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ABSTRACT BOOK

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A new paradigm for anti-parasitoid immunity

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Parasitoid wasps comprise the most important natural enemies of insects in the wild, with parasitization loads exceeding 50% in natural populations of *Drosophila melanogaster*. Parasitoids obligately kill their hosts, and hosts have evolved mechanisms to overcome this severe selective pressure. In the accepted model of parasitoid response, hemocytes encapsulate and neutralize the wasp embryo through melanization reactions that produce noxious reactive oxygen species. In *D. melanogaster*, hemocytes called lamellