhoGEFs (RhoGEF2, RhoGEF3, and Pebble) during cell wound repair. We previously showed that AnnexinB9 (AnxB9), a calcium responsive molecule, is recruited to wounds within three seconds and regulates RhoGEF2 patterning via actin stabilization. To elucidate how RhoGEF3 and Pbl patterns are established, we examined the roles of the other two fly annexins: AnxB10 and AnxB11. We find that all three Anxs are recruited to wounds within three seconds and exhibit distinct localization patterns such that they form a pre-pattern to which the RhoGEFs respond. Interestingly, both AnxB10 and AnxB11 are required for RhoGEF3 patterning, leaving open the means by which Pbl is regulated during cell wound repair. We are currently focusing on calcium dynamics following wounding as this is the only known upstream event prior to Anx patterning. In addition to extracellular calcium influx, we find that intracellular calcium release is also important for normal wound repair processes. Notably, Anxs exhibit distinct recruitment patterns in response to wounding following the depletion of extracellular or intracellular calcium sources. Thus, our results suggest that calcium is not only necessary for the initiation of the repair process, but also regulates the subsequent Anxs spatial recruitment patterns. We are currently investigating the molecular machinery functioning within the seconds between calcium influx and Anx patterning.

662B Evaluation of genotoxicity of jimson weed seed (*Datura stramonium* L.) by the Somatic Mutation and Recombination Test (SMART) in *Drosophila melanogaster* wings

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The species *Datura stramonium* L., commonly known as 'jimson weed', is one of the most widely used plants in Mexican Traditional Medicine for its analgesic effects to treat chronic and acute pain. This herb, like many other species in the Solanaceae Family, is characterized by its content of tropane alkaloids that are responsible for the narcotic, hallucinogenic and toxic properties of the plant.
In this study, the genotoxic potential of jimson weed seeds, which are the part of the plant with the highest concentration of tropane alkaloids, was evaluated by the Somatic Mutation and Recombination Test (SMART) in fruit fly (*Drosophila melanogaster*) wing cells which is based on detecting the loss of heterozygosity through recessive markers that are expressed at the level of the trichomes on the surface of the flies' wings. This bioassay generally uses standard (ST) and high bioactivation (HB) crosses, the second one is applied for the detection of promutagens and procancerigens. For this study, the seeds were used in a decoction in four different concentrations (3, 6, 12 and 24%) to detect a possible genotoxic effect; in addition, a co-treatment was carried out where the 24% seeds decoction was administered simultaneously with a mutagenic compound (4NQO) to determine the existence of a possible antigenotoxic effect.
The obtained results allowed to conclude that jimson weed seeds decoction presents a genotoxic effect in all tested concentrations and is not antigenotoxic in both crosses (ST and HB), furthermore it was determined that a metabolic activation is not required to show its genotoxicity.

663C Myotendinous junction formation during flight muscle morphogenesis is perturbed by the environmental toxicant, methylmercury

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Properly built musculoskeletal systems are essential for optimal motor control in humans and flies. In the indirect flight

muscles (IFM) of *Drosophila melanogaster*, the myotendinous junction (MTJ) forms during pupal development to evolve a structure capable of transducing iterative forceful contractions to the exoskeleton and allow flight. We observed that exposing larvae to the environmental toxicant, methylmercury (MeHg), perturbs indirect flight muscle (IFM) morphology seen as a detachment phenotype yielding myospheres at the pupal stage. While the exact mechanisms of MeHg toxicity remain elusive, a prior genome wide association study using the DGRP panel implicated candidate MTJ genes, including *kon-tiki (kon)*, and *inflated* (*if*). Kon encodes a chondroitin-sulfate-proteoglycan-4 (CSPG4) homolog, while if encodes an α-integrin. Both *kon* and *if* are expressed in muscle and are required to form attachments across the myotendinous junction (MTJ). Here, we investigated *kon* for a role in mediating MeHg disruption of muscle development by evaluating morphological and functional phenotypes of the IFM in pupal and adult flies following 0, 5, 10, and 15 µM MeHg exposure via feeding at the larval stage. Transgenic fluorescent reporter flies (*Mef2-Gal4; RFP*, and *E(spl)mδ-Gal4; RFP*) were used to visualize muscle morphology for live-image acquisition. Developmental MeHg exposure induced a dose-dependent increase in myospheres within dorsal bundles of the IFMs, which paralleled reductions eclosion and adult flight behaviors. These effects were selectively phenocopied by altered expression of *kon*. Exposure to 10 µM MeHg elevated *kon* transcript expression at 20 - 36 hrs. APF, encompassing the window of MTJ development that precedes muscle compaction phases of MTJ development and when myospheres first occur. Finally, the myospheres phenotype resulting from 10 µM MeHg was partially rescued in a background of reduced *kon* expression using a targeted RNAi approach. Our findings implicate a component of the MTJ as a target of MeHg toxicity, which broadens the understanding of how motor deficits can emerge from early life MeHg exposure. Ongoing studies aim to discern effect of MeHg on the Kon protein, which will inform subsequent studies of the underlying mechanism that leads to failure of muscle attachment.

664A An examination of the consequences of recent and ancient molecular evolutionary events at the acetylcholinesterase locus of *Drosophila melanogaster*

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The Acetylcholineesterase locus (*Ace*) exhibits one of the strongest selective sweeps in the *Drosophila melanogaster* genome which is perhaps not surprising as it encodes the molecular target of commonly used organophosphate insecticides. Four amino acid substitutions have arisen that, in different combinations, provide resistance to different organophosphates. *Ace* also has an interesting deeper evolutionary story in that most insects have two acetylcholinesterase genes whereas Cyclorraphan flies, like Drosophila, have only one gene. It has been proposed that the loss of the second *Ace* gene in this lineage correlates with the gain of alternate splicing of the terminal exon at the remaining locus. Here we tested this hypothesis using the recent proliferation of fly genomic and transcriptomic datasets.

One splice form of *D. melanogaster Ace* encodes a protein that is post-translationally processed so that a GPI anchor is added and intermolecular disulfide bridges form to create dimers. In contrast, the evolutionarily derived splice form includes an alternative terminal exon that lacks a cysteine residue and so it is thought to encode a monomer. The function of the monomeric form is not known and so we created transgenic lines encoding resistant and susceptible form of the monomer and dimer forms. We wondered whether the susceptible allele in a monomeric splice form could act to sequester organophosphates better than the resistant form which encodes amino acids that restrict the organophosphate from entering the catalytic groove. We present the malathion resistance of these four transgenic lines.

Cyp6g1 is another gene that confers resistance to organophosphates and it also shows strong signals of recent selection. Furthermore, it has been reported that *Cyp6g1* resistance alleles show sexual antagonism as females carrying *Cyp6g1* based resistance have higher fitness than males. Here we present a research proposal to test whether *Ace*, is also subject to sexually antagonistic selection.

665B *Drosophila* as a non-target model system to understand glyphosate toxicology

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Glyphosate, the active ingredient in Roundup® and other herbicides, is the most abundant pesticide in the US and around the world, used by 89% of US farmers. Though its primary target is the shikimate pathway, absent in animals, it increases mortality, is carcinogenic, and interferes with hepatorenal, nervous, reproductive and endocrine systems in a variety of non-

target organisms. Previous research indicated that *Drosophila melanogaster* might be a useful model system to understand glyphosate toxicity, since Roundup® exposure increased their mortality, induced oxidative stress, and reduced body size in females. Our previous work has also demonstrated reduced reproductive output and ovary size, and altered feeding behavior. For this study, we measured the amount of glyphosate in the bodies of flies exposed through food to Roundup® Super concentrate, Roundup® Ready to Use, glyphosate alone, or no herbicide; at two concentrations; during the complete larval period, for 7 days as adults, or both; and compared soft tissue vs. cuticles. Glyphosate concentration was 1) higher in cuticle than soft tissue but highest in whole bodies, 2) 3.7 X higher when exposed to 10 g/L than 2 g/L of Roundup® Super Concentrate, 3) highest when exposed as both larvae and adults, and lowest when only exposed as larvae, and 4) highest when exposed to Roundup® Ready to Use and lowest when exposed to glyphosate alone. Since all flies were harvested for glyphosate measurement at 7 days post-eclosion, the lower concentration in flies exposed only as larvae likely reflects that they had by then been unexposed during their pupal stage and 7 days as adults. Given that glyphosate measured in human urine decreases below limits of detection after 3 days without exposure, concentrations in flies exposed only as larvae are higher than might be expected. Taken in conjunction with our previous results indicating that abdomens, but not ovaries or testes, accumulate more glyphosate than heads or thoraxes, it is probable that the higher concentration in whole bodies, compared to cuticle only or soft tissue only, results from very high concentration in partially digested food in the abdomen. Therefore, the most surprising result is that the co-herbicide, pelargonic acid, or a proprietary ingredient in Roundup® Ready to Use, appears to increase the uptake of glyphosate or reduce its excretion, and the magnitude is more pronounced than for POEA, present in Roundup® Super Concentrate, which has been hypothesized to increase glyphosate accumulation within tissues because of its surfactant properties. Our future work will utilize genetic tools available in *Drosophila* and pharmacological tools to elucidate mechanisms of glyphosate and Roundup® toxicology in non-target organisms.

666C ER-lysosome interactions promote autophagy to regulate synaptic growth.

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Sites of apposition between organelles, often referred to as membrane contact sites (MCSs), are important hotspots for intracellular signaling, lipid metabolism, and organelle biogenesis /dynamics in eukaryotic cells. The endoplasmic reticulum (ER) forms an extensive and dynamic network of MCSs with almost all organelles. MCSs between the ER and endo-lysosomes are particularly abundant, suggesting important physiological roles. These contact sites are also observed in neurons. However, their molecular composition and physiological function in the nervous system is a matter of active research.
PDZD8 is an intrinsic ER transmembrane protein with a synaptotagmin-like mitochondrial lipid-binding proteins (SMP) domain that has been reported to localize to ER-late endosome/lysosome and ER-mitochondria MCSs. The molecular steps involved in the recruitment of PDZD8 to MCSs have been recently elucidated, yet the *in vivo* relevance of PDZD8 to neuronal function remains unclear. We identified *Drosophila* PDZD8 in a candidate screen for uncharacterized conserved regulators of synapse formation and function. We used the CRISPR-Cas9 system to generate null alleles and endogenously tag PDZD8. Interestingly, we find that PDZD8 is expressed at synapses in the central nervous system and the larval neuromuscular junction (NMJ), where it localizes to ER-lysosome MCSs. We show that in *PDZD8* mutants activity-induced synaptic growth, neurotransmitter release, and locomotion are dysregulated. We further show that PDZD8 regulates synaptic growth by autophagosome maturation. Overall, our studies demonstrate a key role for PDZD8 and ER-lysosome MCSs in the regulation of synaptic growth and function.

667A Elucidating mechanisms of *Chromobacterium subtsugae* phenotypic switching during *Drosophila melanogaster* infection

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A pathogen is defined as a bacterium that causes infection to a host. However, we often forget that many pathogenic microorganisms are able to survive in the environment and only when it comes into contact with a host does it cause disease. Ultimately it is the interaction between the microorganism and the host immune response that leads to pathogenesis. There are two types of defense mechanisms a host can employ to fight an infection: resistance and tolerance. The mechanisms behind resistance, the ability to clear a pathogen, have been well-studied within the field of Immunology. However, the mechanisms behind tolerance, a host's ability to limit damage in response to a pathogen, is less understood. To bridge this knowledge gap, I have identified a host-pathogen system to explore tolerance mechanisms and their impacts on the host. The common fruit fly, *Drosophila melanogaster,* provides a tractable host model organism for studying host-pathogen interactions to identify mechanisms that lead to pathogenicity. Here, we report on our identification of a host-pathogen system in which passage through the host leads to a phenotypic switch of the bacterium that alters virulence. We injected *D.*

melanogaster adults with *Chromobacterium subtsugae*, a Gram-negative environmental bacterium known for its quorum sensing production of the purple-pigment violacein. Flies are significantly more susceptible to the wild-type non-pigmented, than the purple pigmented strain. High density of the white pigmented strain can be cultured from flies that succumb to infection. However, we found that some flies survive infection and, in these flies, although injected with the white strain, we isolated purple pigmented colonies. This phenotypic switching event is occurring within the first 48 hours of the infection. This suggests there is a selective pressure in the fly that leads to a phenotypic switch upon introduction into the fly observed as the production of violacein pigmentation. We will discuss genetic changes in *C. subtsugae* populations, as well as the role of the host immune response in this process. Identifying factors that contribute to the evolution of bacteria within a host will add to our knowledge of pathogen dynamics leading to a greater understanding of ecological shifts occurring during infection. Understanding how microbes interact with a host to cause disease will allow us to identify innovative mechanisms to limit the damage microbes cause during infection.

668B *In vivo* overexpression of Hopscotch in the gut modulates insulin-like peptide signaling in virgin female *Drosophila melanogaster* under various nutrient conditions

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Diet-induced obesity in *Drosophila melanogaster* has been linked to decrease in lifespan, feeding behavior alteration, altered energy storage and altered expression of dilp2, which plays a major role in nutrient uptake and energy storage. Flies analogous physiological system with mammals makes them a good model for the study of the JAK/STAT pathway in obesity and insulin resistance. Obesity is linked to insulin resistance, which results in Type 2 diabetes, which has been further linked to elevated JAK/STAT pathway activity in mammals. In mammals, the specific downstream pathway utilized upon activation of JAK/STAT that results in elevated insulin resistance is difficult to determine due to the complexity and redundancy of the pathway, where there are seven STATS and four receptor-associated JAKs. Compared to mammals, flies have a single STAT protein (STAT92E) and a single activating kinase (Hop) which provides a simpler way to understand the mechanism of the pathway in insulin signaling. In this study, an obesity-promoting diet was utilized (high fat diet, HFD; 20% w/v, saturated fat). Overexpression of $Hop^{Tum\text{-}1}$ in the gut was achieved by using the drm-GAL4 driver. The midgut is a major site of energy sensing in flies and is analogous to the small intestine of mammals. The flies were exposed to normal and HFD for five days prior to conducting experiments. Overexpression of *Hopscotch* in the midgut led to a decrease in lifespan, glycogen content, triglyceride content, and reduced feeding quantity. Furthermore, upon being starved on 2% agar after being exposed to HFD and normal diet for five days, a decrease in starvation resistance was observed. The flies were tested for Akt phosphorylation under a variety of nutrient treatments. Overexpression of Hop led to reduced insulin signaling if fed a normal diet or fed a normal diet and starved overnight, but increased insulin signaling if previously fed a high fat diet regardless. Additionally, flies were starved overnight and exposed to either 10% glucose or water to induce release of Dilp2. Here, overexpression led to increase in insulin signaling on water treatment regardless of prior diet, but decrease insulin signaling if fed glucose regardless of prior diet. However, the $Hop^{Tum\text{-}1}$ construct showed strong signs of leaky expression or transvection and further study is needed.

669C Systems biology approaches to build a predictive model of Dorsal/NF-κB signaling network in the Drosophila embryo

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Systems biology faces a conundrum in the realm of computational modeling. On the one hand, models are necessary to integrate all of the experimental data into one explanatory system. On the other, the large number of unknown parameters limits the utility of systems biology models. To overcome this difficulty, we have performed two types of measurements. First, we have measured the whole system, which allows for the full systems biology model to be fit to the data. Second, we have performed localized experiments, which isolate only 1-2 parameters for estimation. As a model system, we have focused on the Dorsal gradient, which patterns the dorsal-ventral (DV) axis of the early embryo.
Dorsal (Dl), which is ubiquitously expressed and is one of three *Drosophila* homologs of NFkB, is translated in the early embryo from maternally deposited dl mRNA. Dl protein binds to the IkB homolog Cactus (Cact), which helps in retaining it in the cytoplasm, and hence, blocks it from entering the nuclei to regulate its target genes. Our aim is to perform detailed measurements of individual biophysical parameters and global morphogen gradient properties in the early *Drosophila* embryo. As an example, we quantitatively measured the nuclear import and export rates of Dl and Dl/Cact complex through a fluorescence recovery after photobleaching (FRAP) assay in which we bleached single nuclei (ventral and dorsal side). These and other localized experiments that provide estimates of isolated parameters allow us to build predictive systems biology models, which will help us to understand the general knowledge of the NF-κB signaling module found in animals from Cnidarians to humans.

670A A role for Ykt6 in patterning during oogenesis in *Drosophila*

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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. Ykt6 is an essential R-SNARE protein implicated in various vesicle trafficking events in organisms ranging from yeast to humans. 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. To identify defects in oogenesis associated with mutations in *Ykt6*, we used a lethal allele, *Ykt6*^{c}, to generate germline clones, using the FRT/FLP *ovo*^{D} system. Females with ovaries homozygous for *Ykt6*^{c} are functionally sterile, and the vast majority of eggs produced from these females have obvious structural defects. These defects include shortened or small eggs that are flaccid and have mild to severe dorsal appendage defects, indicating possible defects in actin organization and in oocyte /follicle cell patterning. To uncover the effects of Ykt6 on patterning in the oocyte, we examined the localization of *bicoid* and *oskar* mRNAs as markers of anterior-posterior polarity. We also monitored the distribution of Gurken protein and downstream readouts of dorsal-ventral patterning. We find that *Ykt6* is required for both anterior-posterior and dorsal-ventral signaling during mid to late oogenesis. Additionally, we will present evidence that Ykt6 is necessary for successful signaling between the germline and follicle cells via Notch mediated pathways.

671B Transferrin 1 is not Essential for Iron Transport

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Transferrin 1 (Tsf1) is an insect hemolymph protein with a high affinity for iron. It is homologous to mammalian serum transferrin, which transports iron into cells via receptor-mediated endocytosis; however, whether or not Tsf1 plays a significant role in iron transport is unknown, and no Tsf1 receptor has been identified. The goal of this study was to determine whether Tsf1 is essential for iron transport in *Drosophila melanogaster*. To answer this question, we took two approaches. First, we performed phenotypic analyses to determine whether insects lacking Tsf1 have differences in iron content or iron distribution. Second, we used immunohistochemistry to evaluate whether Tsf1 is present in the endosomes of fat body cells and other tissues. We found that insects that lack Tsf1 are viable and fertile, and they have no major changes in iron content or iron distribution. These results indicate that Tsf1 is not essential for iron transport. In addition, we failed to detect Tsf1 in endosomes in most tissues, suggesting that Tsf1 does not deliver iron to most cells through endocytic uptake. One exception was oocytes. Our data suggest that Tsf1 from hemolymph is transported into developing oocytes via endocytosis and stored in yolk granules. We predicted that Tsf1 may provide oocytes with iron; however, newly laid eggs from Tsf1 null mothers were not iron deficient. Tsf1 may function in immune-related iron sequestration in eggs rather than in iron provisioning. This study suggests that iron transport in insects is likely to be mediated primarily by non-transferrin-based mechanisms.

672C Fat Body p53 Regulates Systemic Insulin Signaling and Autophagy under Nutrient Stress via Drosophila Upd2 Repression

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The tumor suppressor p53 regulates multiple metabolic pathways at the cellular level. However, its role in the context of a whole animal response to metabolic stress is poorly understood. Using *Drosophila*, we show that AMP-activated protein kinase (AMPK)-dependent Dmp53 activation is critical for sensing nutrient stress, maintaining metabolic homeostasis, and extending organismal survival. Under both nutrient deprivation and high-sugar diet, Dmp53 activation in the fat body represses expression of the *Drosophila* Leptin analog, Unpaired-2 (Upd2), which remotely controls Dilp2 secretion in insulin-producing cells. In starved Dmp53-depleted animals, elevated Upd2 expression in adipose cells and activation of Upd2 receptor Domeless in the brain result in sustained Dilp2 circulating levels and impaired autophagy induction at a systemic level, thereby reducing nutrient stress survival. These findings demonstrate an essential role for the AMPK-Dmp53 axis in nutrient stress responses and expand the concept that adipose tissue acts as a sensing organ that orchestrates systemic adaptation to nutrient status.

673A Female-biased upregulation of insulin pathway activity mediates the sex difference in *Drosophila* body size plasticity

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Nutrient-dependent body size plasticity differs between the sexes in most species, including mammals. Previous work in *Drosophila* showed that body size plasticity was higher in females, yet the mechanisms underlying increased female body size plasticity remain unclear. Here, we discover that a protein-rich diet augments body size in females and not males because of a female-biased increase in activity of the conserved insulin/insulin-like growth factor signaling pathway (IIS). This sex-biased upregulation of IIS activity was triggered by a diet-induced increase in *stunted* mRNA in females, and required *Drosophila insulin-like peptide 2*, illuminating new sex-specific roles for these genes. Importantly, we show that sex determination gene *transformer* promotes the diet-induced increase in *stunted* mRNA via transcriptional coactivator Spargel to regulate the male-female difference in body size plasticity. Together, these findings provide vital insight into conserved mechanisms underlying the sex difference in nutrient-dependent body size plasticity.

674B The role of mitochondrial physiology in scaling up temperature effects from molecules to populations

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The thermal performance curve (TPC) provides a mathematical and physiological framework for understanding how temperature impacts the biological rates and processes that govern ectotherm development, growth and fitness, and contribute to population dynamics. Yet, we do not know the mechanisms that translate thermal performance of molecular, cellular and individual-level processes to population dynamics. Here we use an outbred population of the fruit fly *Drosophila melanogaster* to fit TPCs for population growth rate, estimated using life-table analyses, and for its underlying life-history and physiological components, including female fecundity, development rate, survivorship, metabolic rate and mitochondrial function. We find that the activation energy estimated from TPCs increases from the molecular to the population level. Flies develop rapidly at higher temperatures, but this trades off with survivorship and fecundity, generating a TPC for population growth rate that is relatively narrow and sits in between the TPCs for female fecundity and development rate. The rich data gathered for insect life-table analyses reveal a thermal-dependent, maternal-age effect on offspring survivorship; when developed at intermediate temperatures, females can maintain higher offspring survivorship across a larger fraction of their lifespan. We will discuss our findings in the context of the hypothesis that mitochondrial physiology may set organismal and population thermal limits, and present our preliminary results on how TPCs across levels respond to thermal evolution.

675C Functional plasticity of polarity proteins controls epithelial tissue architecture and cancer cell invasion

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Overexpression of the FERM-domain protein EPB41L5 leads to cancer progression through poorly understood mechanisms. We use the polarity protein Yurt, which is the *Drosophila* ortholog of EPB41L5, to model the function and regulation of EPB41L5. Yurt binds to Crumbs to limit the ability of the latter to promote apical membrane growth. However, our results highlighted that the Yurt-Crumbs association plays an active role in promoting cortical tension within the apical domain. Yrt overexpression leads to apical enrichment of Myosin II, a function that requires an intact FERM-domain binding motif within the cytoplasmic tail of Crumbs. The apical kinase aPKC phosphorylates Yurt to prevent its association to Crumbs, thereby promoting Crumbs-dependent apical membrane expansion at the expense of apical tension. In contrast, the kinase Pak1 sustains Yurt membrane localization and apical constriction. Pak1 acts through activation of the phosphatase PP2A, which dephosphorylates Yurt on aPKC target sites. Similarly, we found that PAK1 and PP2A promote EPB41L5-dependent invasion of the breast cancer cell line MDA-MB-231. Our discoveries thus highlight a strategy to treat patients showing overexpression of EPB41L5, namely inhibition of the PAK1-PP2A pathway. Interestingly, several PAK1 inhibitors are currently being tested in clinical trials.

676A Deciphering Mechanisms of *Egfr*-Mediated Cell Survival in the *Drosophila* Eye Using Single-Cell Omics

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Epidermal growth factor receptor (Egfr) signaling has multiple functions during *Drosophila* eye development, including

regulation of cell survival and proliferation, and differentiation of most cell types in the eye. In addition, Egfr signaling is required for the proper rotation of ommatidia in the eye. Specifically, whole-eye loss-of-function of *argos* (*aos*), a negative regulator of Egfr signaling, causes ommatidial misrotations (Brown and Freeman, Dev., 2003). Ommatidial misrotations are also observed with loss-of-function mutations in other *Egfr* signaling components, including *Egfr* and the downstream effector *pointed* (*pnt*) . Although perturbation of Egfr signaling causes ommatidial misrotations, the exact mechanism by which this occurs is not clear. Interestingly, *pnt* overexpression in the R3 and R4 photoreceptors have misrotations (Weber et al., Dev Biol., 2008). In addition, Notch signaling was proposed to activate Aos expression in R4 and knockdown of *Notch* in R4 leads to misrotation (Koca et al., Sci. Rep., 2019). These results suggest that perturbation of Egfr signaling in the R3/4 photoreceptors is sufficient to cause misrotations. To further confirm this, we drove a dominant negative form of Egfr (Egfr DN) in R3 and R4 using *salm-T2A-Gal4* and, as expected, we observed ommatidial misrotations in adult and late larval eyes. To identify genes effecting *Egfr*-mediated regulation of ommatidial rotation, we performed single cell RNA sequencing (scRNA-seq) of wild-type and *salm-T2A Gal4 > UAS-Egfr [DN]* late larval eye discs. By comparing previously published (Webber et al., Dev., 2018) and our own Pnt ChIP-seq data, we identified putative direct Egfr signaling target genes that are expressed in distinct photoreceptor subtypes. In addition, we generated single cell ATAC-seq (scATAC-seq) data on late larval eye discs. We are intersecting our ChIP-seq and scATAC-seq data to identify Pnt-bound loci at a single cell resolution. Moreover, our scRNA-seq of *salm-T2A > Egfr DN* discs yielded several candidate Pnt target genes that may be required for ommatidial rotation. Taken together, we have used single cell approaches to identify downstream targets of Egfr signaling that are involved in ommatidial rotation in the developing *Drosophila* eye. We are testing the roles of these target genes in ommatidial rotation using inducible somatic CRISPR to knock down candidate target genes in specific photoreceptor subtypes.

677B *Cell phase specific regulation of polarity genes by the Rbf-E2F protein complex*

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Specification of cellular polarity is vital to normal tissue development and function. Conserved throughout metazoans, planar cell polarity (PCP) genes are also implicated in diverse human birth defects. For instance, mutations in the polarity genes *VANGL1/2* in humans have been associated with neural tube closure defects. Extensive work has shown how PCP proteins are regulated, however relatively little is known about potential transcriptional regulation of these genes. Our earlier study revealed an unexpected role for the Rbf retinoblastoma corepressor protein in transcriptional regulation of PCP genes. Here we analyze the physiological relevance of the role of E2F/Rbf proteins in the transcription of the key core polarity gene *Vang*. Targeted mutations to a E2F site within the *Vang* promoter disrupts binding of Rbf/E2F proteins in vivo, leading to polarity defects in wing hairs. E2F regulation of *Vang* is supported by the requirement for this motif in a reporter gene. Interestingly, the promoter is repressed by overexpression of E2F1, a transcription factor generally identified as an activator. Consistent with the regulation of this polarity gene by E2F and Rbf factors, expression of *Vang* and several other polarity genes is found to peak in G2/M phase in cells of the embryo and wing imaginal disc, suggesting that cell-cycle signals may play a role in regulation of these genes. Our findings suggest that the Rbf/E2F complex mechanistically links cell proliferation and polarity. Rb protein is commonly mutated in many different types of cancer, and we propose that mutations in Rb that deregulate both proliferation and cell polarity would be causal in driving cancer and metastasis.

678C Uncovering functional roles in development for differentially expressed essential ribosomal protein eRpL22-like

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The *Drosophila melanogaster* eRpL22 ribosomal protein family contains two structurally divergent & developmentally essential paralogs: eRpL22 and eRpL22-like - the latter exhibits tissue-specific expression across development; the former is ubiquitously expressed. Multi-tissue co-localization comparison of eRpL22-like and core ribosomal components indicates eRpL22-like may have functional roles both within the ribosome itself and apart from ribosomal processes.

Conditional knock-out of eRpL22-like resulted in many morphological defects within the ovary, including: disruption of the germline stem cell niche, ectopic rounded follicular epithelium cells, oocytes with dual (bifurcated) nuclei, double-anteriorized eggs, and specific spatiotemporal patterns of cell death & oogenesis arrest. This constellation of phenotypes suggests eRpL22-like has a role in ensuring proper cell polarity throughout development.

Sequencing of RNAs enriched on eRpL22 and eRpL22-like polysomes in adult testes revealed differential enrichment of mRNAs (also expressed within the ovary, with homologs associated with human disease) involved in cell polarity, suggesting

that paralogue-specific "specialized ribosomes" translate specific mRNAs. Immunohistochemical characterization of eRpL22-like mutants allows us to tease-apart individual phenotypes, assess downstream protein expression changes of differentially enriched mRNAs, elucidate putative extra-ribosomal functions of eRpL22 paralogues, and potentially reveal a novel model useful for studying specific human conditions arising from a spectrum of polarity defects. These preliminary data broaden the context for investigation of the role of eRpL22-like as an essential player across multiple developmental processes.

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679A Reproductive, developmental and transgenerational variation are shaped by diet-mito-nuclear interactions

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Diet's effect on animal phenotype varies genetically, with known roles for variation in both the nuclear and mitochondrial genomes. However, whether combinatorial "mito-nuclear" genetic variation determines diet's impact is understudied. Specific nutrients underlying diet-mito-nuclear variation remain to be established; integrative analyses of fitness impacts are lacking; and whether mito-nuclear pairs with prior coevolutionary history outperform novel combinations remains equivocal, with implications for human mitochondrial transfer therapies. Here, we characterise diet-mito-nuclear regulation of fitness traits comprehensively in *Drosophila melanogaster*, measuring both quantity and quality of offspring. In a replicated panel of flies with fully-factorial variation in mitochondrial and nuclear genomes, enriching either dietary essential amino acids or lipid elicited genotype-specific effects. Mito-nuclear genotype predicted diet's fecundity effect, for which we infer a behavioural basis. Offspring development time and rate were modulated similarly. Furthermore, a transgenerational effect of parental diet on offspring development was shaped by mito-nuclear genotype, suggesting and epigenetic mechanism. Integrative analysis indicated diet-mito-nuclear regulation of trade-offs between offspring quality and quantity. Overall, responses to diet did not parse by coevolved versus novel mito-nuclear pairings, but evaluating alternative models of the data nevertheless showed that models without diet-mito-nuclear terms were inferior descriptors of biological variation: together, these results indicate that shuffling mito-nuclear pairings can lead to significant phenotypic changes on specific diets, which may be hard to predict based on mito-nuclear pairing. Altogether, these results show that regulation of fitness by specific nutrients depends on mito-nuclear genotype, that mito-nuclear variation may manifest only on specific diets, and that such variation can have transgenerational consequences. The results imply that real-world biological variation should account for this complex axis of variation.

680B The evolution of the centromere-associated retrotransposon *G2/Jockey-3* in *Drosophila simulans* clade species

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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 and CENP-A ChIP-seq. The centromeres in *Drosophila* are organized as islands of complex DNA rich in retrotransposons and embedded within large blocks of tandem satellite repeats. The centromere islands of *D. melanogaster*, while unique to each chromosome, share one particular non-LTR retrotransposon named *G2/Jockey-3*. Studies in other organisms including plants, mammals, and fungi have detected centromere-associated retroelements suggesting a role in centromere function, maintenance, or establishment. However, *G2/Jockey-3* is not exclusive to centromeres in *D. melanogaster* and our work indicates natural selection does not act to conserve centromeric insertions. This suggests that *G2/Jockey-3* is acting like a selfish genetic element rather than an important component of centromeres. Interestingly, *G2/Jockey-3* is also enriched in the centromeric regions of *D. simulans*, a sister species to *D. melanogaster*. Centromeres are known to evolve rapidly but it is unclear if centromere-associated transposable elements evolve in a similar manner or if the elements are conserved components of centromeres between species. Transposable element evolution is dynamic and *G2/Jockey-3* may have different roles in other species. We used heterochromatin-enriched assemblies of three species within the *simulans* clade to discover the patterns, ages, and diversity of *G2/Jockey-3* outside of *D. melanogaster*. We find *G2/Jockey-3* in all three species and are unable to detect full-length elements in other species suggesting *G2/Jockey-3* is a relatively new transposable element. Conservation within the sequence is limited to the open-reading frame while the 5'-end is highly divergent. We also discovered a diversification of *G2/Jockey-3* that is unique to the *simulans* clade where many of the copies are much younger than those in *D. melanogaster*. *G2/Jockey-3* is an active and dynamic element within the *simulans* clade in comparison

to *D. melanogaster* and could have implications for centromere evolution in *Drosophila* and the role of retrotransposons in centromeres.

681C Experimental Evolution Selecting for Adaptation to a High-Sugar Diet in Drosophila melanogaster

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Experimental evolution strategies have been shown to dramatically alter both phenotypic and genotypic characteristics of populations under laboratory selective pressures. In this project, an outbred population made from disparate wild-caught Drosophila was subjected to a control or high-sugar (HS) feeding paradigm. HS feeding reduces both the lifespan and healthspan in adult Drosophila. Therefore, survivors of HS feeding were mated to produce the next generation, allowing selection for protective alleles. Alleles that increase survival, glucose homeostasis, and fecundity are hypothesized to be favored under the HS diet. We found that all selected populations increased tolerance to HS feeding during our selection. Four control and four HS-selected populations will be compared with deep genome sequencing to identify specific, enriched loci that may have conferred phenotypes protective against the negative sequelae of caloric excess. Individual genes that have been identified as potentially protective will undergo manipulation by CRISPR and/or *UAS*-dependent *RNAi* and overexpression to test their roles in mitigating diabetes-like phenotypes. Together, these approaches may identify genes and pathways of interest for combating overnutrition.

682A Host genomes prioritize silencing active TE variants by altering piRNA content.

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Transposable elements (TEs) are selfish genetic elements that act as “genetic parasites”. They can invade host genomes and rapidly increase in copy number until eventually being suppressed by the host’s genomic immune system, piRNAs. The piRNA pathway employs a small RNA mediated silencing to regulates the expression of TEs by producing antisense small RNAs (piRNAs) that bind to sense TE transcripts, which are then degraded into sense piRNAs. As TEs expand in copy number they also acquire polymorphisms, which may lead to the formation of new lineages or subfamilies. TE lineages may be in conflict with each other by competing for the same genomic niche, and may be in conflict with the host by evolving to evade piRNA silencing. We sought to analyze the variation of TE lineages within natural populations to characterize the diversity of TE families and to explore potential conflict between TEs and their hosts. To do this we developed a method to infer haplotypes of SNPs in TEs from short reads by leveraging population genomic datasets, and applied it to a set of 41 recently active TEs in *D. melanogaster* using 85 short-read libraries from the Global Diversity Lines (GDL). We used public PacBio data as well as simulations to validate and benchmark our haplotype inference method. We found strong population structure in the haplotype variants of the *Roo* element, as well as other TEs, that is driven mostly by population-specific expansions of single haplotypes. We then determined which TE haplotypes likely retain transpositional activity by using theoretical expectations of an active element’s copy-number distribution to classify haplotypes as putatively active or inactive. Using this approach, we detected an active population-specific haplotype for *I-element* that appears to have recently invaded individuals in East Asia. Using piRNA data we found that not only is this haplotype being regulated by piRNAs, but it also a source of antisense piRNAs. In general, active haplotypes had higher sense and antisense piRNA read depth than inactive haplotypes. Furthermore, we found that the copy number of active haplotypes, but not inactive ones, is positively correlated with antisense piRNA read depth. These data suggest that the host may adapt to the invasion of active variants by producing antisense piRNAs that target those variants with heightened specificity.

683B Exploring the function and structure of heterogeneous ribosomes in the gonads of *Drosophila melanogaster*

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The spatio and temporal regulation of mRNA translation is vital to a variety of developmental processes (e.g. *osk* mRNA during embryogenesis). At the heart of translation, ribosomes perform protein synthesis and were long thought of as invariable macromolecular machines that lacked any intrinsic ability to regulate translation. However, studies from the last two decades have highlighted that the cellular pool of ribosomes displays significant heterogeneity in both rRNA and ribosomal protein (RP) content. The biological significance of this ribosome heterogeneity remains to be fully understood. However, the disruption of ribosomal genes can produce tissue-specific phenotypes, implying that such ribosomes may specifically translate a subset of mRNAs, and therefore have specialised function. For example, ribosomal mutations in *Drosophila melanogaster* result in the *minute* phenotype, which includes reduced fertility.

We have analysed the protein composition of ribosomes from various tissues of *Drosophila melanogaster* by quantitative mass spectrometry. We discovered nine RP paralog switching events within *Drosophila* gonads, five in the testes and four in the ovaries. Of the paralog switching events, 4 paralogs are located near the mRNA channel in the small ribosomal subunit, while switching events in the large subunit are mostly towards the back of the ribosome, including the testis-specific RpL22-like. Using Cryo-EM, we have resolved testis and ovary ribosome structures to 3.9 Å and 3.4 Å, respectively, confirming the RpL22-like switch in testis. Surprisingly, cryo-EM revealed an extra density at the mRNA channel of the testis monosome that was not present in the actively translating testis polysome or in the ovary. We have identified this density as an ortholog of mammalian IFRD1, which may be important for translational regulation in the testis. Preliminary RpL22 and RpL22-like RNAi experiments suggest that they possess different functional roles during gametogenesis. Future work will focus on investigating the role of heterogeneous ribosomes in the testis, where ribosome heterogeneity was most diverse.

684C The OTUD6 deubiquitinase catalytically regulates ribosomes in response to cellular stress

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Ribosomes are dynamically regulated in response to stress conditions to prevent aberrant protein synthesis. Protein ubiquitination is an essential posttranslational modification that regulates protein translation and ribosome function. OTUD6 is an ovarian tumor (OTU) family deubiquitinase that is highly conserved from yeast (OTU2) to humans (OTUD6A&B). To study Drosophila OTUD6, I used CRISPR/Cas9 to create epitope-tagged wild-type and catalytically dead (DUB-dead) endogenous OTUD6, and a null allele. The null is an early embryonic lethal. OTUD6 DUB-dead and partial loss-of-function mutants are markedly sensitive to oxidizing (paraquat) and alkylating (mms) agents that cause cellular stress. Oxidizing and alkylating stressors impact protein translation and ribosome function. The mms sensitivity of DUB-dead OTUD6 is rescued by mutations in two cooperating nucleolar 60S ribosome biogenesis factors, RPF2 and RRS1. Thus, OTUD6 is a negative regulator of 60S ribosome biogenesis. In wild-type, OTUD6 is localized to both the cytoplasm and the nucleolus. DUB-dead OTUD6 and treatment of wild-type with mms causes OTUD6 to vacate the nucleolus and change distribution in the cytoplasm. Moreover, OTUD6 mutants display delayed development and increased lifespan, consistent with altered ribosome function and protein translation. Thus, OTUD6 deubiquitination regulates ribosome function in response to cellular stress.

685A Dissecting the Genetic Basis of Variation in Cocaine and Methamphetamine Consumption in *Drosophila melanogaster*

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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. 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 for consumption of both drugs, in both sexes. 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 control flies and identified 3,033 variants significantly associated with increased consumption that reside in 1,962 genes following Bonferroni corrections for multiple tests. These genes are enriched for many biological processes including nervous system development, axon guidance, and memory. We assessed the effects of ubiquitous RNA interference (RNAi) on drug consumption for 22 candidate genes, of which 13 showed a significant increase or decrease in consumption in at least one sex. We constructed new AIPs which were homozygous for target alleles in five intergenic SNPs and five genes, tested average consumption for each population and observed extensive sexual dimorphism and differences in genotype- and condition-specific effects after five generations of random mating and after greater than twenty generations of random mating. Supported by U01DA041613.

686B Gene-environment interactions shape transcriptomic and organismal responses to combined ethanol and temperature environments in the fruit fly *Drosophila melanogaster*

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Organisms acclimate and adapt to complex environments in which multiple abiotic stressors may interact with genetic variation to determine the degree of stress experienced by individuals. The fruit fly *Drosophila melanogaster* encounters ethanol during development and shows latitudinal patterns of ethanol tolerance. The expansion of *D. melanogaster* into temperate latitudes that experience cooler and more variable environments is coincident with the evolution of a much higher ethanol tolerance that is considered to be adaptive. We characterized variation in whole-transcriptome responses of multiple *D. melanogaster* wild-type genetic strains from temperate latitudes to larval ethanol exposure (0 or 6%) at two temperatures (16 C and 25 C) by RNA-sequencing. We identified genes that exhibited genotype-by-environment (GxE), ExE and GxExE interactions. We found that diverse pathways and co-regulated networks of genes including lipid and phospholipid metabolism, drug metabolism, autophagy, and mitochondrial function responded to developmental ethanol and temperature exposure, and that some of the transcriptional responses were via the indirect effects of ethanol to delay development and generate cellular stress in certain genetic strains. Ethanol exposure appeared to cause oxidative stress through dysregulation of fatty acid metabolism, increased production of free radicals, and downregulation of scavengers of reactive oxygen species. Finally, we will synthesize these data on gene-expression plasticity with the plasticity that we have observed in the developmental and ethanol tolerance phenotypes of *D. melanogaster* larvae under the same environmental conditions.

687C Expression QTL mapping of tissue-specific copper response in the DSPR

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Heavy metals are pervasive environmental contaminants that negatively impact human health, but a small number of them also serve necessary biological functions. Copper is one such metal. As a critical biological molecule, copper supports enzymatic function and stabilizes oxygen transport. However, toxic leaching of copper through mining, agriculture, and waste mismanagement can cause organ dysfunction and impair learning and neurological development in humans. Understanding the genetic basis of the copper toxicity response can help illuminate genetic risk factors that increase susceptibility to heavy metal stress. Using a large *Drosophila melanogaster* mapping population (*Drosophila* Synthetic Population Resource, DSPR), we recently demonstrated that allelic variation in copper and other metal-responsive genes influences copper resistance. Here, we expand on this work using expression quantitative trait locus (eQTL) mapping to examine variation in the gene expression response to copper stress. We collected head and midgut tissue samples from control and copper-exposed flies from 96 unique DSPR lines. Differential expression analysis revealed stark differences in gene expression due to tissue and due to interactions between tissue and treatment (control versus copper exposed flies). Analyses of gene clusters with tissue-specific responses to copper exposure are enriched for metal response genes. eQTL mapping of gene expression under control and copper conditions as well as for the change in gene expression as a result of copper exposure (copper response eQTL) revealed hundreds of genes with tissue-specific local *cis*-eQTL and many distant *trans*-eQTL that implicate “hotspots” for genes that are candidates for the control of metal-responsive genes. One copper response *trans*-eQTL hotspot observed in midgut tissue falls near *MTF-1*, a metal-responsive transcription factor. *MTF-1* regulates copper- and metal-response genes including metallothioneins and chaperone proteins, both of which we have empirically linked to the copper toxicity response in the DSPR through RNA-seq and traditional QTL mapping. Together, our data build a nuanced description of the roles and interactions between allelic and expression variation in copper-responsive genes, providing valuable insight into the genomic architecture of susceptibility to heavy metal toxicity as well as many candidate genes for future functional validation.

688A Impact of exercise on weight and climbing ability in *D. melanogaster*

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Currently, it is not well understood why some individuals experience the desired response to an exercise treatment, while others show no response. Interestingly, differences in exercise response occur not only in humans but also in animal models used to study exercise, including *Drosophila melanogaster*. Here, we investigate the factors controlling exercise response using the Drosophila Genetic Reference Panel (DGRP), a collection of wild-derived, inbred strains suitable for genome-wide association studies. We used the core set of DGRP strains to profile the response to a 5-day rotational exercise treatment in both sexes. As response measures, we assayed weight change and change in climbing index as an assessment of physical fitness. We find that both animal weight and climbing index differ strongly between males and females and between the different genetic backgrounds represented in the DGRP. We find that there is a complex set of molecular pathways controlling weight and climbing performance. Based on the gene ontology analysis, the CNS plays a role in the weight change with exercise, in particular, signaling from the CNS. Additional analyses revealed that weight in Drosophila is driven by two factors,

animal size, and body composition, as the amount of fat mass versus lean mass impacts the density. Thus, while the CNS appears to be important for weight and exercise-induced weight change, signaling pathways are particularly important for determining how exercise impacts weight. Our analyses suggest that complex and non-overlapping genetics pathways control responses to exercise and that it might not be possible to optimize exercise treatments for multiple outcomes.

689B Testing implications of the omnigenic model for the genetic analysis of loci identified through genome-wide association

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Organismal phenotypes usually have a quantitative distribution and their genetic architecture can be studied by genome-wide association (GWA) approaches. In many of such studies it has become clear that many genes of moderate or small effects contribute to the phenotype. Hence, the attention has turned towards the loci falling below the GWA cut-off, which may contribute to the phenotype through modifier interactions with a set of core genes, as proposed in the omnigenic model. One can thus predict that moderate effect GWA derived candidate genes and randomly chosen genes should have a similar likelihood to affect a given phenotype when they are tested via gene disruption assays. We have tested this hypothesis by using an automated phenotyping system for *Drosophila* pupal phenotypes. We first identified candidate genes for pupal length in a GWA based on the *Drosophila* Genetic Reference Panel and showed that most of these candidate genes are indeed involved in the phenotype. We then randomly chose genes below the GWA threshold and found that three quarters of them had also an effect on the trait with comparable effect sizes. We further tested the effects of these knockout lines on an independent behavioral pupal trait (pupation site choice) and found that a similar fraction had a significant effect as well. Our data thus confirm the implication that a large number of genes can influence independent quantitative traits. Our results suggest that current strategies for the genetic confirmation of GWA hits need to be revisited.

690C The pioneer transcription factor Zelda regulates *taranis* during wing imaginal disc regeneration.

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Regeneration is a complex process, required for the replenishment of lost and damaged tissue. Regeneration in wing imaginal discs is controlled by damage-induced signals such as Reactive Oxygen Species (ROS). ROS signaling triggers additional signaling, including activation of the Jun N-terminal Kinase (JNK), which is required for the repair and proliferation of the damaged tissue. However, our lab has recently described a regeneration-specific mechanism that appears to be independent of JNK signaling. Specifically, we showed that the gene *taranis* is upregulated in the *Drosophila* 3rd instar wing imaginal disc during late regeneration. Lower levels of Taranis in mutants (*tara*[1]/+) cause the posterior selector gene *engrailed* to be overexpressed and then silenced after damage due to JNK signaling, resulting in posterior-to-anterior cell fate transformations in the regenerating wing imaginal disc. Taranis prevents these aberrant cell fate changes by stabilizing *engrailed* expression. However, little is known about the signaling events that activate *taranis* during late regeneration, or how Taranis acts to regulate *engrailed* expression. We have found that the transcription factor Zelda is also upregulated during late regeneration in the wing imaginal disc. Our preliminary data suggest that Zelda regulates *taranis* expression. We are currently working to determine the signaling pathways upstream of *zelda* and *taranis* in late regeneration, and we are developing tools with which we can determine how these genes collectively counteract the effects of JNK signaling on *engrailed*.

691A Hippo Signaling's Role in Neuronal Dendrite Regeneration after Injury

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Dendrites are the specialized projections of neurons that receive, integrate and deliver signals to the cell soma, which can then send outputs in the form of action potentials. Dendrites are perturbed in several disease contexts including stroke and traumatic brain injury, and they have also been shown to degenerate prior to neuronal cell death in neurodegenerative disease. Although limited in number, studies assessing the regeneration of dendrites have uncovered molecular mechanisms not shared with the regeneration of axons (Stone et al. 2014). Furthermore, factors that play a role in dendrite arbor development and maintenance have been implicated in dendrite regeneration (Thompson-Peer et al. 2016). Previously, one group has shown the protein kinase Warts (Wts) regulates dendrite arbor maintenance (Emoto et al. 2006). Wts is part of the Hippo signaling pathway that regulates the cytoplasmic-nuclear shuttling of the transcriptional coactivator Yorkie (Yki). Given that Yki is essential for the regeneration of intestinal, cardiac, and hepatic epithelia following injury (Shaw et al. 2010, Xin et al. 2013, Fan et al. 2016), we are investigating whether Yki, the central effector for the Hippo signaling pathway, plays a role in the development, maintenance, or regeneration of dendrites after injury. We hypothesize that aberrant expression of Yorkie

will reduce the extent to which neurons regenerate after dendrite injury. Using genetic mutants, RNAi, and overexpression, we here present our results so far on the role of Yki in dendrite regeneration. In order to assess dendrite regeneration, we use a two-photon laser to injure the dendrites of class IV da neurons of the larval peripheral nervous system. Dendrites were completely removed at ~72 hours after egg lay, neurons were imaged ~24 hours after injury to confirm complete removal of dendrites, and a final imaging session ~72 hours after injury was used to assess regeneration. Our preliminary data in yki[MB09079] mutants show a reduction in total dendrite length and branch number of regenerated neurons compared to uncut controls. This is a promising hint that the Hippo pathway may play an important role in dendrite regeneration.

692B Necrosis-induced-apoptosis promotes regeneration in *Drosophila* wing imaginal discs

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Cell death is essential for the proper regeneration of tissues following injury. Signals that propagate from dying cells dictate how the surrounding tissue responds to damage by further promoting cell death or proliferation and recovery. The imaginal discs of *Drosophila* have contributed much to our understanding of this phenomenon. For example, apoptotic cells have been shown to release *egr* to adjacent cells, activating the c-Jun N-terminal Kinase (JNK) pathway. It is thought that the relative level of JNK activity can promote either pro-apoptotic or pro-survival environments, depending on the context. Although the crosstalk between apoptotic cells and their neighbors has been extensively characterized, much less is understood about how tissues respond to non-apoptotic modes of cell death, such as necrosis. Necrosis is thought of as a rapid, disordered cell death in which cell membrane integrity is lost and intracellular contents are released to the external environment. This type of death is implicated in many human diseases including stroke, heart attack, infections, and cancer. To investigate the effect of necrosis on regeneration, we developed a genetic ablation system that drives necrotic cell death in the *Drosophila* wing imaginal disc. This loss of tissue is demonstrably unlike apoptotic cell death as necrotic markers like propidium iodide, strongly label the pouch in these discs. Importantly, ablation occurs in the absence of caspase activity, further validating the necrosis-like cell death of this system.
With this new model of necrosis, we show that wing imaginal discs regenerate comparably in response to both apoptotic and necrotic cell death, as assayed by adult wing size. Interestingly however, we have found that necrosis leads to a unique regenerative response. Immunofluorescent staining reveals significant apoptosis is induced at a distance from the wound, which we have called necrosis-induced-apoptosis (NiA). Importantly, unlike other damage-associated apoptosis in the wing disc, NiA appears to be independent of JNK signaling. Moreover, NiA may be required for regeneration following necrotic tissue death, as limiting this apoptosis impairs regeneration by preventing the formation of a blastema. This research suggests that wing disc tissue relies on apoptotic signaling to direct regeneration following necrosis, although the mechanism that activates this NiA remains to be identified.

693C Regulation of Damage-Responsive Maturity-Silenced enhancers in *Drosophila*

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Regeneration is a complex process that occurs in a variety of different organisms. In early larval stages, the imaginal discs of *Drosophila* – the precursors to the adult appendages – have a significant capacity to regenerate that is lost as the organism matures. Using a genetic ablation method established by our group we have found that several genes involved in disc regeneration are regulated by damage-responsive and maturity-silenced (DRMS) enhancers. These regulatory elements are activated upon damage to induce regenerative gene expression, but epigenetically silenced as the organism approaches pupariation. The goal of my research is to investigate what specific signals activate DRMS enhancers and how they become progressively silenced as discs mature. The genes *wg* and *Wnt6* are activated during regeneration and controlled by a single DRMS enhancer ($DRMS^{Wnt}$). We found that JNK signaling is necessary for the activation of $DRMS^{Wnt}$ but not sufficient, since developmental JNK signaling does not activate the enhancer. Using a GFP reporter for $DRMS^{Wnt}$ we found that reducing JAK/STAT pathway activity decreases damage-induced activation of the reporter, suggesting a role for JAK/STAT in the activation of the enhancer. This was confirmed using an *ex vivo* culture method and a chemical inhibitor of JAK/STAT signaling. Currently, we are working to determine if the activation of the DRMS enhancer by STAT is direct or via an intermediate, and whether STAT activity leads to the initial activation of the enhancer or maintains it during regeneration. We are also testing whether this genetic relationship regulates other DRMS enhancers we have identified.
Lastly, we are using genetic ablation and knockdown assays to detect genes that are required for DRMS silencing with maturity in an attempt to extend regenerative capacity. Our results have identified a number of Polycomb group genes that potentially contribute to regeneration via different mechanisms. By understanding the necessary inputs required for the activation, and subsequent silencing, of DRMS enhancers our work aims to understand how a regeneration program is regulated during development, findings that could ultimately be used to induce regeneration in non-regenerative tissue.

694A Wounding induces polyploidization and cellular hypertrophy

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Epithelial tissues are frequently subject to wounding. Small wounds that result in the loss of only a few cells can be repaired simply by the migration of neighboring cells into the damage site. However, large wounds resulting in the loss of hundreds of cells requires additional behaviors to recover the functionality of the tissue. Studies from the Losick lab have demonstrated that in the post-mitotic adult *Drosophila* epithelium, puncture wounding results in the formation of large syncytial cells via cell-cell fusion that cover the wound site. These syncytia contain and are often surrounded by nuclei that undergo endocycling, characterized by sequential S-phase entry without division. The increased ploidy, achieved by undergoing fusions and endocycling, allows the barrier function and synthetic capacity of the tissue to be reestablished. Inhibiting either endocycling or cell-cell fusion delays wound closure.

In contrast to adult post-mitotic tissue, the pupal notum epithelium is a mitotic tissue. Although we expected that wounds to this tissue would result in increased mitosis, during live imaging experiments of wound repair we frequently observed enlarged nuclei, altered cell cycle regulation, and cell-cell fusions. Rigorous ploidy analysis requires DAPI staining, but the pupae is refractory to dissection and fixation so I developed a novel dissection protocol to access this tissue. With this protocol I determined that wound proximal nuclei undergo endocycling to become polyploid. These endocycling nuclei are also included in the observed syncytial cells. Since the notum is capable of mitosis, these findings suggest that wounding is specifically inducing endocycling and cell-cell fusions and indicates these behaviors provide some benefit to repair. To explore the role of polyploidy following wounding I have validated the chromatin marker Histone-GFP as an *in vivo* measure of ploidy. By combining this chromatin marker with an apical border marker, I will characterize the spatial and temporal pattern of cell-cell fusions and endocycling following wounding. This ploidy 'roadmap' will provide clues for the potential benefits these behaviors provide during closure.

695B Critical feed-forward loop involving JNK/AP1 signaling and Ets21C activates a regeneration-specific transcriptional program necessary for imaginal disc regeneration

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The capacity to regenerate damaged tissues varies greatly between organisms and even between different tissues in the same organism. A fundamental question for the field of regenerative biology is if a genetic program exists specifically for regenerative growth. Evidence for such a regeneration-specific program would come from identifying genes that are required during regeneration but that are dispensable for normal growth and development. Additionally, such a program may be inactive in tissues that cannot regenerate. Regeneration of *Drosophila* imaginal discs is characterized by blastema formation, a zone of localized cell proliferation and increased cellular plasticity. There is evidence that cells throughout the disc respond to tissue damage and that cells outside of the blastema may also participate in regeneration. To identify the genetic pathways that regulate regeneration at different locations in the disc, we utilized single-cell transcriptomics to profile cells from both undamaged and regenerating tissues. Mapping the cells back to our virtual model of the wing disc allowed us to investigate gene expression changes with spatial resolution. We identified transcriptional responses globally throughout the tissue, as well as localized responses at different distances relative to the damage site. The most dramatic transcriptional changes occurred within the blastema cells, which express many genes that are not detectably expressed in undamaged discs. To search for a transcriptional regulator that could initiate a regeneration-specific program, we looked for transcription factors that were specifically expressed within the blastema and identified the Ets family transcription factor Ets21C. Using *Ets21C* mutants and RNAi, we found that Ets21C is necessary for imaginal disc regeneration while being dispensable for normal development. Ets21C is a downstream target of the JNK/AP1 pathway and jointly with AP1 forms a network motif, known as a coherent type 1 feed-forward loop. Together Ets21C and AP1 induce the expression of several critical effectors of regeneration, including the *Insulin-like peptide-8 (Ilp8)*, *Matrix metalloproteinase 1 (Mmp1)*, and *asperous (aspr)*, as well as other novel genes uncovered by our single-cell analysis. Our model suggests that Ets21C is critical for cells to interpret a sustained JNK/AP1 signal as pro-regenerative and that Ets21C initiates a regeneration-specific gene regulatory network that is required for imaginal disc regeneration.

696C The basolateral polarity module promotes slit diaphragm formation in drosophila nephrocytes, a model of vertebrate podocytes

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Kidney filtration of the blood requires highly specialized cells known as podocytes. Podocyte slit diaphragms (SDs) are

intercellular junctions that contribute to size-selective filtration, which excludes larger proteins from the urine. Abnormalities in SDs cause proteinuria and nephrotic syndrome. Podocytes have been shown to exhibit apicobasal polarity, which can affect fundamental aspects of cell biology, including intercellular junction formation. Indeed, in humans and rodent models, mutations in genes encoding apical polarity proteins cause defects in SD formation and nephrotic syndrome. While these observations seem to suggest key roles for apicobasal polarity in podocytes, there is no evidence that basolateral polarity proteins contribute to SDs. In fact, studies of basolateral polarity proteins in mouse podocytes revealed no obvious functional role, however we speculate this may be due to genetic redundancy in vertebrates. Thus, the role of apicobasal polarity in podocytes remains unclear.

To explore the potential role of basolateral polarity proteins in SD formation, we examined the consequences of disrupting basolateral polarity proteins in Drosophila nephrocytes, which possess SDs remarkably similar in molecular composition and function as those in mammalian podocytes. Using confocal and electron microscopy we characterized SD integrity following loss of basolateral polarity proteins. Our study identified the basolateral polarity proteins Dlg, Scrib, Lgl, and Par-1 as novel regulators of nephrocyte SDs. Loss of basolateral polarity proteins also compromised the central function of nephrocytes—filtration of the fly hemolymph. We also performed genetic interaction studies, which suggest the basolateral polarity proteins work together, in concert with apical polarity proteins, to regulate SDs by promoting normal endocytosis and trafficking of SD proteins.

Given the recognized importance of apical polarity proteins and SD protein trafficking in podocytopathies, our findings connecting basolateral polarity proteins to these processes significantly advance our understanding of podocyte biology.

697A Expanding the network of seminal fluid proteins contributing to the long-term postmating response in *Drosophila melanogaster*

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Drosophila melanogaster females exhibit a wide array of postmating responses, largely induced by seminal fluid proteins. One such seminal fluid protein, Sex Peptide (SP), induces a number of important postmating responses in mated females (i.e. increased egg production, gut growth and re-tooling, sperm release from storage, and changes in several behaviors). Interestingly, SP remains in the female for ~10-14 days after mating, allowing it to continue to affect her physiology and behavior long-term. This effect is due to SP's binding to sperm and being retained with them in storage, and then its active portion being released from sperm, to affect the female. Our lab has identified a network of seminal proteins that bind SP to sperm, but it is as yet incomplete. To investigate the molecular nature of SP's association with sperm and its cleavage from them, I am taking two approaches. First, I am using genetic approaches, such as RNAi and CRISPR-Cas9, to test novel sperm-bound seminal proteins (identified in a collaborative study with the Dorus/Pitnick labs; Wittington, Singh et al. in preparation) to determine which are involved in binding SP to sperm, as assessed by probing for disrupted postmating responses and by Western Blotting for SP. Second, using a proteomics approach, I tagged an uncleavable SP mutant with a C-terminal TAP tag to pull down proteins to which SP is bound in the mated female, with the goal of identifying SP's target site on sperm. Together, these experiments will expand our current understanding of exactly how SP binds to sperm.

698B The Role of Insulin Signaling in Sex Dimorphism of Reproductive Senescence

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Drosophila is an important model for exploring the genetic mechanisms underlying sexual dimorphism in senescence and ageing. Senescence can have large effects on reproductive physiology and subsequent fitness, as organs and tissues with reproductive roles also senesce with age. This phenomenon is known as reproductive senescence. Reproductive senescence leads to decreased fecundity and fertility as males and females age. The insulin and insulin-like growth factor signaling (IIS) pathway play an important role in ageing and in modulating reproduction through effects on gametogenesis and mating behavior. This same pathway contributes to sexual dimorphism in physiology and behavior and therefore they may also contribute to sex differences in reproductive senescence. One way to investigate the role of insulin is to measure the effect of perturbation of this pathway on reproductive output such as egg production and quality in both sexes at different ages. In this study, the gene-switch system was used to express a dominant negative allele of the insulin receptor in young and old males and females. Egg production and hatch rate were assayed for each treatment. We found distinct patterns of responses to the perturbation, with age and sex effects. This is consistent with age and sex differences in the response to insulin signaling that may play a role in sex difference in reproductive senescence. Further work will examine how regulatory responses change with age in reproductive tissues, and how this corresponds to changes in fecundity and fertility.

699C Does 4-nonylphenol affect the fertility of Drosophila?

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Endocrine disrupting chemicals (EDCs) are commonly found dispersed in the environment and may pose a threat to human health. These chemicals come from many everyday products, including plastic containers and detergents, and some can persist for long periods of time in soil and water. EDCs interfere with hormone biosynthesis, metabolism, or action, and can negatively affect reproduction. We have focused specifically on the effects of 4-nonylphenol, an EDC that is particularly prevalent in the environment. 4-nonylphenol has been shown to have a definitive feminizing effect on fish. However, its impact on the fertility of *Drosophila* is currently not as clear. The objective of this study is to determine if 4-nonylphenol influences the fertility of either male or female *Drosophila* compared to controls.

700A Ras/MAPK signaling autonomously controls adult adipocyte cell size but does not remotely influence oogenesis in *Drosophila*

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Approximately 40% of adults in the United States are obese. Obesity-induced adipocyte dysfunction, metabolic syndrome and altered tumor metabolism produces a permissive environment for cancer maintenance and progression. Adipocytes, the primary cellular component of fat tissue, are nutrient-sensitive and secrete adipokines that control physiology, including metabolism, appetite, and insulin sensitivity. Despite the intricate relationship between dietary input, adipose tissue and peripheral organ function, we are at the tip of the iceberg regarding our understanding of the cellular and molecular mechanisms underlying adipose communication to other tissues. The *Drosophila* ovary receives nutritional signals from the fat body with multiple nutrient-sensing pathways functioning in adipocytes to control distinct stages of oogenesis. Insulin/insulin-like growth factor signaling (IIS) within adult adipocytes remotely controls oocyte production at distinct stages of oogenesis. The PI3K/Akt1 axis in adipocytes promotes germline stem cell maintenance via SGG, Drosophila GSK3beta, and early germline survival via an unidentified Akt1 target. Using adipocyte-specific manipulation of IIS pathway components, we find that a second axis downstream of the insulin receptor, the Ras/MAPK signaling pathway, acts cell autonomously to control adipocyte size. However, Ras/MAPK signaling within adipocytes does not regulate germline stem cell maintenance or the survival of early and late germline stem cell progeny – germline cysts and vitellogenic egg chambers, respectively. Future studies will assess the ovary intrinsic role of InR/Ras/MAPK activity as well as identify SGG/GSK3beta and Akt1 substrates within adipocytes required to communicate to the ovary. These studies highlight the complex mechanisms that underlie inter-organ communication.

701B Octopamine modulates sperm preference in female *Drosophila melanogaster*

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In polyandrous internally fertilizing species, a multiply-mated female can exert preference on the stored sperm that she uses to fertilize her eggs. The female's ability to assess sperm quality and compatibility is essential for her reproductive success, and represents an important aspect of postmating sexual selection. In *Drosophila melanogaster*, previous studies demonstrated that the female nervous system plays an active role in sperm preference, and suggested a role for octopamine (OA), a potent neuromodulator with known functions in female reproduction, in this process. Here, we report that OA signaling is essential for female sperm preference. Doubly-mated OA-null *Tdc2* mutant females have different sperm storage and usage dynamics than control females, storing more first-male sperm and retaining more sperm over the course of a ten-day assay. These defects are attributable to the activity of octopaminergic *Tdc2* neurons, as females with inhibited *Tdc2* neurons show sperm storage and usage dynamics similar to OA-null females'. Inhibiting *Tdc2* neuronal activity also causes females to produce a higher-than-normal proportion of first-male progeny. This difference is attributable to preferential use of first-male sperm, as opposed to increased initial storage of first-male sperm. Intriguingly, the lack of *Tdc2* neuronal activity also causes stored sperm to clump soon after the second mating. Hence, OA signaling may be required for proper sperm movement in storage after a second mating, and for preferential use of second-male sperm to produce progeny. These results suggest that OA signaling allows a multiply-mated female to exert sperm preference, and identify a new role for the female nervous system in postmating sexual selection.

702C Elucidating the effect of JAK/STAT signaling on initiation of individualization

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In *Drosophila* spermatogenesis, individualization happens near the end of spermiogenesis when 64 individual spermatids are formed from the spermatid bundle. While the morphological events that take place during the synchronous differentiation

of spermatids are well known, the underlying signals that lead to these changes have not been completely understood. Disruption of JAK/STAT pathway activity in cyst cells impairs individualization, suggesting that cell signaling is required for spermiogenesis.

The aim of this project is to identify effectors regulated by JAK/STAT signaling that initiate individualization. Using RNA-seq, we have examined expression profiles from testes in which JAK/STAT signaling has been impaired prior to individualization by expressing the negative regulator Eye-transformer/Latran (ET/Lat) and compared to testes dissected from wild type flies. Estimates of transcript abundance of the control and experiment profiles were calculated using RSEM (Li 2011). edgeR and EBSeq were later on used to perform differential expression analysis. Using edgeR to conduct the differential expression analysis on the raw counts provided by RSEM, we identified approximately 420 genes that were differentially expressed more than a log2 1.5-fold change upon the arrest of JAK/STAT signaling at elongation. The differentially expressed genes identified in the RNA-Seq analysis were analyzed to understand if they affect initiation of individualization. These experiments will provide insights into the soma-germline relationship and how signaling between them results in synchronous differentiation of spermatids.

This project has also led to the identification of genes that show high variability in expression among biological replicates. To further study this, a number of publicly available RNA-seq experiments on Drosophila testis tissue were analyzed to calculate their inner-replicate variance. This study will uncover genes with variable expression and their potential biological roles in determining cellular development.

703A An RNA-sequencing time series to investigate Sex Peptide-mediated transcriptome changes in the head of *Drosophila melanogaster* females

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Seminal fluid proteins are male-derived proteins that are transferred to the female during mating, and there is strong evidence that they enhance reproductive success. In *D. melanogaster*, the best-studied of these proteins is Sex Peptide (SP). SP initiates a suite of long-term responses in females, both physiological and behavioral. Several studies have shown that gene expression changes in females are altered in the absence of SP, shedding light on the identity of SP-responsive genes. However, published studies have sampled few time points or examined a mix of tissues. Thus, current data make it difficult to pinpoint key regulators that act downstream of SP to mediate specific post-mating responses. Here, we used an RNA-sequencing time series of female heads to identify potential downstream effectors of SP. Heads were chosen since they include the brain and fat body, two tissues likely to play a role in post-mating responses. We sampled heads of virgin females and females mated to SP null or heterozygous control males at 10 time points within the first 24 hours after mating. Comparing the expression profiles of control-mated females with those of SP null-mated females highlighted 172 genes whose profiles differed significantly. Clustering analysis indicated mostly transient expression changes over time, with a maximum or minimum expression at 8-12 hours after mating. Of these 172 genes, 95 were upregulated in control-mated females. These upregulated genes were significantly enriched for factors involved in translation and phototransduction, the latter being a class of genes previously found to be SP-responsive. We also detected 19 differentially expressed antisense lncRNAs. These RNAs have not previously been implicated in post-mating responses and might have a regulatory role or indicate the presence of active enhancers. Even though SP has far-reaching effects on females, our results so far indicate that, within the first 24 hours after mating, SP alters the expression of only a limited number of genes in the head. These genes might be important regulators of downstream targets or cellular processes that mediate SP-dependent responses. We plan to use gene network analyses to further elucidate those pathways and prioritize validation of candidate regulators of post-mating responses that act downstream of SP in the female head.

704B An anciently conserved protein is required for sperm motility in *Drosophila melanogaster*

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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. A CRISPR/Cas9-generated null allele of *dsg1* was

homozygous lethal, confirming the gene has additional roles beyond reproduction, as is true of its ortholog in other animals. To study Dsg1 protein localization, we are taking two approaches. First, we have raised peptide antibodies to two epitopes of the protein and are characterizing their performance in western blots and immunofluorescence experiments. Second, we are using CRISPR to create a dsg1-GFP fusion allele at the endogenous *dsg1* locus and are investigating GFP localization in testes and sperm. Finally, to identify potential interacting partners of Dsg1, we used fluorescence microscopy to study the localization patterns of other known sperm motility proteins in *dsg1* knockdown flies. 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.

705C Regulators of binding and release of the seminal Sex Peptide (SP) with sperm, in mated *D. melanogaster* females

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The seminal fluid protein sex peptide (SP) induces a myriad of physiological and behavioral changes in mated females, redirecting resources and energy to achieve efficient fertility. These post-mating changes last long-term, since SP persists in the female due to its binding to sperm. Some male-derived seminal proteins ("LTR-SFPs") are known to be necessary for this binding. SP normally binds to the sperm that it entered the female with, but we found that SP-free sperm stored within females can bind SP introduced by a subsequent male. Further, those experiments showed that LTR-SFPs "prime" sperm to bind SP. We find that sperm in ejaculate bind SP much more weakly than sperm within females, suggesting that priming of sperm may improve inside the female reproductive tract. This, in turn, suggests that female molecules participate in facilitating SP binding to sperm. To determine the source of these molecules, we tested whether secretions from tissues of the female reproductive tract are needed for SP's binding to, or retention on, sperm. We targeted expression of the ER stress-inducer Rh1G69D to ablate spermathecal secretory cells, and tested Hr39 mutants, which have defective spermathecal secretory cells and/or parovaria, for effects on SP-sperm binding. Neither treatment affected binding of SP to sperm; we are currently testing whether they regulate its retention on sperm, and testing effects of secretions of other female reproductive tissues on sperm-SP binding. We are also working on ways to test whether non-protein components of the female reproductive tract, such as pH, affect SP-sperm binding or retention.

706A Toxicological Effects of Roundup® on *Drosophila melanogaster* Reproduction

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Farmers within the U.S. and worldwide increasingly use herbicides, particularly glyphosate, the active ingredient in the most widely used herbicide, Roundup®. Glyphosate, although effective in agricultural practice, adversely affects the reproductive and endocrine systems of non-target organisms, including fecundity, development of reproductive organs, and hormone levels. This study utilized the fruit fly, *Drosophila melanogaster*, to explore the effects of Roundup® exposure on reproductive anatomy, building on previous research showing that exposure increases mortality, induces oxidative stress, and reduces body size in females. At either 2 or 4 hours after eclosion, flies were exposed to either organic medium, medium containing one of two commercial Roundup® formulations, or medium containing a pelargonic acid based herbicide, Scythe®, at different concentrations of active ingredient. One Roundup® formulation includes pelargonic acid in addition to glyphosate, the other POEA, a surfactant. After an exposure period of 7 days, survival was observed, females were weighed, and their ovaries and oocytes were dissected and measured. Survival was not affected by Roundup® with POEA, and only slightly by the other treatments. This was expected since we chose concentrations of active ingredients that were sub-lethal in a previous study. Body weight was only significantly affected by Roundup® with pelargonic acid at 2 hours of eclosion, with the greatest effects occurring at 4 g/L of combined active ingredients, the highest concentration used. More notably, both Roundup® formulations and Scythe® affected ovary development at all concentrations tested, causing reduced ovary volume with fewer mature oocytes compared to the organic control. Again, the greatest effects on ovary volume and oocytes were seen at 4 g/L of combined active ingredients. This is consistent with results in other species suggesting that glyphosate and other herbicides disrupt endocrine signaling. These effects were greater in flies exposed within 2 hours of eclosion compared to 4 hours of eclosion, which suggests a critical period of increased ovarian sensitivity to glyphosate, which may begin during the larval or pupal period but ends shortly after eclosion. These results support multi-species evidence that glyphosate-based herbicides, in addition to pelargonic acid-based herbicides, have toxic reproductive effects and interfere with normal development of the reproductive system of non-target organisms.

707B Essential mRNA Regulatory Functions of the TRIM-NHL Protein Brat

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TRIM-NHL proteins share a conserved domain architecture and play crucial roles in stem cell biology, fertility, and development. Recently, multiple TRIM-NHL proteins were shown to recognize specific RNA motifs and structures via the NHL domain. Functional and genetic analyses revealed that TRIM-NHLs negatively regulate protein expression from the mRNAs that they bind. However, whether the RNA-binding function of TRIM-NHL proteins is necessary for their biological roles remained to be determined. In addition, how TRIM-NHL proteins negatively regulate protein expression is unknown. To investigate these key questions, we focused on the *Drosophila* TRIM-NHL protein Brain Tumor (Brat). First, RNA-binding defective mutations were introduced into the endogenous *brat* locus in flies via CRISPR/Cas9 genome engineering. Our phenotypic analysis demonstrates that the key residues necessary for RNA-binding in vitro are essential for larval development. RNA-binding defective mutations phenocopy the lethality observed in loss-of-function *brat* mutations, implying the essential function of Brat is to bind and regulate mRNAs. To elucidate the molecular mechanisms by which Brat controls protein expression, we used cell-based reporter assays to identify three domains of Brat that autonomously repress target mRNAs. We then identified components of the RNA decay and translation machinery that are necessary for Brat-mediated repression. Collectively, our findings provide crucial insights into the molecular mechanism and function of Brat in vivo.

708C A temperature-sensitive mutation reveals Drosophila TRMT61A is essential for adult viability and fertility, with partial rescue by a non-inherited maternal wild-type allele

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tRNAs undergo a variety of post-transcriptional modifications. Among these, methylation of the adenine at position 58 (m^1A58) is among the most conserved, found in bacteria, archaea, and eukaryotes. Depletion of the enzyme complex responsible for the modification, TRM6/TRM61, results in death of yeast and mammalian cells, but reversibility of the modification also plays an important role in regulation of protein synthesis. We mapped a temperature-sensitive lethal mutation from a stock of wild origin, and found it to be a missense mutation in *CG14544*, the *Drosophila* orthologue of TRM61A, the nuclear-targeted version of TRM61. Preliminary experiments reveal that the level of modified tRNAs in flies hemizygous for the missense mutation (*m/-*) is lower than in wild-type controls (+/-), with greater reduction when flies are raised at higher temperature. Shifting flies from the permissive (21°C) to restrictive (28°C) temperature greatly reduced longevity, and resulted in a rapid loss of fertility in both sexes that well preceded mortality. Mutant flies raised at an intermediate temperature were also more ethanol sensitive than wild-type controls. Surprisingly, all of these effects were greater when *m/-* flies came from crosses of females homozygous for the missense mutation (*m*/*m*) to males with a copy of the wild-type allele (+/-) than when they came from the reciprocal cross. Moreover, initial results indicate that the level of modified tRNAs depended on cross direction, being higher in the flies from mothers with the wild-type allele. These results suggest that a significant amount of maternally deposited TRM61A (or, less likely, modified tRNAs and/or *CG14544* mRNA) can persist into the adult stage.

709A RNA-binding protein Alan shepard regulates whole organism adiposity via isoform-specific functions within the fat body of *Drosophila melanogaster*.

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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. Here, we investigate the role of the RNA binding protein Alan shepard in fat storage homeostasis within the fat body (FB; homologous tissue to adipose and liver). We find that knockdown of *shep* in neurons phenocopies the high-fat phenotype of the *shep* mutant and drives changes in two complex metabolic behaviors, feeding and activity. By contrast, knockdown of *shep* in the fat body results in a lean phenotype. We find that *Drosophila* brain and FB express tissue-specific combinations of *shep* splice isoforms, suggesting differential utilization of *shep* by the two tissues to regulate energy homeostasis. The fat body expresses the Shep mRNA isoforms A, B, E, F, and H. We find that fat body-specific overexpression of the Shep-E isoform increases organismal fat levels, whereas neither Shep-A nor B overexpression alters adiposity. By overexpressing a mutated Shep-E carrying substitutions in the RNA Recognition Motif, we find that RNA-binding activity is necessary to generate the fat phenotype resulting from Shep-E FB overexpression. This result suggests that Shep likely mediates metabolic processes within FB cells through its binding to RNA targets. Finally, we find that Shep transcript and protein levels are regulated in a nutrient-dependent manner: varying the diet results in tissue- and isoform-specific changes in Shep levels. These results suggests that Shep is regulated by a nutrient-sensing pathway to effect metabolic adaptation.

710B The role of *Drosophila* germ granules in regulating mRNA stability during germ cell development

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Germ granules, membraneless organelles containing mRNAs and proteins required for germline development, are a characteristic feature of germ cells. In *Drosophila,* germ granules form at the posterior of the oocyte and are subsequently segregated to the germ cell progenitors, called the pole cells, which bud from the posterior of the embryo. After pole cell formation, the germ granules increase in size and persist at least until the pole cells coalesce in the gonad. The persistence and evolutionary conservation of germ granules suggest that they play an important role in RNA regulation during germ cell development, but their properties and roles are not fully understood. We found that the germ granules begin to associate with mRNA degradation factors in the pole cells at the same time that the germ granules begin to increase in size. This association coincides with a drastic decrease in the levels of two granule mRNAs, *nos* and *pgc*. However, other germ granule mRNAs, such as *cycB*, appear to be protected from this degradation throughout embryogenesis. This finding suggests that the germ granules may play a dual role in the pole cells, selectively protecting some mRNAs while promoting the degradation of others. Furthermore, the apparent coordination between the onset of mRNA degradation and the increase in germ granule size may indicate that there are additional changes to the germ granules' composition and physical properties during this period that allow them to take on a more complex role in regulating mRNA stability. Since germ granules are only one example of an RNA-rich membraneless organelle, understanding their function in regulating RNA stability can provide general insights into the roles of membraneless organelles in post-transcriptional regulation.

711C Mistranslating tRNAs cause developmental defects but extend lifespan in a sex-specific manner in *Drosophila melanogaster*.

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Proteostasis requires the dynamic regulation of all proteins within cells, including their production, modification and degradation. Loss of proteostasis affects cell viability and is a hallmark of many disease states. Mistranslation, the incorporation of amino acids not designated by the genetic code, naturally occurs at low levels in all cells but can be dramatically increased by transfer RNA (tRNA) variants, leading to loss of proteostasis. My goal is to understand how tRNA variants that mistranslate affect development, viability, and behaviour of *Drosophila melanogaster*.

Previously, we characterized serine tRNA ($tRNA^{Ser}$) variants in yeast that mis-incorporate serine at proline codons. These tRNAs induce a heat shock response and slow growth. I have engineered similar $tRNA^{Ser}$ variants and integrated them into the genome of the fruit fly, *Drosophila melanogaster*. One mistranslating $tRNA^{Ser}$ variant, that mis-incorporates serine at proline codons at a frequency of ~5% in yeast, increased development time of the flies by nearly a full day and caused significantly fewer embryos to reach adulthood. Adult flies containing this mistranslating tRNA also presented with significantly more developmental deformities (gross leg, wing, or abdomen defects) than control flies or flies expressing a weaker mistranslating $tRNA^{Ser}$ variant that mistranslates serine for proline at a frequency of ~ 0.5% in yeast. Interestingly, there is a significant sex effect of mistranslation. Female flies expressing mistranslating tRNA variants were twice as likely to present with deformities compared to males. Further, expression of both low and high mistranslating $tRNA^{Ser}$ variants increased median lifespan of males relative to wildtype, while only the low mistranslating $tRNA^{Ser}$ variant increased lifespan in females.

This work shows that mistranslating tRNA variants have diverse effects on *Drosophila melanogaster* and suggest that male and female flies differ in their ability to withstand the resulting proteotoxic stress. Transcriptomics and proteomics are being performed to identify key pathways responsible for the lifespan increase and sex differences. My research demonstrates the broad impact of mistranslating tRNA variants in a multicellular organism. As humans have ~65 tRNA variants per person (including some with the potential to mistranslate), and several human diseases have been linked to mistranslation, this research is significant for our understanding of how tRNA variants affect human health.

712A Characterizing the mRNA components of the oosome in the wasp *Nasonia vitripennis*

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Specification of germ cell fate during embryogenesis is an essential process in sexually reproducing organisms to ensure the correct transmission of parental genetic information to offspring. In many cases it involves germ plasm, a specialized cytoplasmic organelle composed largely of mRNA and RNA-binding proteins that drives germ cell fate determination. There is great variability in morphology and composition among organisms that contain germ plasm. For example, in *Drosophila* the germ plasm consists of many relatively small granules that remain associated with the posterior pole of the egg until they are

taken into individually budding poles cells. In contrast, in the wasp *Nasonia,* germ plasm assembles into an extremely large, dense structure, called the "oosome". The oosome migrates anteriorly to 50% egg length, before returning to the posterior pole where a single large bud containing multiple nuclei emerges during pole cell formation. While much is known about the structure and assembly of the fly polar granules, how the structure of the oosome compares is as yet unknown. Here we describe our progress in determining the spatial arrangement of several of the localized mRNAs of the oosome using super-resolution microscopy, and single molecule *in situ* hybridization. These results will be compared to the known homotypic clustering and dynamics of the fly polar granules.

713B A Dual-activity Topoisomerase Complex Interacts with piRNA Machinery to Promote Transposon Silencing and Germ Cell Function

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Topoisomerase 3 beta (Top3b) is the only dual-activity topoisomerase in eukaryotes that can change topology for both DNA and RNA. Emerging evidence suggests that Top3b is required for multiple RNA and DNA metabolisms including transcription, replication, and resolving DNA/RNA hybrid (R-loop) in cells. Furthermore, Top3b forms a complex with RNA binding proteins Tudor domain containing 3 (TDRD3) and Fragile X Mental Retardation Protein (FMRP) to regulate mRNA translation.
Top3b mutation in mice exhibits reduced lifespan and fertility, chromosomal abnormality, and abnormal neurodevelopment while *Top3b* mutation in human has been linked to schizophrenia, autism, epilepsy, and cognitive impairment. Such evidence indicates that Top3b has an important role in aging and mental health. However, the mechanism of how Top3b maintains normal life-span and mental health remains largely unclear.
We have recently shown that the Top3b-TDRD3 complex interacts with the siRNA machinery to facilitate heterochromatin formation in fly heads. Heterochromatin maintains and represses transcription of genes and transposable elements (TEs) within its regions, and loss of heterochromatin results in de-repression of TE. TEs are mobile genetic elements that can cause genomic instability by uncontrolled expression and transposition. Additionally, mobilization of TEs has been shown as a driver for aging, age-associated inflammation, and neurodegeneration. Therefore, one mechanism by which Top3b and TDRD3 functions in aging and neuronal function could be through the regulation of TEs via the siRNA machinery.
In addition to siRNAs, PIWI-interacting RNAs (piRNAs) is the other major class of small RNAs that mediate suppression of TEs in gonads. Here, we present evidence that the Top3b-TDRD3 complex interacts with piRNA machinery to promote ovary development and silencing of TEs. First, Top3b and TDRD3 form stable complexes with the piRNA machinery, including PIWI, in gonads of both mouse and *Drosophila*. Second, mutation of either *Top3b* or *Tdrd3* results in reduced fertility due to defective oogenesis and embryogenesis, as well as de-silencing of multiple transposons in ovary. Third, *Top3b* and piRNA nuage components *maelstrom (mael), vasa, aubergine (aub)* and *ago3* genetically interact to suppress expression of TEs and promote ovarian functions. Notably, the double mutants exhibit defective piRNA biogenesis in dual strand piRNA clusters. Together, our data reveal a novel role of the Top3b-TDRD3 complex: regulation of TE silencing and germ cell function via interaction with piRNA machinery.

714C A region of SLBP outside the mRNA-processing domain is essential for deposition of histone mRNA into the Drosophila egg

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Metazoan histone mRNAs are the only eukaryotic mRNAs that are not polyadenylated. Instead they end in a 3' end Stem loop (SL). Processing of the histones pre-mRNAs is accomplished by an endonucleolytic cleavage after the SL. The stem loop binding protein (SLBP) binds to the SL, and SLBP is a key factor in all steps of the life cycle of histone mRNAs. We are studying the role of SLBP in *Drosophila melanogaster* in vivo. In *Drosophila* each histone gene contains a cryptic polyA site after the histone processing site, and when histone pre-mRNA processing is defective histone mRNAs are polyadenylated. Using FLY-CRISPR Cas9, we obtained a 30 nucleotide deletion ($SLBP^{\Delta 30}$) in the N-terminal domain (NTD) of SLBP. The 30 nt deletion removed 10 aa from the N-terminal domain of SLBP in a region of unknown function distinct from the processing domain. The $SLBP^{\Delta 30}$ mutant was viable but females were sterile. They laid eggs, but the eggs didn't hatch, because they didn't store histone mRNA in the egg. In Drosophila, nurse cells produce large amounts of histone mRNA at the end of oogenesis which is translated and stored in the egg to allow the development of the embryo until zygotic histone gene transcription turns on. The stored histone mRNA is produced in absence of DNA replication. Despite having normal amount of SLBP, the $SLBP^{\Delta 30}$ mutant expresses small amounts of polyadenylated histone mRNA at all stages. In the ovary histone mRNA expression is normal in the rapidly replicating

nurse cells throughout oocyte development but very little histone mRNA is expressed at the end of oogenesis after nurse cell replication is completed. Immunofluorescence data shows that the SLBP$^{\Delta 30}$ protein is mainly localized in the cytoplasm at this stage, suggesting the deleted region is important for nuclear import of SLBP at the end of oogenesis. The histone locus bodies present at the histone genes are also defective, and do not activate transcription of the histone genes. These results suggest that defective nuclear import of SLBP$^{\Delta 30}$ may lead to a defect in HLBs and histone gene transcription.

715A Sex-specific alternative splicing in *Drosophila melanogaster*

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Alternative splicing can greatly increase the transcriptome and proteome complexity of an organism. It can also be an important mechanism for sexual dimorphism, as it allows males and females to express different proteins. While differential gene expression and genome location have been vastly studied to explain sexual dimorphism in both *Drosophila* and mammals, the extent to which different splice forms are found in male and female tissues, and their direct contribution to sexual differentiation, are still not fully understood. Previous work in *Drosophila* has looked at this question using only short RNA-seq reads, which makes it difficult to call different splice forms, as it requires an assembly step that may fail to discriminate between similar isoforms. Here, we use long read sequencing to not only identify spliceforms that are sex-specific but also to quantify the frequency of known sex-specific spliceforms in *D. melanogaster*, such as those for genes transformer, *fruitless* and *sex-lethal*. We sequenced male and female heads, mid-guts, and gonads using PACBio SMRT long-read sequencing to fully analyse exon usage and detect unique transcripts in different organs and sexes. Our study provides a more detailed characterization of alternative splicing in these tissues of *D. melanogaster* than was previously available, and allows us to carefully assess the extent to which splicing differs between sexes in soma and gonads of this sexually dimorphic model organism, an important step towards understanding the role of alternative splicing in sexual differentiation. Our pipeline allows for the identification of splice forms that are unique to each sex and tissue as well as check for the diversity found in them.

716B The Effects of N6-methyladenosine (m6A) on RNA Metabolism in Neural Cells

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N6-methyladenosine (m6A) is the most abundant RNA modification in eukaryotic mRNAs and is particularly enriched in the central nervous system. This modification influences mRNA translation and stability but the degree to which these effects differ between neural progenitors and neurons is not fully defined. To investigate if m6A has distinct distributions and functional consequences in neuroblasts versus neurons, we performed mRNA decay measurements in larval neuroblasts and neurons using two approaches: EC-tagging, in which mRNA pulse/chase was targeted to neuroblasts or neurons, and 4-thiouridine-based pulse/chase using brains of mutant larvae (*insc-gal4>aPKCcaax*) with a neuroblast over-proliferation phenotype ("neuroblast-enriched") or in wildtype brains. These approaches yielded transcriptome-wide mRNA half-life measurements for neuroblasts and neurons. We also performed m6A-RNA Immunoprecipitation combined with RNA-sequencing (meRIP-seq) in neuroblast-enriched brains and will compare these data to meRIP-seq using wildtype brains to map common and neuroblast-specific m6A sites. Combining mRNA stability and m6A mapping data will allow us to identify transcripts that are differentially modified in neuroblasts, transcripts that are similarly modified across cell types, and the likely effects of these modifications on mRNA stability in neuroblasts and neurons. The effect of m6A on candidate differentially regulated transcripts will be investigated by performing cell type-specific mRNA decay measurements using EC-tagging in control and m6A methyltransferase (*ime4*) null larvae. Ultimately, we hope to discover whether m6A distribution varies during neural differentiation and whether the functional consequences of this modification differ in neural progenitors versus neurons.

717C Codon optimality and transfer RNA dynamics during neural differentiation

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Neural development requires precise deployment of genetic information and this is partly achieved through regulation of mRNA translation and decay. Pioneering work in yeast revealed that these processes are linked via codon optimality: optimal codons support rapid ribosome translocation and are enriched in stable mRNAs while non-optimal codons slow ribosome translocation and promote mRNA decay. We previously demonstrated a link between codon optimality and mRNA stability in *Drosophila* (Burow et al., Cell Reports, 2018). The codon optimality-mRNA decay model assumes that optimal codons are decoded by abundant tRNAs and non-optimal codons are decoded by less abundant tRNAs, thus causing ribosome stalling and triggering decay. Multiple lines of evidence support this model but *in vivo* quantification of tRNAs is missing in most cases. To overcome this knowledge gap, we have established the previously described "Hydro-tRNASeq" method (Gogakos et

al., 2017) in *Drosophila*. We initially obtained *in vivo* tRNA profiles from late-stage whole larvae. This work yielded 10 million reads that almost exclusively mapped to tDNA genes. As expected, tRNA abundance correlated well, but not precisely, with tDNA gene copy number. Importantly, tRNA levels correlated closely with codon optimality values obtained in whole embryos. These results demonstrate the efficacy of Hydro-tRNASeq in *Drosophila* and support the hypothesized relationship between tRNA abundance and codon optimality. To investigate this relationship in the context of neural development, we obtained mRNA decay measurements from larval neuroblasts (NBs) and neurons and calculated codon optimality in each cell type. This work revealed cell type-specific optimal codons: 11 codons that correlate with increased stability in neuroblasts and 8 codons that correlate with increased stability in neurons. We identified transcripts from each cell type that are enriched in NB or neuron-specific optimal codons (≥ 20% codon content) and gene ontology analysis of these transcripts revealed shared (e.g. cytoplasmic translation) and unique (e.g. "neuron projection morphogenesis" only in neuron mRNAs) gene ontology categories. We are currently performing Hydro-tRNASeq in NB-enriched brains and wildtype brains to test if differential codon optimality in NBs versus neurons may be explained by differential abundance of the cognate tRNAs. Ultimately we aim to identify the mechanisms that regulate tRNA expression in the nervous system.

718A The role of the extracellular protease AdamTS-B and BMP signaling in wing vein formation

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The Drosophila wing is composed of longitudinal veins (L1-L6) and crossveins, the posterior crossvein (PCV) and anterior crossvein (ACV). AdamTS-B is one of three ADAMTS proteins expressed in Drosophila, specifically in the wing imaginal disc. It was previously shown that loss of AdamTS-B in the wing resulted in extra PCVs or deltas. Moreover, over-expression of this protease resulted in a complete absence of the PCV and distal end of the L5 longitudinal vein. The BMP pathway is known to play an essential role in wing vein formation, especially the PCV. The question we are asking is whether AdamTS-B functions through the BMP signaling pathway to inhibit wing vein formation. In order to explore this question, we are performing genetic interaction studies between AdamTS-B and extracellular components of the BMP signaling pathway to examine whether these two proteins function in the same signaling pathway or not. Using the GAL4/UAS system of over-expression, we are using a wing-specific GAL4 line (MS1096-GAL4) to over-express combinations of AdamTS-B and each of the following BMP signaling pathway components: dpp, gbb, and tkv. The anticipated impact of this research will be more knowledge of how this important BMP signaling pathway is regulated. We will also learn more about potential functions of the AdamTS-B gene in other tissues as it is also expressed in the embryonic trachea.

719B Regulation of apical localization and abundance of the Dachs-Approximated-Dlish complex by the protocadherins Fat and Dachsous

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A fundamental question in developmental biology is how size and cell number in organ is regulated during development. The Hippo signaling pathway regulates organ size by restricting activity of the transcriptional co-activator Yorkie, which promotes growth. The protocadherins Fat and Dachsous, which act as receptor and ligand pair, function to promote upstream Hippo pathway activity, thereby restricting growth. Loss of Fat leads to overgrowth and stabilization of the key effector Dachs, an unconventional myosin. Two additional regulators, a palmitoyltransferase Approximated (App) and SH3 domain containing protein Dachs Ligand with SH3s (Dlish) are required for Dachs stabilization and localization at apical junction. Together these proteins form a 'core complex' at the junctional cortex that functions to promote growth.
It remains unclear how Fat and Dachsous suppress growth, though they appear to do this by regulating the accumulation and localization of the core complex at the junctional cortex. Current models emphasize that Fat represses Dachs activity by preventing its cortical localization and promoting its degradation, while Dachsous promotes localization at the cortex by recruiting Dachs. However, this model appears inconsistent with the observation that *fat dachsous* double mutants have a much stronger overgrowth phenotype than either single mutant alone, which suggests that Fat and Dachsous work synergistically to repress growth.
In this study we have focused on the intracellular domains of Fat and Dachsous since these domains interact with the core complex. We used CRISPR-Cas9 to delete the entire ICD of Fat and Dachsous. Loss of the Fat ICD leads to massive overgrowth and accumulation of Dachs at the junctional cortex. In contrast, removing the Dachsous ICD leads to reduction of the core complex at the junctional cortex and undergrowth of the wing. While these results seem consistent with current models suggesting that Dachsous promotes core complex activity, we additionally find that in the absence of Fat removal of the Dachsous ICD has the opposite effect and causes even greater overgrowth than *fat* mutants alone. Analysis of Dachsous trafficking suggests that this function is mediated by co-endocytosis of Dachsous and the core complex. Taken together, our results indicate that both Fat and Dachsous can function to either repress or promote growth in a context dependent fashion

and that these activities are mediated by their intracellular domains.

720C Examining the Role of Adipocyte CD98hc in Regulating Oogenesis

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Communication between different organ systems is a vital part of regulating physiological responses to a shifting environment. Changes in nutrient availability can cause shifts inter-organ signaling in *Drosophila* and how nutrient sensing modulates communication between organs is not completely understood. We are investigating the nutrient sensing pathways that lie between the adipocytes and the ovary. The ovary is a nutrient responsive organ that relies on macronutrients like protein to maintain oogenesis. Amino acid transporters are plasma membrane transport proteins responsible for mobilizing amino acids through different cells. Amino acid transport in adult adipocytes has been shown to be required for proper ovarian germline stem cell maintenance. Using the *Gal80^{ts}/UAS/Gal4* system, we exerted RNA interference-mediated knockdown of *CD98hc*, an amino acid transporter specific to leucine, in adipocytes of adult *Drosophila* females. Reduction of *CD98hc* in the adipocytes led to a loss of germline stem cells, a looser sheath covering the ovarioles, and higher occurrences of cell death during vitellogenesis. We are currently addressing if CD98hc-dependent amino acid transport within adipocytes is critical for germline stem cell maintenance, germline cyst development, and progression through vitellogenesis. We also want to examine how the knockdown of *CD98hc* in the adipocytes affects adipocyte morphology and lipid storage. By addressing these topics, we would like to elucidate the signaling pathways that regulate nutrient sensing between adipocytes and other nutrient sensitive tissues.

721A Negative feedback couples Hippo pathway activation with Kibra degradation independently from Yorkie transcription

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The Hippo signaling pathway regulates tissue growth in many animals. Multiple upstream components are known to promote Hippo pathway activity, but the organization of these different inputs, the degree of crosstalk between them, and whether they are regulated in a distinct manner is not well understood. Kibra activates the Hippo pathway by recruiting the core Hippo kinase cassette to the apical cortex. Here we show that the Hippo pathway downregulates Kibra levels independently of Yorkie-mediated transcriptional output. We find that the Hippo pathway promotes Kibra degradation via SCF^{Slimb}-mediated ubiquitination, that this effect requires the core kinases Hippo and Warts, and that this mechanism functions independently of other upstream Hippo pathway activators including Crumbs and Expanded. Moreover, Kibra degradation appears patterned across tissue. We propose that Kibra degradation by the Hippo pathway serves as a negative feedback loop to tightly control Kibra-mediated Hippo pathway activation and ensure optimally scaled and patterned tissue growth.

722B Polygenic adaptation drives rapid evolution of pre- and post-mating reproductive isolation

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The emergence of new species is one of the most fundamental questions in biology. Several theoretical speciation models consider adaptation an important driving force of speciation. Because most adaptive traits have a polygenic basis, it is necessary to revisit the speciation processes from a polygenic perspective. One key aspect of polygenic adaptation is genetic redundancy, which allows for different solutions to the same selection pressure. Since the influence of genetic redundancy on speciation processes is not yet understood, we addressed this question by exposing 10 replicate *Drosophila simulans* populations to a hot temperature regime. Within 200 generations, both pre- and post-mating reproductive isolation occurred. We propose that the assortative mating between ancestral and evolved populations results from a quantitative modification of cuticular hydrocarbons (CHCs) across all replicate populations. The post-mating incompatibility among evolved replicates could be caused by the replicate-specific up-regulation of different reproduction-related genes. We conclude that in less than 200 generations, polygenic adaption resulted in reproductive isolation related to two different speciation processes: ecological speciation and mutation order speciation.

723C Speciation in the laboratory - has 30 years of divergent selection resulted in reproductive isolation between laboratory populations of *Drosophila melanogaster*?

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In this study we ask if three decades of divergent life history selection on age at reproduction has resulted in the evolution of reproductive isolation (RI) between laboratory fruit-fly populations. Evolved differences in body size, an important sexually selected trait in Drosophila melanogaster, and the increased ability of larger flies to cause and resist mate harm suggested the potential for prezygotic barriers driven by sexual selection, sexual conflict and potential physical incompatibilities. We tested for premating, postmating-prezygotic and postzygotic (viability and fitness) reproductive isolation between 3 replicate population pairs. Although a simple prediction would be preference for larger size, creating directional isolation, our results indicate that both these populations show homotypic mate choice; indicative of prezygotic RI driven by sexual selection and sexual conflict. Hybridization between the focal populations resulted in the production of viable adult flies with intermediate size and developmental traits. We observed a suggestive but statistically non-significant trend of fitness decline in F2 generations of hybrids, but no significant evidence suggesting the evolution of postmating-prezygotic or postzygotic RI. This is in accord with extant literature that posits that premating RI evolves well before postmating prezygotic RI, postzygotic sterility or inviability.

724A Escargot controls somatic stem cell maintenance through the attenuation of the Insulin Receptor pathway in *Drosophila*

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Adult stem cells coordinate intrinsic and niche-derived cues in order to maintain the proper balance between self-renewal and differentiation. In addition, stem cell behavior is influenced by systemic cues, such as acute and chronic changes in metabolism. In the *Drosophila* testis, both self-renewal and differentiation signals influence stem cell behavior; however, the precise mechanisms stem cells use to integrate these signals remain elusive. Here we show that Escargot (Esg), a member of the Snail-family of transcription factors, regulates the maintenance of somatic cyst stem cells (CySCs) by attenuating the activity of the pro-differentiation insulin receptor (InR) pathway. DamID was used to provide insight into putative targets of Esg in the testis, which revealed that Esg bound close to control regions of two genes involved in the insulin signaling pathway, *InR* and the insulin antagonist *ImpL2.* Both *in vitro* and *in vivo* assays suggest that Esg positively regulates the expression of *ImpL2*, while also attenuating the expression of *InR*. Furthermore, Esg-mediated repression of the InR pathway is required to suppress CySC loss in response to removal of protein from the diet (protein starvation). Characterizing the mechanism(s) by which Esg senses changes in metabolism and, in turn, regulates cell fate decisions will provide insight into the metabolic regulation of stem cell behavior.

725B Integrity of the nuclear lamina is required for asymmetric stem cell divisions

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Tissue homeostasis depends on the maintenance of stable stem cell populations. Following each division, stem cells give rise to one daughter that maintains a stem cell identity and a second daughter that differentiates, a process known as asymmetric cell division (ACD). Events controlling ACD are poorly understood. We recently discovered that the asymmetrically dividing germline stem cells (GSCs) in the Drosophila ovary employ a non-canonical mode of mitosis, one in which the nuclear envelope (NE) and nuclear lamina (NL) remain intact but permeable. This unusual mitotic mode sensitizes GSCs to defects in NL proteins, evidenced by findings that loss of the NL protein emerin/Otefin compromises ACD, coupled to defects in the centrosome position and mitotic spindle structure. Strikingly, centrosome defects persist in interphase *emerin/otefin* mutant GSCs. Indeed, these interphase GSCs carry centrosomes that remain embedded in the NE and NL and carry large amounts of pericentriolar material. As a result, astral microtubules are nucleated, producing dramatic NL deformation at the site of insertion. Notably, loss of emerin/Otefin activates a Chk2-dependent checkpoint, which blocks germ cell differentiation and causes accumulation of stem-like cells that eventually die. Our current efforts are focused on understanding the linked between mitotic defects and Chk2 activation in *emerin/otefin* mutants. We found that Chk2 activation induces detachment of the spindle-anchoring spectrosome from the GSC-niche interface and mis-orientation of the nuclear-enclosed mitotic spindles, due to Chk2-dependent downregulation of the polarity protein Bazooka (Baz)/Par-3. These data link blocked differentiation to the inappropriate orientation of the mitotic spindle. Notably, in *chk2, emerin/otefin* double mutants, structural defects in centrosome structure remain, suggesting that centrosome defects trigger Chk2 activation and GSC loss. This hypothesis is

being tested using RNAi to eliminate components of pericentriolar material and assess effects on checkpoint activation and GSCs loss. Taken together, these studies illustrate how NL components contribute to ACD and tissue homeostasis.

726C *Drosophila* midgut tissue replacement involves a homeostatic switch from static homeostasis to pulsed turnover

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Division of intestinal stem cells (ISCs) in the adult *Drosophila* gut ensure homeostasis in a tissue that has to cope with continuous mechanical, chemical and biological challenges. Understanding how this tissue homeostasis is achieved through regulation of ISCs proliferation is vital to elucidate the mechanisms that become dysregulated in many diseases such as colorectal cancer or Inflammatory Bowel Disease (IBD).
Several recent lineage tracing studies have shown that ISC' clones expand and/or contract by neutral competition between symmetrically dividing ISCs. Under the neutral drift model, ISCs would give rise to two daughter cells whose fate is resolved through competition between proximate cells following division. However, the mathematical modelling necessary to interpret the clonal behaviour relied on a critical assumption - ISCs within a stem compartment share the same dividing rate or λ value. Yet, this assumption of a constant turnover rate is at odds with other observations which have shown, when using a non-temperature inducible tracing method, turnover is not uniform across stem cell compartments. In this scenario, ISC division rate uniformity seems incompatible with having spatially different tissue replacement rates.
Our study aims to understand how the intestinal tissue is sustained by ISCs that divide at the same rate within the stem compartment while having different replacement rates among compartments.To reconcile both observations, we propose a quiescence-division switch model for intestinal tissue replacement in the adult *Drosophila* midgut. Under homeostasis, ISCs could only be quiescent (rate of division = zero, λ≈0) - or dividing (λ>0) and this switch can be triggered by a temperature change.
Using inductive heat shock (HS) of 37°C for 60 min, we observed that a temperature increase triggers a switch from quiescence to division in ISCs, inducing pulsed turnover in the adult fly midgut. We characterised this switch model in terms of cell cycle regulation as measured by expression of Cyclin A, Polo and PCNA, and phosphorylation of Histone H3. We also showed that this mild heat stress, unlike other external stress such as directed enterocyte ablation or enteric infection stimulates ISC division not as a compensatory or regenerative mechanism in response to tissue disruption, but a form of tissue turnover.
In conclusion, the quiescence/division switch model for intestinal turnover could arise as a way to reconcile the model of neutral competition and the coexisting of slow and fast turnover areas in the adult *Drosophila* midgut.

727A The FERM domain protein Okapi limits follicle stem cell number by regulating Hippo signaling in Drosophila ovaries

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Follicle stem cells (FSCs) in the Drosophila ovary generate three somatic cell lineages that are essential for egg development, including the interfollicular stalk cells (IFS) that connect follicles. Here, we show that the FERM domain protein Okapi (Oka) has an important function in regulating the number of FSCs. Loss of Oka resulted in a strong increase in the number of FSC-like cells and IFS cells. Cell-type specific knock down of *oka* indicated that the FSC and IFS overgrowth phenotype is caused by a loss of Oka specifically in the FSC lineage and not in other cell types of the germarium. A similar FSC and IFS overgrowth phenotype was reported for a disruption of several signaling pathways, including the Hippo (Hpo) pathway, which limits nuclear translocation and activity of the transcriptional co-activator Yorkie (Yki). In *oka* mutants, we observed nuclear Yki in a substantially larger proportion of FSCs than in wild-type ovaries. Further, reduction of Yki or overexpression of Hpo strongly reduced the FSC overgrowth and caused formation of normal IFS in *oka* mutant ovaries. However, in contrast to *hpo* mutant FSCs, which are known to outcompete and displace wild-type FSCs in mosaic ovaries, *oka* mutant FSCs were lost over time in the presence of wild-type FSCs. Together, our results suggest that Oka regulates the number of FSCs and IFS cells by regulating Hpo/Yki signaling, and contributes to the competitiveness of FSCs through an unknown mechanism. As Oka associates with adherens junctions, we are investigating whether loss of Oka reduces competitiveness of FSCs due to defective adherens junctions as has previously been reported for the loss of DE-cadherin.

728B Transcriptomic analysis of feminizing somatic stem cells in the *Drosophila* testis reveal putative downstream effectors of the transcription factor Chinmo

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One of the best examples of sexual dimorphism is the development and function of the gonads, ovaries and testes, which produce sex-specific gametes, oocytes and spermatids, respectively. The development of these specialized germ cells requires sex-matched somatic support cells. The sexual identity of somatic gonadal cells is specified during development and must be actively maintained during adulthood. We previously showed that the transcription factor Chinmo is required to ensure the male sexual identity of somatic support cells in the *Drosophila melanogaster* testis. Loss of *chinmo* from male somatic gonadal cells results in feminization: they transform from squamous to epithelial-like cells that resemble somatic cells in the female gonad but fail to properly ensheath the male germline, causing infertility. To identify potential target genes of Chinmo, we purified somatic cells deficient for *chinmo* from the adult *Drosophila* testis and performed next-generation sequencing to compare their transcriptome to that of control somatic cells. Bioinformatics revealed 304 and 1,549 differentially upregulated and downregulated genes, respectively, upon loss of *chinmo* in early somatic cells. Using a combination of methods, we validated several differentially expressed genes, which will be presented in this poster.

729C Very low levels of specific Grainy head isoforms regulate *Drosophila* midgut intestinal stem cell differentiation

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The continuous regeneration of the adult *Drosophila* intestine is mediated by a population of multipotent intestinal stem cells (ISCs). In this tissue, mature cells are constantly lost and replaced by new cells produced by ISCs. This process is tightly regulated by both intrinsic and extrinsic factors. In this study, we sought to investigate how this process can be regulated by genes expressed at extremely low cellular levels, specifically the transcription factor *grainy head (grh). grh* is alternatively spliced to produce 8 mRNA transcripts, which, depending on the splicing of exons 4 and 5 produces two protein isoforms, Grh.N and Grh.O. In cell lineage tracing experiments, loss of both isoforms results in a mild reduction of progeny arising from a single ISC, however, loss of only the Grh.O isoform, which has previously been characterized as neural specific, results in more severe reduction of progeny numbers. Loss of Grh.O also resulted in a decreased ability to maintain ISCs. Ectopic expression of Grh.O in ISCs only did not result in a phenotype that differed from controls whereas expression in enteroblasts (EBs) caused ectopic proliferation and production of cells that contained markers of both ISCs and differentiated enterocytes. Converesly, GRH.N over expression resulted in the loss of the ISC and EB population. We hypothesize that Grh functions to maintain ISC numbers by balancing activity of Grh.O and Grh.N isoforms. *grh* is normally expressed at very low levels in the midgut and may interact with other regulators of gene expression expressed at low levels. We could suppress the pehnotype associated with ectopic expression of Grh.O by co-expressing another transcription factor also normally found at very low levels, Zfh-1. We also obtained suppression by reducing the activity of miR-8. Our data suggest that Grh functions at two stages of intestinal homeostasis to maintain the undifferrentiated sate of ISCs but also to promote differentiation.of EBs.

730A Control of the identity of intestinal stem cells by the Polycomb goup epigenetic regulators.

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The *Drosophila* intestine is a monolayer epithelium sheltering two types of differentiated cells (enterocytes, enteroendocrine cells) that derive from intestinal stem cells (ISC). These ISC provide a tissue regeneration capacity on a daily basis, as well as after external aggressions, making the *Drosophila* gut an excellent model to study stem cell behavior and regeneration processes.
Polycomb group (PcG) proteins are important epigenetic regulators of gene expression. Two main complexes, PRC1 and PRC2, are widely involved in gene silencing. Canonical PRC2 is recruited to the chromatin by Polycomb responsive elements and is responsible for the trimethylation of H3K27. In turn, PRC1 is recruited to depose the H2AK119ub mark, leading to repression of gene expression through chromatin compaction. However, PcG regulations are very dynamics and more complex, as highlighted by studies showing mechanisms that are independent of PRC2 or catalytic subunits. All the complexity and the large range of implication of PcG proteins in gene regulation is not well understood.
In *Drosophila melanogaster*, canonical PRC1 is composed by the core complex made of Posterior sex comb (Psc) and the catalytic subunit Sex comb extra (Sce), associated with the chromatin binding protein Polycomb (Pc), Sex comb on midleg (Scm), and Polyhomeotic (Ph). It was shown that Psc acts redundantly with its paralog Suppressor of zeste 2 (Su(z)2) to restrict follicle stem cell proliferation of the follicular stem cells in the ovary and of the cyst stem cells in the testis. It was also recently shown that PRC1 could act as tumor suppressor independltly of PRC2 in the eye discs.
Here, we study the function of *Psc* and *Su(z)2* in ISC. Using MARCM, we observed that their loss of function in ISC leads to neoplastic tumor formation. The neoplastic cells lose the stem cell markers and keep dividing without the capacity to generate differentiated cells. We are currently investigating the role of the other components of the PcG complexes, as well as the signaling pathways and gene regulatory networks controlled by Psc/Su(z)2 to block tumor formation. Our results will provide a

better understanding of the epigenetic mechanisms controlling the ISC identity in the stem cell niche of the *Drosophila* gut.

731B Sbf and Rab21 controls autophagic flux to modulate drosophila intestinal cell fate.

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The intestinal epithelium faces many stresses caused by food ingestion and its proximity to microorganisms among others, explaining its rapid renewal. Mechanisms, such as autophagy, can be induced by immune or environmental stressors to control gut homeostasis. Dysfunctional autophagy has been associated with inflammatory bowel pathologies. However, its specific roles in intestinal stem cell remain poorly investigated. Since this intracellular catabolic mechanism also plays a role in the maintenance of stemness or the differentiation of dermal and hematopoietic cells, we wondered if it may contribute to the renewal of the intestinal epithelium. To investigate this question, two new autophagy regulators were studied: the small GTPase Rab21 and the myotubularin-related protein Sbf. During starvation, Sbf activates Rab21 to induce the endosomal sorting of the lysosomal SNARE Vamp7, required for autophagosome-lysosome fusion. Moreover, Rab21 was recently identified in a genome-wide screen as a potential intestinal stem cell regulator. **Taken together, we hypothesized that a dysfunctional autophagy caused by the depletion of either *Sbf or Rab21* would disturb drosophila intestinal cell fate.**

Drosophila intestinal epithelium renews very quickly every 5-7 days. Intestinal stem cells (ISCs) divide into specific progenitors, either pre-enterocytes or pre-enteroendocrine cells that differentiate into nutrient-absorbent enterocytes or hormone-secretive enteroendocrine cells respectively. To inhibit autophagy, we used the thermosensitive Gal4-UASt system to express interfering RNA against *Sbf*, *Rab21* or *Vamp7* (positive-control) specifically in adult ISCs and/or progenitor cells, along with GFP to allow the quantification by confocal microscopy of depleted cells. Our results showed that autophagy inhibition specifically in ISCs or in the progenitor of enterocytes does not affect their number but surprisingly increases the proportion of enteroendocrine cells. Using the ReDDM (Repressible dual differential stability markers) lineage tracing method, we confirmed that the depletion of either *Sbf*, *Rab21* or *Vamp7* in ISCs and both progenitor cells increased the number of newly formed-enteroendocrine cells as well as progenitors of enteroendocrine cells, without disturbing the ratio between ISCs and progenitors.
Thus, **our data suggest that autophagy regulates the differentiation of intestinal cells into enteroendocrine cells.**

732C The tumor suppressors *merlin* and *expanded* integrate signaling inputs to regulate stem cell division in the *Drosophila* testis stem cell niche

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The ability of adult organisms to maintain tissue homeostasis is mediated by small groups of adult stem cells that support the tissues they reside in. Adult stem cells are regulated by signals from their microenvironment, the stem cell niche. One of the best-characterized model niches is that of the *Drosophila* testis, which contains two stem cell populations that cluster around a group of nondividing somatic cells called the hub. The germline stem cells (GSCs) produce cells that differentiate into sperm, while the somatic cyst stem cells (CySCs) produce cyst cells, which guide germline cell differentiation. Recently, contact inhibition, mediated by the tumor suppressor and human disease gene Merlin/NF2, was proposed as a mechanism that regulates proliferation of CySCs and their coordination with GSCs. In other *Drosophila* tissues, Merlin acts redundantly with the related tumor suppressor protein, Expanded. We investigated the role of these genes, and found that inhibition of either *merlin, expanded,* or both genes together in the cyst lineage resulted in an increase in the number of CySCs, a corresponding decrease in the number of GSCs, and inappropriate division of cyst lineage cells outside the niche. More severe phenotypes were observed in the double mutant, suggesting partial redundantly of these genes in this tissue. Surprisingly, expression of a nonphosphorylatable, constitutively active allele of *merlin* showed some similar phenotypes to the loss-of-function testes. We found that the *merlin* loss-of-function phenotype was associated with increased dpERK accumulation, whereas the gain-of-function phenotype was associated with decreases in both dpERK signaling and the differentiation-promoting Tor pathway. Our findings support a model in which the function of *merlin* and *expanded* is to balance and integrate multiple signaling inputs, allowing for dynamic regulation of signaling within the cyst lineage cells. This work was supported by NIH R15 GM102828 to J.L.

733A Experimental validation of downstream targets of two known master regulators of intestinal stem cells in the *Drosophila melanogaster* posterior midgut

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Many of our organs contain adult stem cells (ASCs) in charge of replenishing cells lost to damage, disease or normal tissue turnover. Like their embryonic counterparts, ASCs can divide asymmetrically, giving rise to a new copy of themselves (i.e. self-renewal) and a sister cell that commits to differentiation into a specific cell type.
Decades of research have led to the identification of so-called "master regulator" (MR) genes, i.e. pleiotropic genes whose loss or gain of function mutations simultaneously affect diverse aspects of normal ASC biology. Interestingly, genome-wide screens related to MR genes have identified hundreds of putative targets that could serve as their downstream effectors. Notably, the target sets for different MR genes in a given ASC type are usually non-overlapping, suggesting two non-mutually exclusive modes of regulation: 1) each MR gene acts through a unique group of effectors, which converge downstream in function; or 2) several MR genes co-regulate a common set of shared targets, which then execute the corresponding downstream regulatory functions.
To begin addressing this question, we used intestinal stem cells (ISCs) in the *D. melanogaster* posterior midgut. We first integrated genome-wide DNA mapping data for two known MR genes in these cells: the Snail family transcription factor Escargot (Esg) and the signal transducer protein STAT. The long-term goal of our project is to compare the relative contribution to ISC regulation by genes that are regulated by Esg only, STAT only, or both. However, we first needed to experimentally validate the *in silico* predictions of regulation. To this end, we carried out RT-qPCR analyses of gene expression in response to ISC-specific RNAi-mediated knockdowns of Esg, STAT or both. Our results thus far have shown that the bioinformatic predictions have a 50% false positive rate (i.e. half the genes predicted to be regulated by either MR did not significantly change in expression in response to the knockdowns), but only a 10% false negative rate (i.e. only 10% of the genes predicted to be regulated by neither showed a significant change in expression). More importantly, only rarely did the patterns of gene expression caused by the MR knockdowns match theoretical predictions, once again underscoring the deep prevalence of additional complexity layers that evade simple modeling based on DNA mapping data alone.

734B Regulation of blood cell transdifferentiation by oxygen sensing neurons and cGMP signaling

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Transdifferentiation generates specialized cell types independent of stem or progenitor cells. Despite the high interest in this unique phenomenon, it remains largely unknown how transdifferentiation is regulated in vivo and how it is linked to environmental sensing. We address these questions using a simple model of hematopoietic sites (hematopoietic pockets) in the *Drosophila* larva, which also serves as paradigm for blood cell transdifferentiation from macrophage-like plasmatocytes to crystal cells (Corcoran et al. bioRxiv 2020). Interestingly, we discovered that in this system, blood cell transdifferentiation is promoted by the activity of specific neurons in the caudal sensory cones; their ectopic activation by TrpA1 increases transdifferentiation, while their genetic ablation, or sensory neuron silencing via transient expression of Kir2.1, reduce transdifferentiation. We identified a positive role for environmental oxygen, the atypical guanylyl cyclase Gyc88E, and cGMP signaling in these neurons in the control of plasmatocyte-to-crystal cell transdifferentiation, while long-term hypoxia has negative effects. This conclusion is supported by evidence from crystal cell quantification and transdifferentiation under conditions of hypoxia, Gyc88E silencing, silencing and gain of function of cGMP-degrading phosphodiesterases, and chemical manipulation of cGMP levels including feeding of the cGMP boosting drug Viagra (sildenafil citrate). Downstream of cGMP, based on in vivo RNAi and chemical inhibitor injection, we discovered a key role for the cyclic nucleotide-gated ion channel subunits CngB and CngL in blood cell transdifferentiation. Emphasizing the biological significance of the process, we uncovered a functional connection between neuronally-driven crystal cell production and the immune response against infection with gram-positive bacteria. Overall, our findings reveal an unexpected link between environmental oxygen sensing and blood cell transdifferentiation, a principle that may be conserved across species and organ systems where environmental sensors and tissue precursors coincide.

735C Stress-responsive *miR-958* adjusts BMP signaling via *cabut* to regulate stem cell expansion in the Drosophila intestine

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Adult organisms must sense, respond, and adapt to environmental fluctuations. In high-turnover tissues such as intestine, these adaptive responses require rapid changes in gene expression that, in turn, likely involve post-transcriptional gene control. However, intestinal tissue specific microRNA mediated regulatory pathways remain unexplored. Here we report

the role of an intestinal specific microRNA, *miR-958*, that non-cell autonomously regulates stem cell numbers during tissue homeostasis and regeneration in the Drosophila adult midgut. We identify its downstream target *cabut*, the Drosophila ortholog of mammalian KLF10/11 transcription factors, which mediates this *miR-958* function by promoting paracrine enterocyte-to-stem cell BMP signaling. We also show that mature *miR-958* levels transiently decrease in response to stress, and that this decrease is required for proper stem cell expansion during tissue regeneration. In summary, we have identified a novel post-transcriptional mechanism that modulates BMP signaling activity within Drosophila adult intestinal tissue both during normal homeostasis as well as tissue regeneration to regulate intestinal stem cell numbers.

736A Microbes affect gut epithelial composition through immune-dependent regulation of intestinal stem cell differentiation

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A central problem in gut biology is understanding the complex and reciprocal interactions between a host and its microbiome. It is increasingly evident that while hosts influence microbial composition in the gut, gut microbes in turn can alter host physiology. Our results suggest that microbes not only influence the function of the midgut but alter its entire structure. We first demonstrate that microbiota and pathogens both influence intestinal stem cell (ISC) differentiation. Surprisingly, while microbiota push differentiation into enterocytes, pathogens stimulate entero-endocrine cell (EE) fate and long-term accumulation of EEs in the epithelium. We further uncover a new role for the Imd pathway in ISCs, where it modulates ISC differentiation and lineage. Progenitor specific transcriptomic and DamID analyses reveal that the evolutionarily conserved Drosophila NFKB modulates stem cell lineage by directly regulating differentiation factors. Furthermore, we found by functional genetics that the JAK/STAT pathway promotes opposite stem cell fate in response to epithelial damage. Altogether, we propose a model in which the balance of activity of pathways involved in microbial pattern (PAMP) recognition such as Imd and damage (DAMP) recognition such as JAK-STAT influences ISC differentiation, epithelial composition and gut physiology. Our work therefore identifies the cross talk between immune and developmental pathway in stem cells at the center of the communication network between a host and its microbiota.

737B The Mechanism of Niche to Stem Cell Conversion in the *Drosophila* Ovary

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Adult stem cells are maintained within specialized microenvironments, or niches, that are typically created by niche cells. These niche cells provide signals to stem cells within the niche to promote self-renewal and inhibit differentiation. The escort cells (ECs) located in the *Drosophila* ovary support the follicle stem cells (FSCs), and both reside at the anterior of the tissue in a structure called the germarium. Previous studies have identified several signals produced by ECs that are essential to maintain FSC fate. However, the cellular behavior of ECs and their relationship to FSCs in the adult ovary is not well understood. We recently generated a single-cell atlas of the *Drosophila* ovary that revealed three distinct populations of ECs: anterior ECs (aECs), central ECs (cECs), and posterior ECs (pECs). We used G-TRACE to determine the lineage of each of these EC populations and found that ECs generally only produced other ECs under standard laboratory conditions. In contrast, upon 24 hours of starvation, pECs, but not aECs or cECs, were capable of converting to FSCs and producing follicle cells. To investigate the mechanism by which ECs convert to FSCs, we performed a targeted RNAi screen of genes known to be involved in cell signaling and proliferation. We found that RNAi knockdown of either *bendless* (*ben*) which is an E2-ubquitin-conjugating enzyme known for its role in JNK signaling, or *Retinoblastoma* (*Rbf*), which regulates cell growth and proliferation, is sufficient to induce EC to FSC conversion in well-fed conditions. Our current studies are aimed at investigating how *ben* and *Rbf* regulate this process. In addition, we are working to further characterize how the number and cell cycle profile of the aEC, cEC, and pEC populations change in response to starvation. Collectively, these studies will provide new insight into the natural response of a stem cell niche to a physiological stress such as starvation.

738C The RNA Binding Protein SWM is required for *Drosophila* intestinal progenitor cell maintenance.

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The adult *Drosophila* intestinal epithelium hosts a heterogenous cell population including a pool of actively proliferating stem cells known as intestinal stem cells (ISCs). Being able to adapt to rapid environmental changes through tightly controlled programs of cell proliferation and differentiation, the adult intestine serves as an excellent model system to study stem cell mediated tissue homeostasis. Compared to the two differentiated intestinal cell types, known as enterocytes (ECs) and enteroendocrine cells (EEs), ISCs along with their immediate daughter cells, enteroblasts (EBs) maintain: (1) a high level of nascent protein synthesis, (2) a dense population of free ribosomes, and (3) microscopically visible cytoplasmic messenger-

ribonucleoprotein particles (mRNPs) including processing bodies. These observations strongly suggest that gene expression in ISCs and EBs, known collectively as intestinal progenitor cells, is tightly regulated at the posttranscriptional level. Although mRNA processing and nuclear export play vital roles in posttranscriptional gene regulation, how these processes are involved in maintaining proper mRNA regulation and translation in intestinal progenitors is not well understood. Resulting from a candidate RNAi screen of RNA processing and export factors, I found that depletion of the RNA binding protein Second mitotic Wave Missing (SWM) in intestinal progenitors lead to an accumulation of poly(A)+-RNA in nuclei, suggesting RNA processing or export was disrupted. Furthermore, RNAi-mediated knock down of *swm* resulted in a severe loss of progenitor cell but not differentiated cell types, indicating that SWM plays important roles specifically in progenitor cells. Progenitor cells are physically attached to the basement membrane and this attachment is crucial for proper progenitor cell function. Upon *swm* depletion, progenitors detached from the basement membrane, shifted towards the luminal side of the epithelium, failed to express ISC/EB markers, and lost stem cell characteristics such as proliferation. To characterize the molecular basis of these cellular phenotypes, I profiled the intestinal mRNA targets of SWM using CLIP-seq (crosslinking-immunoprecipitation sequencing). Interestingly, SWM associated with transcripts encoding proteins involved in epithelial cell adhesion and related processes. Based on these data, we hypothesize that SWM directly binds and regulates processing and/or export of mRNAs involved in epithelial cell adhesion and thereby maintains proper progenitor cell functions. Knowing these direct mRNA targets of SWM, future work will elucidate how SWM is involved in post transcriptional regulation of mRNA to maintain stem cell function.

739A Sphingolipid metabolism regulates intestinal stem cell homeostasis

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Lipid metabolism is an essential part of developmental and growth processes. Cellular processes including lineage determination of the stem cells, proliferation, as well as growth are regulated through lipid metabolic pathways during development. Sphingolipid metabolism regulates proliferation, growth, apoptosis, inflammation, senescence as well as tumorigenesis in adult mice. However, the molecular mechanisms involved in sphingolipid metabolism mediated intestinal stem cell (ISC) homeostasis and tumorigenesis are yet to be worked out. We do not know if the different by-products in the anabolic or catabolic processes of the ceramide have different functions in regulating ISC fates in the adult midgut. To check if any of the sphingolipid metabolic enzymes are involved in the ISC homeostasis, we have knocked down these enzymes within the progenitor cells. Our preliminary results indicate that key enzymes in ceramide synthesis process such as *schlank*, and *lace* are essential for ISC proliferation in the basal condition. However, increased levels of ceramide in the basal condition suppress ISC proliferation. Since EGFR signaling pathway is an important driver for ISC proliferation, we probed if the activated EGFR pathway would regulate the sphingolipid metabolic genes. Transcript analysis of the Spitz activated EGFR pathway using *RNAseq* shows that EGFR pathway can both upregulate and downregulate genes that are involved in the ceramide biosynthetic pathway. These results suggest that different enzymes and products of the sphingolipid metabolic pathway can act as signaling molecules and interact with major stem cell signaling pathways such as EGFR signaling to affect ISC homeostasis.

740B A spatiotemporally controlled establishment of asymmetric CENP-A at sister centromeres during cell cycle of stem cells

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Many stem cells utilize asymmetric cell division (ACD) to produce a self-renewed stem cell and a differentiating daughter cell. How non-genic information could be inherited differentially to establish distinct cell fates is not well understood. Here, we report a series of spatiotemporally regulated asymmetric components, which ensure biased sister chromatid attachment and segregation during ACD of Drosophila male germline stem cells (GSCs). First, sister centromeres are differentially enriched with proteins involved in centromere specification and kinetochore function. Second, temporally asymmetric microtubule activities and polarized nuclear envelope breakdown allow for the preferential recognition and attachment of microtubules to asymmetric sister kinetochores and sister centromeres. Abolishment of either the asymmetric sister centromeres or the asymmetric microtubule activities results in randomized sister chromatid segregation and stem cell defects. Together, these results provide the cellular basis for partitioning epigenetically distinct sister chromatids during stem cell ACDs.
However, how asymmetric sister centromeres are established with identical genetic sequence is not well understood. I performed Super-Resolution Chromatin Fiber (SRCF) assay to visualize newly replicated sister chromatids. I found that one sister chromatid inherited more CENP-A compared to the other, suggesting pre-existing (old) CENP-A is recycled asymmetrically

during DNA replication. Further, the inner-kinetochore protein CENP-C shows a similar asymmetric pattern, the sister chromatid with more CENP-A shows more CENP-C than the other sister chromatid, indicating a relay of asymmetry from centromere to kinetochore. I found that the CAL1 chaperone specific for CENP-A is required for both old CENP-A recycling during S phase and new CENP-A incorporation during S/G2 phase in male GSCs. Compromising CAL1 activity abolishes both CENP-A and CENP-C asymmetry and results in mis-determination of GSC and progenitor germ cell fate, resulting in both GSC loss and early-stage germline tumor phenotype. Together, these results show the molecular mechanisms underlying the establishment of asymmetric sister centromeres in GSCs and opens new directions to study these phenomena in other biological contexts.

741C A chromatin recognition mechanism separates endogenous and ectopic chromatins after induced cell fusion

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Cell-Cell fusion is a highly regulated biological event involved in important processes such as fertilization, placenta formation, muscle formation and bone homeostasis. Misregulation of cell-cell fusion has been proposed to be involved in the initiation and progression of cancer, but direct evidence in support of this theory is currently missing. In addition, the cellular consequences of cell-cell fusion are not understood. Here, we use the developing larval *Drosophila melanogaster* brain to define the cellular consequences of induced cell-cell fusion. We designed a protocol to induce fusions between fly neural stem cells (neuroblasts;Nbs) and adjacent differentiating ganglion mother cells (GMCs) with high temporal and spatial precision. We use live cell imaging to monitor and quantify spindle formation dynamics, chromatin alignment and segregation and cell fate decisions in hybrid cells. We identified that the endogenous (Nb) and ectopic (GMC) chromatins in Nb-GMC hybrid cells align but remain separate at the metaphase plate when the fusion is induced early in the Nb cell cycle. We reasoned a single spindle that directs both sets of chromatins or double spindles, one for each set of chromatins drives this metaphase alignment in the hybrid cell. We indeed observed double spindles in hybrid cells with the endogenous and ectopic chromatins alignment. These double spindles keep the endogenous and ectopic chromatins separate at least through anaphase onset. We hypothesized that the differences in kinetochore proteins account for this differential recognition of endogenous and ectopic chromatins by the respective spindles. While testing this hypothesis we have identified that the chromatins in Nbs stay close to the apical centrosome with the aid of microtubules (MT). We reasoned that this proximity of Nb chromatins to apical centrosome is key to for differential recognition and separation of ectopic and endogenous chromatins by respective spindles in a hybrid cell. We indeed identified that abolishing the microtubule organizing (MTOC) activity of Nb apical centrosome could result in premature mixing of the endogenous and ectopic chromatins before spindle nucleation. This further eliminates any separation between the two sets of chromatins as observed in WT hybrid cells. Finally, to test whether cell fusion can initiate cancer, we transplanted brains containing hybrid cells into wild type hosts but did neither detect tumor formation, nor reduced lifespan of the host flies. In conclusion, we report here that the endogenous and ectopic chromatins in the hybrid cell align at metaphase plate with the aid of double spindles and stay separate through anaphase onset by a spindle asymmetry mechanism.

742A *chinmo*-mutant germline stem cells cause biased inheritance by fencing off the niche and evicting their neighbors

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Stem cell competition is a selective process that determines stem cell quantity and quality during development and tumorigenesis. However, little is known about the genes and mechanisms that control this process. We find that *Drosophila* male germline stem cells (GSCs) homozygous mutant for *chinmo*, which encodes a putative transcriptional repressor, outcompete non-mutant neighbor GSCs for niche access. Eventually, this results in a testis whose GSC pool is comprised exclusively of *chinmo*-mutant cells. We demonstrate that *chinmo*-mutant GSCs evict non-mutant GSCs by ectopically secreting the heparin sulfate proteoglycan Perlecan (Pcan) into the niche milieu. This leads to the formation of an ectopic extracellular matrix (ECM) around the endogenous niche, which hinders normal GSC-niche interactions and causes a loss of non-mutant neighbor GSCs. Ectopic expression of Pcan by otherwise wild-type GSCs mimics the effects of *chinmo*-mutant GSCs. Removing *Pcan* from *chinmo*-mutant GSCs inhibits formation of the ectopic ECM and prevents non-mutant GSCs from leaving the niche. These data indicate that Pcan is necessary and sufficient for the competitive properties of *chinmo*-mutant GSCs.

How do *chinmo*-mutant GSCs stay in altered niche? We found that Pcan-interacting proteins Dystroglycan (Dg) and βPS integrin are increased at the interface between *chinmo*-mutant GSCs and niche cells. Depletion of either *Dg* or *βPS integrin* from *chinmo*-mutant GSCs reduces their adhesion to the niche and their competitive abilities. Moreover, supplying non-mutant neighbor GSCs with increased Dg allows them to stay in the altered niche.

Testes with *chinmo*-mutant GSCs frequently become monoclonal with all germline cells being *chinmo*-mutant. As a result, the *chinmo*-mutant allele should be inherited more often than the *chinmo* wild-type allele. Indeed, we show that offspring of aged males with *chinmo*-mutant GSCs inherit the *chinmo*-mutant allele at super-Mendelian frequencies (> 50%). While it is unlikely that *chinmo*-mutant GSCs will occur in nature due to essential developmental functions of Chinmo, any mutation that co-opted these competitive properties (Pcan secretion and Dg upregulation) could increase its representation in the next generation and cause gene drive.

743B Live characterization of *Drosophila melanogaster* female germline stem cells

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The germline stem cells (GSCs) present in the *Drosophila melanogaster* adult ovary have long been used as a suitable model to study stem cell (SC) behaviour inside its natural microenvironment or niche. GSCs generally divide asymmetrically, as they give rise to one daughter SC that remains in the niche and to a sister cell that enters oogenesis and differentiates into the oocyte and the nurse cell cluster. A large body of research using the fly ovary has allowed the deciphering of different processes that take place in the niche such as cell fate determination, division orientation, intercellular communication, etc. However, because of the long duration of a GSC's cell cycle (around 20 hours), some descriptions were limited by the difficulties of performing long-term ex vivo analysis of these processes. We have used a combination of genetic tools (such as FlyFUCCI and a myriad of fluorescent proteins) and an ex vivo culture protocol that permits filming live GSCs for long periods of time (at least 10 hours) to describe in detail several processes that occur during GSC proliferation. We will present our recent results that define the behaviour of the spectrosome - the membranous organelle that is essential for proper orientation and division of the GSCs – and its growth throughout the cell cycle, the correlation of cell cycle progression with changes in spectrosome morphology and the behaviour of centrosomes during GSC proliferation.

744C Childless Gambino: A novel regualtor of testis stem cell niche homeostasis

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Gondal stem cells are essential for sexual reproduction and homeostasis of reproductive tissue. For these stem cells to retain functionality, they must actively maintain their sexual identity and self-renew. We have identified the novel gene, Childless gambino (Chigno), as a binding partner of Chinmo, a gene which is necessary for sex maintenance and cyst stem cell (CySC) self-renewal in the *Drosophila* testis. To investigate Chigno's function, tissue-specific gene knockdown was performed using the Gal4-UAS system, and aspects of CySC behavior including self-renewal, differentiation, and sex maintenance were assayed. When Chigno function is reduced in somatic cells of the testes, there is an over-proliferation of CySCs, hub expansion, and aberrant sperm production which worsens with age. Additional analyses suggest that these changes arise because Chigno plays a critical role in regulating both CySC and hub cell identity. Chigno has a mammalian homolog, PIN2/TERF1, that is also expressed in testis. Therefore, gaining a better understanding of Chigno's function in regulating behavior of somatic cells in fly testes will provide valuable insight into human health issues including gonadal cancer and infertility.

745A Role of glial niche in regulating Neural Stem Cell fate

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Stem cell does not always function in isolation. They are surrounded by cells that serve as a niche and help them communicate with their surroundings. This cross-talk between signals intrinsic and extrinsic to stem cells is also essential in regulating its correct fate. Drosophila neural stem cells (NSCs) or NBs are popular models to understand how this communication occurs. In a screen we have identified several genes that non-autonomously influence the NSC homeostasis. During different stages of development, the NSCs can switch fates back and forth from proliferation to quiescence. We are exploring the extent to which the glia niche is involved in decision-making for various stem cell fates. We have found that knockdown of the candidate genes through RNAi affect the cortical glia network around the NSCs which is essential for NSC survival and is required for its proliferative potential. We are currently exploring several signaling pathways that could be pivotal in establishing the cross-talk between NBs and their microenvironement.

746B From spikes to intercellular waves: tuning intercellular Ca^{2+} signaling dynamics modulates organ size control

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Calcium (Ca^{2+}) signaling is a fundamental pathway for the propagation of information in eukaryotic cells. Recent experimental studies in developing *Drosophila* wing imaginal discs, a premier model for studying cell signaling during development, showed four different patterns of Ca^{2+} activity occurring on a tissue level. These include single cell Ca^{2+} spikes, intercellular calcium transients, propagating tissue-level Ca^{2+} waves, and a global "fluttering" state. However, the biophysical mechanisms that govern the dynamics and transitions between multicellular calcium signaling classes remain elusive. Here, we used a combination of computational modeling and experimental approaches to show that there are two different populations of cells in the wing disc pouch connected through gap junctional proteins. These include a small number of initiator cells and standby cells. Initiator cells are predicted to exhibit higher levels of Phospholipase C activity and produce more inositol trisphosphate, a key molecule that triggers the release of Ca^{2+} from the endoplasmic reticulum into the cytosol under uniform agonist stimulation. We show that the strength of hormonal stimulation and the fraction of initiator cells jointly determine the predominate class of Ca^{2+} signaling activity in a tissue. Further, single-cell Ca^{2+} spikes are stimulated by insulin signaling while intercellular calcium waves are dependent upon levels of Gq activity. Phenotypic analysis of perturbations to Gq signaling compared to insulin signaling supports Ca^{2+} signaling as a readout of the growth state of a tissue, and perturbations impacting calcium signaling tune the final size of organs.

747C Elucidating cellular contributions to tau-mediated neurodegeneration using *Drosophila* and single cell transcriptomics

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Alzheimer's Dementia (AD) is neuropathologically defined by aggregations of amyloid-beta plaques and tau neurofibrillary tangles in the brain, with tau pathology being most strongly correlated with cognitive decline. Systems biology approaches profiling post-mortem human AD brain tissue have highlighted involvement of key processes such as innate immunity and translational regulation. However, interpretation of cross-sectional post-mortem gene expression data is confounded by co-morbidities and mixed brain pathologies at autopsy. Moreover, characterizing longitudinal, age-related changes in Alzheimer's dementia (AD) is of particular importance because clinical manifestation of AD is a decades-long process, and age is the strongest known risk factor. We previously completed RNA-sequencing and proteomics of several highly tractable, longitudinal, *Drosophila* models of tauopathy to characterize tau- and age-specific perturbations. However, cellular heterogeneity confounds interpretation of bulk tissue data. We thus leverage single cell RNA sequencing (scRNA-seq) to explore cell-specific contributions to tau- and age-mediated gene expression. Whole brains of adult *Drosophila* pan-neuronally expressing a mutant variant of human Tau (R406W) and controls were dissociated for scRNA-seq (10x Genomics Chromium) at 1-, 10-, and 20- days of age. Perturbations in cellular composition and gene expression were quantified. To address cross-species conservation of tau-mediated scRNAseq signatures, we leverage existing human AD brain scRNA-seq data. As expected, we highlight evidence for tau-mediated degeneration of Kenyon cells, a neuronal population in the Drosophila "mushroom body" that is required for learning and memory, along with a concomitant increase in glial cell numbers (e.g. gliosis). Gene set enrichment analysis reveal overrepresentation of acetylcholine receptor regulation pathways in vulnerable mushroom body neurons. Interestingly, regularized regression analyses highlight innate immune activity as predictive of tau-related neuronal reduction. Cluster-specific transcriptional signatures between flies and humans demonstrate strong correlations among inferred cell identities. Our results comprise a powerful single cell transcriptomic resource for studying tau-mediated disruption of gene expression and dynamic age-dependent changes at cellular resolution in a tractable model system that is amenable for high throughput functional validation.

748A New genetic tools for functional studies of paralogs and other redundant genes

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When multiple genes have redundant or partially overlapping functions, individual loss-of-function studies of such genes typically fail to reveal a phenotype. Here, we describe a growing collection of transgenic *Drosophila* lines for performing pairwise double-knockout experiments using multiplex CRISPR, as well as a strategy for characterizing the co-expression of gene pairs using knock-in split-Gal4 reporters, and an online tool for identifying paralogs of any gene-of-interest, called Paralog Explorer. Our initial transgenic collections consist of sgRNA-expressing fly lines targeting pairs of paralogous ligands for various signaling pathways, paralogous kinase/phosphatases, uncharacterized paralogous gene pairs with human orthologs,

and paralogs for which null mutations have been observed segregating in wild-caught *Drosophila* populations. The fly stocks and online resources generated as part of this project will support studies that have the potential to shed new light on gene function and redundancy in *Drosophila*.

749B New methods towards large-scale genetic interaction screens in *Drosophila* cells

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A genetic interaction occurs when the simultaneous perturbation of two genes produces a phenotype that is not predictable based on the individual perturbation of each gene alone. Analysis of genetic interactions in organisms such as yeast and *Drosophila* has been transformative to our understanding of how cells and organisms function. For example, by allowing the assembly of signalling pathway models or mapping the mechanisms underlying the cell cycle. Furthermore, genetic interaction screens are often used to identify new drug-targets for the treatment of human diseases. Unfortunately, it is not currently possible to perform genome-wide genetic interaction screens because of the large number of pairwise gene pairs to be tested (approximately 100 million in the *Drosophila* genome). This limits our ability to exploit this powerful approach in *Drosophila* cells.

Our research is aimed at developing new screening methods to allow unbiased, genome-scale genetic interaction screens in *Drosophila* cells. Here, we will describe two new screening assays designed to increase the scale at which genetic interaction screens can be performed. The new assays build on the Variable Dose Analysis (VDA) method that we developed in 2017. The VDA method uses RNAi to generate a gradient of expression of the target gene across a population of cells and assesses how phenotypes change with expression level. This leads to a dose curve readout for each target gene screened, improving detection of genetic interactions by increasing signal to noise ratio and allowing analysis of essential genes. We have already applied this method to find effective drugs to kill Tuberous Sclerosis Complex tumour cells as well as to screen for synergistic drug combinations. Using an interdisciplinary approach combining automated, miniaturised liquid handling, machine learning and improved vector design, we developed two new variants of the VDA method. The first, called high-throughput VDA (htVDA) allows screens to be performed with four-fold increased speed and 10-fold reduced reagent requirements while maintaining high data quality. The second variant, called VDA-plus, enhances data quality by approximately 12-fold compared to standard RNAi assays, allowing much more sensitive screens to be performed.

These new methods allow us to perform more sensitive and larger scale genetic interaction screens, bringing us closer to our goal of unbiased genetic interaction analysis.

750C A novel image analysis pipeline, MAPPER, unmasks differential regulation of *Drosophila* wing features

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Phenomics requires quantification of large volumes of image data, necessitating high throughput image processing approaches. Existing image processing pipelines for *Drosophila* wings, a profound model for studying morphogenesis, are limited in speed, versatility, and precision. To overcome these limitations, we developed MAPPER, a fully-automated machine learning based pipeline that quantifies high dimensional phenotypic signatures, with each dimension representing a unique morphological feature of *Drosophila* wings. MAPPER magnifies the power of *Drosophila* genetics by rapidly identifying subtle phenotypic differences over ample images from varying magnifications and imaging orientations. To demonstrate its widespread utility, we used MAPPER to reveal new insights connecting patterning and growth across *Drosophila* genotypes and species. The morphological features extracted using MAPPER identified the presence of a uniform scaling of proximal-distal axis length across four different species of *Drosophila*. Observation of morphological features extracted by MAPPER from *Drosophila* wings from varying insulin signaling pathway perturbations revealed the presence of a morphogen gradient across the wing blade. Additionally, the batch processing of samples with MAPPER revealed a key function for the mechanosensitive Ca^{2+} channel, Piezo, in regulating bilateral symmetry and robust organ growth. Further, the computational platform MAPPER is built on and its open-source nature allows it to be used as a tool for all researchers requiring rapid analysis of large volumes of imaging data. Overall, MAPPER provides new capabilities to rigorously and systematically identify genotype-to-phenotype relationships in an automated, high throughput fashion.

751A Development of a fly model to probe the functions of inorganic polyphosphates

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Inorganic polyphosphate (polyP), which consists of chains of orthophosphate residues of varying chain lengths, is found in all living organisms alongside other phosphate-rich biomolecules such as ATP and inositol phosphates. The biological functions of polyP have been extensively studied in prokaryotes and unicellular eukaryotes, however, their functions in metazoans are largely underexplored. In fact, polyP has been referred to as a molecular fossil. In recent years polyP has been shown to be implicated in blood clotting and bone mineralisation in mammals. Moreover, several functions of polyP are predicted due to its presence in various cell types and its specific binding with numerous proteins. However, the major limitation in testing the functions of polyP in metazoans is the lack of knowledge of the genes involved in polyP synthesis and turnover, which restricts the modulation of polyP levels in vivo. We have developed a Drosophila model to study the functions of polyP. Here we show that polyP exists in flies and that its levels are developmentally regulated during embryogenesis. Further, through phylogenetic analysis, we identified Prune as a putative exopolyphosphatase. Indeed, we observed significantly higher levels of polyP in prune mutants as compared to wildtype flies. Prune is a mitochondrial protein known to hydrolyze cAMP. Mutations in *prune* are linked to a variety of processes such as mitochondrial biogenesis, eye pigmentation and neurodegeneration. Based on our data, we surmise that prune mediated regulation of polyP may attribute to some of the prune phenotypes and will help understand polyP biology in metazoans**.**

752B Gene Disruption Project (GDP) update: using in vitro synthesized homology donor constructs for CRISPR mediated homologous recombination

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Despite increasing knowledge about genomes and genetic variation, experimental knowledge about gene function is still limited. Model organisms offer numerous paradigms to bridge this knowledge gap and Drosophila offers the strongest genetic tool set to study gene function. The Drosophila Gene Disruption Project (GDP) aims to provide genetic tools to study the function of most genes that are conserved between human and fly. Currently, the GDP uses CRISPR mediated homologous recombination to integrate Swappable Integration Cassettes (SIC) in the introns of genes (CRIMIC). SICs typically are comprised of a mutagenic region (e.g. Splice Acceptor-T2A-GAL4-polyA) and a dominant marker (e.g. 3xP3-GFP) flanked by attP sites for Recombinase Mediated Cassette Exchange (RMCE). This generates a severe loss of function allele, while expressing a GAL4 with the expression pattern of the targeted gene. To date, the GDP has integrated SICs in introns of more than 2,000 genes and generated RMCE cassettes to convert these SICs into diverse genetic tools. In addition, we are generating a comprehensive UAS-human cDNA transgenic fly library to facilitate gene function studies. These UAS-human cDNA transgenes can be driven by T2A-GAL4 alleles that we create to rescue associated phenotypes, replacing the fly gene with the orthologous human gene thus “humanizing” the fly.
To function as gene and protein traps, SICs with a splice acceptor must be inserted in an intron between two coding exons. About 50% of the Drosophila genes either do not have an intron or their coding introns are too small (< ~100 bp) to allow efficient splicing into the SIC. We developed methods to replace the coding region of these genes with a Kozak-GAL4-3XP3GFP cassette generating a null allele, while expressing GAL4 from the endogenous locus.
Our previous CRIMIC method used large homology arms (>1kb) to the targeted locus to integrate SICs. More recently, we showed that in vivo linearization of the donor constructs enables incorporation of large SICs using short homology arms (100-200 bps). Shorter homology arms make it feasible to commercially synthesize homology donors and minimize the cloning steps in donor construct generation. In addition, synthesis of custom plasmid backbones that contain the gRNAs in the backbone obviates the need to inject a separate plasmid encoding the gRNA thus increasing the transgenesis rate. These upgrades will enable efficient targeting of nearly 100% of conserved fly genes.

753C REDfly: The Regulatory Element Database for Drosophila and other insects

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The REDfly database provides a comprehensive curation of experimentally-validated *cis*-regulatory modules (CRMs, "enhancers") and transcription factor binding sites (TFBSs) for *Drosophila* and other insects. The database, which contains data on more than 43,000 regulatory sequences, seeks to include all functionally tested sequences, both with and without observable regulatory activity, so that all experimental data are available for exploration. These data have numerous uses ranging from detailing the regulatory structure of a single locus to large-scale studies of the regulatory genome to providing training and/or validation data for machine-learning analyses of gene regulation. A key REDfly feature is extensive expression pattern annotation for each CRM's activity using structured anatomy ontologies, which allows for detailed searching of the data at varying levels of granularity. The growth in regulatory sequence deletion experiments, for example by using CRISPR technology, has led to an increase in sequences tested for regulatory necessity, as compared to the sufficiency studies provided by traditional reporter gene experiments. Our new "CRM_segment" data category now better handles data from such experiments. New this year has been the incorporation of additional insect species, beginning with the malaria mosquitoes *Anopheles gambiae* and *Aedes aegypti*, and the beetle *Tribolium castaneum*. More species, including additional *Drosophila*, will continue to be added throughout the year. Future plans include updating REDfly's search and download features to better serve our users, improved JBrowse integration of our data, and curation of silencers in addition to enhancers. REDfly is freely accessible at http://redfly.ccr.buffalo.edu and can be followed on Twitter at @REDfly_database.

754A FlyMet: an online metabolomics atlas and resource for *Drosophila*

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An organism's metabolome contains all the compounds that are produced by its metabolism. In all multicellular organisms, the metabolomes of different tissues are likely to differ significantly, reflecting the different specialized jobs they perform. Using *Drosophila* as a model, we have produced an atlas of 19 reference tissue metabolomes, obtained by separately micro-dissection of adult (male and female) and third instar larval *Drosophila melanogaster*. Using liquid chromatography-mass spectrometry (LC-MS), a snapshot of each tissue's metabolome was taken in the form of a list of LC-MS peaks, with each peak concisely represented as a tuple of mass-to-charge (m/z), retention time (RT) and intensity values (I). To present this complex data, we have developed FlyMet (www.flymet.org): a database and Web application that provides user-friendly visualization of metabolite profiles across *Drosophila* tissues. Additionally, as a part of the FlyMet project, we will use metabolomics to help characterize putative enzymes and add phenotypic information to uncharacterized genes.

755B Generation of *Drosophila* attP cell lines using CRISPR-Cas9

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The generation of *Drosophila* stable cell lines have become invaluable for complementing *in vivo* experiments and as tools for genetic screens. Recent advances utilizing attP/PhiC31 integrase system has permitted the creation of *Drosophila* cells in which recombination mediated cassette exchange (RMCE) can be utilized to generate stably integrated transgenic cell lines that contain a single copy of the transgene at the desired locus. Nonetheless, the technique, besides laborious and the inclusion of extraneous elements, is limited to a handful of cell lines of embryonic origin, Kc167, Sg4, Ras-attP-L1 and Ras-attP-L2. Nonetheless, with well over 100 characterized *Drosophila* cell lines available, including an ever-increasing number CRISPR/Cas9 modified cell lines, a more universal methodology is needed to generate a stably integrated transgenic line from any one of the available *Drosophila* cell lines. Here we describe a toolkit and procedure that combines CRISPR/Cas9 and the PhiC31 integrase system to achieve this possibility. Utilizing CRISPR/Cas9 we generated and isolated single cell clones containing an Actin5C::dsRed flanked by attP sites into the cellular genome of Kc167 and S2R+ cell lines that mimic the *in vivo* attP sites located at 25C6 and 99F8 of the *Drosophila* genome. Furthermore, we utilized RMCE to test the functionality of our inserts utilizing two independent GFP expressing constructs flanked by attB sites that permit RMCE and insert any genetic construct of interest. Lastly, to demonstrate the universality of our methodology and existing constructs, we have successfully integrated Actin5C::dsRed flanked by attP sites into two different imaginal disc cell lines, DmBG2-c2 and DmBG3-c2. Overall, the reagents and methodology reported here permit the efficient generation of stable transgenic cassettes with minimal change in the cellular genomes in existing *Drosophila* cell lines.

756C Investigating the relationship between central and peripheral circadian clocks with novel tissue-specific, GAL4-inducible *period* gene reporter lines

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Daily rhythms in physiology and behavior are produced by a circadian system consisting of central clock cells located in the brain and peripheral clock cells located in various peripheral tissues. Clock cells track time of day through the presence of a molecular clock that functions as a transcriptional-translational feedback loop in which the transcriptional activators CLOCK (CLK) and CYCLE (CYC) drive expression of the *period* (per) and *timeless* (*tim*) genes, and PER and TIM proteins in turn inhibit CLK/CYC activity. Molecular clocks must be coordinated across tissues, but the manner through which central and peripheral clocks communicate with one another to achieve this coordination is not well understood. We reasoned that the ability to track clock gene expression in specific tissues of living flies would facilitate an investigation into the relationship between different clock-containing tissues. Previous efforts to accomplish this in *Drosophila* have relied on reporter constructs in which regulatory elements of clock genes have been used to dictate expression of a luciferase reporter enzyme, the activity of which can be monitored using a luminometer. Although these reporter lines have been instrumental in our understanding of the circadian system, they generally lack cell specificity, making it difficult to compare molecular clock oscillations between different tissues. Here we report the generation of several novel lines of flies that allow for GAL4-inducible expression of a luciferase reporter construct for the *period* gene. We have expressed these reporter constructs selectively in different tissues, including the brain and fat body, to assess the presence and relative strength of *per* gene oscillatory rhythms. By comparing *per* expression patterns in central and peripheral clock tissues in the presence and absence of environmental light-dark cues, we will shed light on how molecular clocks are synchronized by light cues and by communication between clock-containing tissues.

757A Resources and services at the Vienna Drosophila Resource Center (VDRC)

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The Vienna Drosophila Resource Center (VDRC, www.vdrc.at), part of the Vienna Biocenter Core Facilities, is a professionally organized, non-profit bioresource center which aims to promote scientific discoveries in Drosophila. We primarily maintain unique transgenic Drosophila melanogaster stocks and DNA resources and distribute them both locally and worldwide. We additionally aim to acquire, create and develop new VDRC resources and services, according to the emerging new technologies and needs of the international Drosophila research community.
The VDRC is Europe's main Drosophila resource center. Since 2007 we have distributed more than 1.3 million stocks to nearly 3,000 registered users in 52 countries. We currently maintain over 25,000 unique transgenic fly stocks and nearly 14,000 DNA plasmids.
Our RNA interference (RNAi) lines, collectively covering over 91% of annotated protein coding genes, remain our core focus. In combination with the appropriate GAL4 driver lines, this unique resource enables large scale gene knockdown screens, making it possible to carry out loss-of-function experiments in essentially any tissue or cell at any developmental stage from embryo to adult. To facilitate RNAi screens, we offer the opportunity for researchers to perform their screens directly in our facility or to make use of our screening service.
Further stock collections include (1) the Heidelberg CFD sgRNA library (HD-CFD) for generating CRISPR-mediated loss-of-function mutations in a germline restricted, ubiquitous or tissue specific fashion; (2) Vienna Tiles (VT) enhancer-GAL4 driver lines, typically used to drive restricted expression of UAS lines; (3) Tagged FlyFos TransgeneOme (fTRG) library - proteins engineered with multi-epitope tags at the C-terminus, for use in a variety of downstream applications.
We additionally provide fly extract for cell culture, fly food and stock keeping services and are actively seeking donations of stocks/resources that are likely to be of broad future interest to the Drosophila community. For discussions about our resources and services, please contact office@vdrc.at.

758B Efficient Cas9 and Cas12a-based resources for tissue-specific CRISPR mutagenesis in Drosophila

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CRISPR/Cas gene editing allows for targeted mutagenesis with unprecedented ease. We are currently generating and optimising large-scale resources for tissue-specific CRISPR gene editing in Drosophila. To facilitate conditional gene disruption mediated by the widely used Cas9 nuclease we have generated a large-scale sgRNA library targeting over 2000 genes. In order

to prevent artefacts commonly associated with excessive amounts of Cas9 protein, we also developed a series of novel UAS-Cas9 transgenes, which allow fine tuning of Cas9 expression to achieve high gene editing activity with significantly reduced toxicity. In addition, we have established an orthogonal gene editing system based on the RNA-guided endonuclease Cas12a. Cas12a can directly utilize compact crRNA arrays that are substantially easier to construct than Cas9 sgRNA arrays, facilitating multiplex genome engineering. We are currently further optimising and expanding these tools and will discuss our current progress.

759C Generation of CRISPR based genome engineering resource to uncover principles of cellular organization and tissue architecture by lipid signaling

Deepti Trivedi[1], Vinitha CM[1], Vishnu Janardan[1], Raghu Padinjat[1] 1) National Centre for Biological Sciences.

Drosophila is a highly attractive eukaryotic model organism used to understand the functions of gene. Its adaptability from earlier approaches of gene modification techniques like mutations, insertions and deletions to recent genome engineering technologies used to precisely target a gene has made the fly a sought-after model organism in the modern biology era. Over past decade our facility (Fly Facility, NCBS, India) has provided services, including a reliable in-house fly stock maintenance service, microinjection facility to generate transgene insertions, and CRISPR based genome engineering services. In a collaborative project with Prof Raghu Padinjat›s lab, the facility has generated CRISPR based resources to target a set of genes involved in phosphoinositide (PI) signaling.

Since PIs are key regulators of cellular organization in eukaryotes, genes that tune PI signaling are implicated in many human disease mechanisms including cancer and diabetes. Biochemical analyses and studies in cultured cells have identified a large number of proteins that can mediate PI signaling. Here, we describe a set of CRISPR-based genome engineering tools that allow the manipulation of each of these proteins with spatial and temporal control during metazoan development. We demonstrate the use of these reagents to deplete a set of 103 proteins individually in the *Drosophila* eye and identify several new molecules that control eye development. Our work demonstrates the power of this resource in uncovering the molecular basis of tissue homeostasis.

Our facility provides CRISPR based gene editing to enable precise genome editing in addition to genetic screening services and we are interested to interact with scientists who would be interested to use our services for the same.

760A Protein manipulation in *Drosophila* using nanobodies that recognize short peptide tags

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Nanobodies have emerged as powerful protein-binding tools that can uncover function. Using functionalized protein binders, proteins of interest can be visualized, degraded, delocalized, or post-translationally modified *in vivo*. Here, we report the use of two short peptide tags, 127D01-tag and VHH05-tag, and the corresponding nanobodies, 127D01-Nband VHH05-Nb, for *in vitro* and *in vivo* studies in *Drosophila*. In S2R+ *Drosophila* cells, proteins labeled with the 10 aa 127D01-tag or 15 aa VHH05-tag could be visualized and monitored in their native surroundings using fluorescent protein-labeled nanobodies. These two tags and their nanobodies also worked well in S2R+ cells as primary antibodies for western blots and co-immunoprecipitation. Expression of the nanobodies in vivo was non-toxic and was effective at detecting various 127D01-tag or VHH05-tag tagged cytoplasmic or secreted proteins. Furthermore, we also showed that these two tag/Nanobody systems can be used for protein trapping and for E2 ubiquitin-conjugating enzyme-based protein degradation. Our data indicate that these two short peptide tags and their nanobodies systems can be used for labelling and manipulating proteins, and thus will be useful tools for protein functional studies in *Drosophila*.

761B Expanding the toolkit for dual control of gene expression

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Having tools and resources for independent control of gene expression in two different tissues in the same animal is emerging as a major need. This type of study is made possible by technologies combining the GAL4/UAS and a second binary expression

system such as LexA/lexAop or QF/QUAS. Here we describe the efforts of the Transgenic RNAi Project (TRiP) to fill the gap in reagents for combinatorial studies by building reagents that facilitate combined use of the GAL4/UAS and a second binary systems in various tissues. Focusing on genes with well-characterized tissue-specific GAL4 expression patterns, we are generating a set of LexA-GAD and QF2 insertions by CRISPR knock-in. We have also built constructs that encode QF2 and LexA-GAD transcription factors in a single vector, with each coding sequence flanked by FRT sites. Following successful integration into the fly genome, the vector expresses both drivers in the same pattern. If desired, one of the two coding regions can then be excised with Flp, resulting in flies that express only QF2 or LexA-GAD. We have initiated evaluation of these systems for in vivo gene knockdown and will generate a compatible library of shRNA lines targeting conserved signaling pathways as a community resource. Together, these QF2/LexA-GAD and QUAS/lexAop vectors and fly lines will provide a new set of tools for researchers who need to activate or repress two different genes in an orthogonal manner in the same animal.

762C Investigating *robo1* and *robo2* expression in a *Drosophila* injury model

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To 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. Our objective is to assess gene expression changes due to trauma in the ventral nerve cord of *Drosophila* embryos. Cellular trauma and the severing of critical neural connections affects biological processes such as the formation and maintenance of axonal trajectories and synaptic connections, and cell cycle regulation of neuronal subtypes. We severed the ventral nerve cords of late-stage embryos using a fine glass needle and analyzed the expression changes using RNAseq. Our data from injury and mock samples showed that 702 genes are significantly altered in our injury model. We found that *robo3* expression is upregulated. The Roundabout receptor family contributes to axon guidance, among other developmental processes. Our objective is to use qPCR analysis to determine if expression of *robo1* and *robo2* is also altered upon injury. Using the monoclonal antibody against Robo (13C9) we can also monitor the effect of injury on a subset of the axons in the CNS. Preliminary analysis allows us to detect injured nerve cords, but it is difficult to visualize the extent of the injury using epifluorescence. We plan to use confocal microscopy to allow us to better visualize the injury. We will also test the effect of varying the time between the injury and harvesting RNA for qPCR analysis or preparation of embryos for cellular analysis. This will allow us to determine if recovery time after trauma has an effect on RNA and protein expression of the *robo* genes.

763A Mechanisms for culling of cells with DNA damage after exposure to ionizing radiation in *Drosophila* wing discs

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Ionizing radiation (IR) can induce cell death which is useful in treatment of cancer where it is employed to treat approximately half of patients. Irradiation of cells results in DNA damage and events such as loss of heterozygosity (LOH). However, undesirable effects such as oncogenesis may be promoted by the functional loss of a tumor suppressor due to LOH. To track LOH throughout development we used a cell-autonomous fluorescence-based system and focused on studying the larval wing disc. The fluorescence system uses the QF/QS transcriptional module to detect LOH, which may be used in larval, pupal and adult stages and in parallel with the GAL4/UAS system to express RNAi against genes of interest. These studies identified two distinct phases of LOH cell culling and p53-dependent as well as p53-independent mechanisms (Brown et al., PLoS Genetics, 2020, PMID: 33075096). Currently, we are using the QF/QS system to perform an RNAi screen where engrailed>GAL4 controls the expression of RNAi. This allows us to compartmentalize RNAi to the posterior half of the wing disc and to compare its effect on LOH cell culling to contralateral anterior half as control. We are interrogating genes involved in DNA repair, cell cycle checkpoints, Apoptosis-induced-Proliferation, JNK and p53 signaling, and cell competition. The results of these studies will be presented.

764B Drosophila TNFRs, Grindelwald and Wengen, form high and low affinity hexamers with Eiger/TNF and promote distinct cellular functions

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The *Drosophila* tumour necrosis factor (TNF) ligand-receptor system consists of a unique ligand, Eiger (Egr), and two receptors, Grindelwald (Grnd) and Wengen (Wgn), and therefore provides a simple system for exploring the interplay between ligand and receptors, and the requirement for Grnd and Wgn in TNF/Egr-mediated processes. We here report the crystallographic structure of the extracellular domain (ECD) of Grnd in complex with Egr, a high-affinity hetero-hexameric assembly reminiscent of human TNF:TNFR complexes. We show that ectopic expression of Egr results in internalisation of Egr:Grnd complexes in vesicles, a step preceding and strictly required for Egr-induced apoptosis. We further demonstrate that Wgn binds Egr with much reduced affinity and is localised in intracellular vesicles that are distinct from those containing Egr:Grnd complexes. Altogether, our data provide insight into ligand-mediated activation of Grnd and suggest that distinct affinities of TNF ligands for their receptors promotes different and non-redundant cellular functions.

765C A photo-switchable dendrite degeneration and repair model in *Drosophila melanogaster*

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Neurodegeneration occurs when the nervous system is injured, aging, or in a disease state. It leaves survivors with permanent disabilities and is a huge burden on society. While much has been learned about how to improve neuronal survival and axon regeneration, what happened in the dendrite, the hub receiving and processing information for neurons, remains largely unknown. Here, we seek to elucidate the underlying mechanisms of dendrite degeneration and repair in *Drosophila* peripheral nervous system using light-activated caspase-3 (Smart et al., 2017) to injure neurons in contrast to using laser to sever dendrites as in previous studies. With the light-activated caspase-3, we can control the degree of damage by adjusting the intensity and the duration of the light. We characterized the caspase-3 induced dendrite degeneration and repair in different classes of da neurons and investigated the effects of mouse Wallerian degeneration slow (Wld^S), dSarm1 and Axed in the caspase-3 induced degeneration and repair at both morphological and functional levels. We found that neurons with Wld^S maintain more and longer dendrites from the caspase-3 induced dendrite degeneration. Moreover, Wld^S can improve the recovery in the impaired thermal nociceptive behavior induced by chronic low caspase-3 activation. Knockdown of dSarm1 preserves more dendrite structure, while knockdown of Axed does not change the dendrite structure following the caspase-3 induced dendrite degeneration. Both dSarm1 and Axed are required for the thermal nociceptive behavior in uninjured animals and knockdown either of them does not improve the functional impairment induced by chronic low caspase-3 activation. Altogether, our work provides a new model to study dendrite degeneration and repair. With which, we were able to uncover the roles of Wld^S, dSarm1 and Axed in dendrite degeneration and repair.

766A Drosophila as a model to elucidate cell type-specific viral envelope protein trafficking pathways

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Arbovirus transmission by insects requires that viruses ingested by insect vectors transit in a highly regulated manner in the gut and salivary gland. Arboviruses initially require apical-to-basal transit through gut cells, but then require basal-to-apical transit through salivary gland cells to enable vectoring into non-insect hosts. Strikingly, we found that the inversely oriented transit through these two key tissues is reflected by the trafficking of viral envelope proteins (baculovirus GP64 and the arbovirus vesicular stomatitis virus G) in transgenic flies expressing only GP64 or G outside the context of virus infection. This suggests that viral envelope proteins exploit the complexity of host trafficking pathways to facilitate virus egress from these different cell types in the appropriate orientation. This also implies that there are inherent differences in the repertoire or function of vesicular trafficking factors between gut and salivary gland cells. In order to identify cellular trafficking factors involved, we are using both a cell-based RNAi screen and *in vivo* manipulation. We first performed a targeted *in vitro* RNAi screen of 250 genes known to be involved in various aspects of vesicular trafficking. Using a stable Drosophila cell line that conditionally expresses the baculovirus envelope protein GP64, we identified 12 cellular genes (Rab GTPases, Rab effectors, clathrin adaptors and SNAREs) that were important for GP64 transport and display on the cell surface. In order to investigate viral envelope protein trafficking in polarized gut and salivary gland cells, we have also established a cellular map of all Rab proteins in these two tissues and we are assessing the role of genes discovered from our *in vitro* screen by analysis of gut and salivary glands of transgenic flies (conditionally) expressing GP64 or G.

767 Larry Sandler Award Lecture

768 The metaphysical dilemma: Academic Black women

Janelle Williams[1] 1) Widener University

Daughter, sister, wife, mother, these titles are typically attributed to women. Academic, researcher, doctor, professor, scholar, these titles are typically attributed to men. African-American, Black, Black American, Colored and Negro are terms used to describe Americans in the Black (socially constructed) racial group. What though, if you identify with all of the descriptors? According to author Ntozake Shange, to be Black, a woman and an academic is a metaphysical dilemma, which is described as the reality beyond what is perceptible to the senses. This corollary suggests that Black women who are academics deviate from what is standard. Which begs the question, are Black women an anomaly in academia? If so, is it realistic for a Black woman to aspire to be an academic in America?

Statistics say no. According to a **2016** study released by the National Center for Education Statistics, Black women held just **three** percent or 45,000 of the 1.5 million faculty positions in degree-granting postsecondary institutions. Seventy-six percent of the faculty positions including professors, associate professors, assistant professors, instructors, lecturers, adjunct professors and interim professors are held by White men (41 percent) and White women (35 percent). While we cannot speak for the entire three percent of Black women in faculty positions, as Black women who identify as academics, we can confidently say that academic aspirations are, will, and should continue to be realistic. Conversely, we would be remiss if we did not acknowledge that such aspirations, unfortunately come with institutional racism, marginalization, misogyny, scrutiny, and vulnerability.

Understanding the dilemma set before us, informally, Black women are often asked – How have you managed to navigate your roles, embrace your identity, and push past the obstacles (both real and imagined)? The short answer is resilience. The long answer involves accepting hard truths and applying practical tactics. This presentation will offer lessons learned along our journey to the professoriate, both as a doctoral student and faculty member, with full transparency, in hopes to help those who will also face this metaphysical dilemma.

769 Teaching Critical Thinking and Information Literacy in Introductory STEM Courses

Mays Imad

One of the core competencies outlined in the 2011 AAAS "Vision and Change" report is the ability for students to use quantitative reasoning to understand and interpret data. The report further recommends that students can understand the relationship between science and society. While not explicitly stated, critical thinking skills are a subtle theme in the report. The report does not include, explicitly or implicitly, the need for students to develop qualitative reasoning, including logical and ethical reasoning skills. This presentation will underscore that in order for STEM graduates to become autonomous thinkers and effective problem solvers, it is necessary to acquire the ability to critically evaluate arguments and dialectically reason within different points of view.

We will then deliberate on the meaning of critical thinking as it pertains to critically evaluating arguments and information and examine the value of logical-reasoning. We will consider the current research and practical implications surrounding undergraduate critical thinking skills. In our STEM courses, what does it mean to think critically? To reason logically? How can we assess it? Do we currently assess whether our students can distinguish between facts and opinions in their daily lives? Do we engage our students in ethical reasoning or dialectical reasoning within different points of view? We will interrogate various solutions toward highly-trained STEM graduates to evaluate and synthesize information, and collaborate to create solutions that are well-reasoned, innovative and interdisciplinary.

770 IndigiData: empowering Indigenous genomic and data science education

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Data increasingly drives innovations in research related to science, technology, engineering, mathematics (STEM) and bioinformatics. However, Indigenous students are severely underrepresented in these fields, contributing to a digital divide associated with who has access to data and skills needed to extract knowledge from those data. Culturally-consistent genomic

and data science education can narrow this divide by creating an inclusive learning environment that is tailored to the specific cultural needs of Indigenous students. We describe the formation and development of **IndigiData**, an Indigenous-centered summer workshop that aims to introduce Indigenous participants to fundamental concepts and methods in genomics, data science, and bioinformatics. Our pedagogy places a strong emphasis on bioethics, empowerment through Indigenous genomic and data sovereignties, and relationality between health, culture, environment, and data that is culturally congruent with Indigenous perspectives and knowledges. Further, we leverage existing Indigenous data scientists to build mentorship networks to enrich future career and research connections. Increasing representation of Indigenous data scientists and research leaders will have a profound impact on building a STEM and bioinformatics workforce for Indigenous nations. We anticipate participants will be empowered to use skills gained through **IndigiData** to influence decisions that directly impact the health and research trajectories of their Indigenous communities.

771 Use of an Inclusive Summative Assessment Increased Deep Learning and Reduced Test Anxiety in an Undergraduate Molecular Cell Biology Course

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STEM fields have traditionally suffered from disproportionate rates of attrition of systemically minoritized groups, beginning at the undergraduate level. Thus, inclusive pedagogies in undergraduate STEM courses are paramount to foster more enduring diversity and inclusion in the field. One critical curricular area for educators to examine is their approach to formative and summative assessments. Of these, summative assessments is often the most challenging for professors to view through an equity lens. Here, I share my approach to an inclusive summative assessment referred to as "the public exam." The public exam, originally envisioned by Dr. Benjamin Wiggins (University of Washington), allows students to view a working draft of an upcoming exam, and even contribute to improving it in advance of its administration. In my molecular cell biology course, the draft exams shared with students included 40 questions. All questions were at the Bloom's Taxonomy levels of apply, analyze, and evaluate, in order to foster critical analysis and application of course content instead of short-term memorization. Students were not aware which questions would end up on the final version of the exams, which only included half of the draft exam questions. Further, students were not aware which questions would become multiple choice or remain short answer on the final version—for multiple choice questions, the answer options were not included on the draft exams. I also created a shared spreadsheet for students to provide feedback or ask clarifying questions on the wording of each question—the intention of this activity was to ensure students understood what each question was asking and to give them the opportunity to suggest specific questions potentially be re-worded. This both ensured that any deficits in my ability to effectively write questions would not hinder my students' success and also sent the important message that I care about their ideas and their success. It is also important to note that confirming questions are interpretable can be particularly important when English is not the first language of a student. I administered an anonymous student survey at the end of the Fall 2020 semester; students indicated that my implentation of the public exam format reduced test anxiety, promoted critical thinking about course content, increased time spent studying, and led to students feeling like the grade they earned was a more accurate reflection of their learning compared to traditional exams.

772 Telling our story: Integrating Culturally Inclusive Practices into the STEM classroom to Cultivate a Sense of Belonging Among Underrepresented Groups

Vida Mingo[1] 1) Columbia College.

Racial inequity in this nation is as old as higher education. The social injustices that was present in the formation of the American society as early as 1776, influenced science and created a culture of exclusion that persists in STEM today. In fact, the American Association for the Advancement of Science (AAAS) stated in its Project 2016 report, science both affects and is affected by society. As a social activity, science inevitably reflects social values and viewpoints. Prior to the twentieth century and well into it, women and Black, Indigenous People of Color (BIPOC) were essentially excluded from most of science by restrictions on their education and employment opportunities. From pseudoscientific arguments that the intelligence of any individual can be predetermined solely based on their gender or skin color has been used as the genetic basis for the ranking of human groups. This ranking system of intrinsic value and mental worth would be used as a proxy for who belongs in STEM, who can succeed, and who should be excluded. This practice has led to a history of abuse, marginalization and a social stigma of inferiority, in addition to inequitable access and barriers to STEM education and careers. As a result, there is underrepresentation of women and BIPOC in the STEM workforce. To meet the needs of the STEM workforce of the future, STEM needs to transform from exclusive to inclusive. One area that can be transformative is helping faculty to integrate culturally relevant STEM practices into their classrooms to help students develop a sense of belonging. One aspect of student

belonging in STEM is how well they feel accepted and their own self-efficacy. One way to address this sense of self-efficacy are culturally inclusive pedagogical practices. The Diversity, Equity and Inclusion Committee of the Genomics Education Partnership is facilitating inclusive STEM practices to help increase faculty engagement in this transformative pedagogical practice. Literature has shown that students who have positive interactions tend to be more engaged and more likely to be remain in their STEM major and become a member of the STEM workforce. The Genomics Education Partnership https://thegep.org is a nationwide collaboration of faculty from more than 100 institutions which aim to integrate active learning into the undergraduate curriculum through Course-based Undergraduate Research Experiences (CUREs) centered in genomics and bioinformatics.

773 Initiating and sustaining early and sustained undergraduate research programs as a mechanism for access, inclusion and academic success

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Miami University received an NSF award (2008-2014), through the Undergraduate Research and Mentoring [URM] program, to improve the retention and academic success of underrepresented groups in the Biological Sciences. Faculty from four departments mentored 51 students, along with graduate students and upper-level undergraduates. Recruitment of students relied on partnerships with the Office of Admissions, and with Orientation programs. Applicants participated in a two-semester "Introduction to Research" sequence, before they chose their home labs. Students learned about ongoing research, obtained hands on knowledge with research skills such as literature searches, conducted laboratory rotations, and participated in larger group meetings. The meetings allowed all student cohorts to experience a community of researchers, enabling their confidence in communicating research and preparation for their intended careers. Assessment data reveal that graduation rates of Under-represented, at-risk students in a Biological Science major was four-fold higher than matched institutional controls. Qualitative data obtained from analyses of surveys and focus groups revealed the importance of mentors, sense of belonging, and career preparation.

To sustain essential program features at the institution, Faculty Learning Communities comprised of faculty, and staff from offices across campus, such as Residence Life, University Libraries, met over a two-year period. As a result, the FYRE program was established- First Year Research Experience, which has been active since 2011. One of the authors [JF] has incorporated her *Drosophila* research on Neuromuscular Development into a FYRE course. In collaboration with the Undergraduate Research Office, FYRE is being adopted by multiple academic success initiatives for minority students, including the NSF funded LSAMP. Another sustained outcome of the NSF award was the establishment of a "Skills and Strategies" course which is taught by Graduate Students in the Biology department and focuses on the success of students in Introductory Biology. A team of graduate students successfully engaged in the scholarship of teaching.

In an institutional ecosystem, the resources of multiple interacting niches such as a research lab, program, department, college, and university can be leveraged through partnerships to broaden the participation of under-represented groups, and to provide avenues for inclusion and access into the research enterprise.

774 Choose Development! A multi-level mentored summer undergraduate research program to diversify STEM

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The Society for Developmental Biology (SDB) has been diversifying the community of developmental biologists by supporting undergraduate students who are members of groups underrepresented in basic/biomedical developmental biology and related research fields. SDB is able to achieve this goal with the Society's unique *Choose Development!* (*CD*) Program (2013-present), which provides a research-intensive undergraduate training experience to undergraduate students at a lab of an established developmental biologist SDB-member, a multi-level mentoring plan for each student, society-wide recognition and enculturation activities at national meetings and continued support in years after participation in the *Choose Development!* Program. Key outcomes of the introductory *Choose Development!* Program include: (1) Of a total of 33 undergraduate Fellows, 63% of Fellows that have graduated (27) have entered a graduate program in developmental biology or closely related field (including one MD/PhD), 11% have entered medical school, and 11% have taken gap years in preparation towards submitting a more competitive application to graduate schools while working in their previous mentors' lab; (2) to date, a total of 10 publications have involved the research of *CD* Fellows, with 8 manuscripts having a Fellow as a co-author and 2 crediting Fellows in the acknowledgements; (3) increased awareness and appreciation across the entire SDB led to proactive actions aimed at diversifying committees, Board of Directors, increased representation from non-R1

institutions, postdoctoral fellows and graduate students on the Board and active searches of underrepresented speakers at annual meetings. These cumulative outcomes of the *CD* Program have provided these Fellows an atmosphere of inclusiveness within the entire SDB and has impacted their continuation in the field. Educational and mentoring activities enhance the two-summer immersion requirement of each participant in the research laboratory of an established SDB member anywhere in the USA. Summer hands-on research experiences by trainees in laboratories that study the development of multicellular organisms at the molecule, cell, tissue, organ and whole organism levels and cover topics ranging from stem cells and nuclear reprogramming to evolutionary developmental and systems biology, and from computational analysis to identify gene regulatory networks involved in morphogenesis and organogenesis to the etiology of disease.

775 Increased Diversity of Post-graduate STEM Training and Careers Requires Intervention and Support Early in Undergraduate Education

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Recent efforts in higher education aimed at increasing the retention of underrepresented students in STEM majors have failed to significantly stimulate diversity and persistence in postgraduate STEM careers. Minority-serving institutions (MSIs) with decades of experience have developed an array of unique programs aimed at alleviating resource inequity by providing opportunities beyond pre-health advising and standard career services. Among these, engagement in research and peer and professional mentorship emerged as key predictors of retention and long term success in STEM. Adaptation of these programs represents a valuable approach to mitigating inequities observed at large research-intensive institutions that have a relative lack of diversity in their student populations. Despite institution-level efforts to increase racial diversity, currently matriculating underrepresented minority (URM), non-traditional, and first-generation students at these institutions continue to experience significant barriers to inclusion in programs that support professional development.
All students majoring in Molecular, Cellular, and Developmental Biology at the University of Colorado Boulder enroll in one of two large-scale introductory-level course-based undergraduate research experiences (CUREs). Student gains are most readily observed for URM, non-traditional, and economically disadvantaged students across multiple educational measures, however, support for students after enrollment is significantly lacking. Leveraging the community created in our CUREs, we designed a discussion-based series aimed at connecting students to peer and professional mentors who have been recently successful in achieving post-graduate goals. This tiered mentorship program provides information about 1) the process of applying to professional or doctoral programs, 2) opportunities in laboratory or medical settings, and 3) funding mechanisms and programs specifically available to underserved students. Special sessions are dedicated to the social aspects of networking and approaching members of the professional STEM community including conversion of a resume to a curriculum vitae, email etiquette, and identifying potential employers. Here we describe the organizational details of this remote discussion series and present feedback from students and mentors that support its role in creating ongoing relationships in the scientific community with particular attention to gains made by traditionally disadvantaged students.

776 Expanding and Unclogging the Pipeline: Programs that Increase Faculty Diversity in STEM

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The lack of diversity in the sciences remains a prevalent issue within the scientific community despite significant efforts in the last decades. Although the number of programs supporting women, underrepresented racial/ethnic groups, and LGBTQ+ communities has increased, these groups are still far from reaching presence levels that represent demographic data. Here, I will describe two examples of programs initiated at the University of Maryland, Baltimore County (UMBC) through the work of multiple people that focus on supporting promising scholars to promote diversity and inclusive excellence in STEM. One is the **Meyerhoff Scholarship Program** that was founded in 1988 to support undergraduate students committed to obtaining PhD degrees and has significantly contributed to UMBC's success in producing the largest number of Black MD/PhD degree-earners in the US. One alumnus of UMBC and the Meyerhoff Undergraduate Program is Dr. Kizzmekia Corbett, who with her team at the Vaccine Research Center at the National Institute of Allergy and Infectious Diseases has been critical in developing the COVID-19 vaccine with Moderna. In 1997, the Meyerhoff Graduate Fellows Program was created, promoting cultural diversity in the biomedical sciences at the graduate level. In addition to the Meyerhoff program, UMBC offers additional programs aimed to support undergrads, such as MARC-USTAR/URISE, Louis Stokes Alliances for Minority Participation (LSAMP), McNair Scholars Program, STEM BUILD @ UMBC, and the Center for Women in Technology. To diversify the faculty level, UMBC created in 2017 the **Natural Sciences Preprofessoriate Fellowship Program** (NSPFP). NSPFP is a structured postdoctoral conversion program that prepares scholars committed to diversity and inclusive excellence for tenure-track appointments within the University System of Maryland. Additionally, UMBC offers the Postdoctoral Fellowship for Faculty Diversity and the AGEP PROMISE Academy Alliance (APAA) to help facilitate the hire of diverse faculty. Efforts to implement UMBC programs

nationwide include partnerships to replicate UMBC's Meyerhoff Program at UCSD and UC Berkeley funded by the Chan Zuckerberg Initiative in 2019 as well to develop programs based on the NSPFP at Dartmouth College in 2020. Whereas the programs described above represent solid, slow steps to promote long term changes, much remains to be done to achieve a diverse scientific community that celebrates a welcoming environment for all citizens.

778 High-speed 3D microscopy of neural activity in behaving larval and adult Drosophila

Elizabeth HIllman

779 Large-scale image segmentation for light and electron microscopy

Anna Kreshuk

780 Making an accessible connectome for biological discovery

Stephen Plaza

781 Tools and strategies to identify new disease causing human genes and variants using Drosophila

Shinya Yamamoto

782A Establishing a Multicultural Inclusive Environment with the GEP-Translate Initiative

Stephen Klusza[1] 1) Clayton State University, Morrow, GA.

The Genomics Education Partnership (GEP) is a 200+ faculty collective centered in creating educational modules and curriculum frameworks to deploy bioinformatics-based CUREs in undergraduate classes to expose students to original genomics research. In order to enhance the undergraduate research experience in *Drosophila* fruit fly and parasitoid wasp genomics, The Understanding Eukaryotic Genes (UEG) modules were created to provide walkthrough protocols in exploring genetic architecture with GEP-curated bioinformatics tools. Videos of each module were also generated to help guide students through the protocols and establish formative experiences with bioinformatics that are reinforced in turn by gene annotation research.

In response to recent events in the United States and a continuing lack of diversity and accessibility in higher academia, the Diversity, Equity, and Inclusion (DEI) Committee was formed with the goals of creating an inclusive environment in GEP for faculty/students alike, and to pursue initiatives that increases equity for all people interested in genomics and *Drosophila* research. The DEI Committee identified an urgent need to translate the UEG modules curriculum from English to Spanish, in order to better serve Spanish-speaking students interested in genomics. With generous funding from the Fly Board, the GEP-Translate initiative is currently focused on translating UEG module protocols and videos to Spanish. In this presentation, we will describe the process being implemented for this initiative, our progress so far, and our future goals to make *Drosophila* research more accessible and equitable to all.

783B IUpcycle – Upcycling Laptops to Give Everyone Equal Access to Technology.

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As COVID-19 crept into the United States and ravaged our homes, communities and learning two graduate students at Indiana University Bloomington (IUB) realized that there was an issue. An astounding number of undergraduate students, graduate students and even staff were doing their day-to-day work on university owned equipment. Therefore, when the university switched to online remote learning those individuals no longer had easy and timely access to technology to finish assignments, record lectures and advance research in their field. Kaitlin Doucette and Allie Smith realized this was an issue and starting planning on how we as a community to secure equitable access to technology for everyone at Indiana University both during and after the COVID-19 pandemic. The IUpcycle program started as a pipe dream with two graduate students with a passion for service and understanding that there was a problem at hand. Smith and Doucette emailed the university putting a call out for donations of used but functional laptops that could be wiped and refurbished (if needed) that could then be upcycled to students, staff or faculty who needed technology but do not have the financial means to purchase said equipment. After the launch of the program in November of 2020 the IUpcycle program has received 20 laptops that have been wiped and refurbished by Doucette and thus far 5 laptops have been given to students in need. The program has secured funding by the College of Arts and Science at IUB as well as co-sponsorships by the Center of Excellence for Women & Technology, the department of biology and Serve IT. The hope for the program is to receive, wipe and upcycle 100 laptops by the end of 2021. In 2021 technology is required for almost everything we do, including writing this abstract, and everyone deserves equitable

access to said technology. This is a program that could be put onto any campus across the United States and Smith and Doucette and happy to share resources on how to get a campus as such off of the ground.

784C How can *Drosophila* help us work for equity and inclusion?

María Alejandra Petino Zappala[1] 1) Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

While most subdisciplines in biology deal with the question of natural diversity, a trade-off also exists in which simplifications and control over the environment are needed to satisfyingly carry out research, particularly in some of them. It may be argued that such necessity has been relaxed by the advancement of available technologies and methodologies. However, there may be an inertia preventing most researchers to really take advantage of these developments. Even with access to the most up-to-date technologies and infrastructure, we still can fall prey to our (unrecognized) assumptions and to the framing of our questions. These shortcomings may lead us to very serious problems when we want to generalize findings to other organisms, especially to humans. However, this is not to say that model organisms such as *Drosophila* can't teach us useful lessons on diversity and lead us towards equity and inclusion.

I want to share the experience of going from the study of *Drosophila melanogaster* development to the search of social determinants of health in humans and to the battle for health equity. Starting with *Drosophila,* even in inbred, homozygous lines raised under controlled conditions, we uncovered a considerable amount of complexity and characterized a flexible genotype-phenotype relationship. Later, I tried to apply some of these insights to the question of why some human groups are far more vulnerable to multifactorial diseases, and how can public policy be aimed to reduce health inequity.

Far from making us fall into determinism, working with *Drosophila* or other model organisms in controlled settings may provide us with an useful basis on which we can build layers of increasing complexity to better understand natural diversity, including that related to the human species, and approach interesting philosophical questions.

Finally, I want to argue that inclusive practices and adequate representation are necessary (although not sufficient) to achieve a more diverse science. However, the path towards equity also must involve thinking and questioning the relationship between power and the construction of knowledge.

785A Molecular Regulation of Centrosome Stability

Ana Pimenta-Marques[1], Tania Perestrelo[1], Patricia Reis-Rodrigues [1], Paulo Duarte[1], Mariana Faria[1], Monica Bettencourt-Dias[1] 1) Instituto Gulbenkian de Ciência, Portugal.

The centrosome is the primary microtubule organizing center (MTOC) in animal cells. It is crucial for cell shape control and polarity in interphase, and spindle pole organization during mitosis. The centrosome is composed by a pair of centrioles surrounded by a network of proteins, named pericentriolar material (PCM). Although centrosomes are known to be very stable in cycling cells, they can be inactivated or even lost in cells undergoing differentiation.

A large body of knowledge exists regarding key molecules and pathways involved in centrosome biogenesis, but little is known about their maintenance. Our previous work has shown that POLO kinase and PCM are critical players in this program. Nevertheless, the mechanism through which they confer stability is unknown. We are now interested in understanding if centrosome stability is under a central pathway mechanism mediated by POLO and PCM, or if there are multiple pathways involved, and which are the consequences of this misregulation.

By studying centrosome disappearance in the female germline, as well as its stability in a Drosophila cultured cell lines (DMEL), our preliminary data suggests that some centriolar proteins are crucial for the centrosome maintenance program both *in vitro* and *in vivo*. *W*e are currently exploring if these proteins and POLO kinase work together to maintain centrosome stability. These results will provide a deeper understanding on the molecular mechanisms by which centrosome stability is maintained, and will help us to tackle down how centrosome numbers are lost or increased in disease.

786B Pan-neuronal expression of human mutant huntingtin protein in *Drosophila* impairs immune response of hemocytes

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Huntington's disease (HD) is a dominantly inherited, late-onset, progressive neurodegenerative disorder characterized by

hallmark chorea, cognitive decline and psychological impairment leading to gradual loss of functional capacity and eventually death. The causative factor behind HD was assigned to an unstable expansion in the polymorphic trinucleotide (CAG) repeat beyond 35 in Interesting Transcript 15 (IT 15) gene encoding ~348 kD Htt protein. The pathological effects of mutant Huntingtin (mHtt) are not restricted to the nervous system but systemic abnormalities including immune dysregulation have been observed in HD patients and animal models.

Indeed, mutant huntingtin (mHtt) is ubiquitously expressed and could induce cellular toxicity by directly acting on immune cells. In the present study, we used transgenic *Drosophila melanogaster* expressing human mHtt exon1 fragment with 93 glutamine repeats selectively in neuronal cells using pan-neuronal driver and assessed its non-cell-autonomous effects on hemocytes population and their physiological functions. We found that non-cell-autonomous expression of mHtt has noxious effects on *Drosophila* hemocytes and increase in crystal cells and plasmatocyte count in late 3rd instar larva and adults. Consequently, we observed that plasmatocytes of diseased flies exhibit reduced phagocytic activity *ex vivo* despite of increase in their number. Furthermore, diseased flies displayed elevated reactive oxygen species (ROS) in circulating plasmatocytes at larval stage and in sessile plasmatocytes of hematopoietic pockets at advanced stage of disease. Interestingly, alteration in phagocytic activity and ROS levels of plasmatocytes have strong correlation with disease progression. These findings indicate that neuronal expression of mHtt alone is sufficient to induce non-cell-autonomous immune dysregulation *in vivo*. Therefore, the identification of new therapeutic targets aimed to immune regulation may prove to be effective in delaying disease progression and the quality of patient's life.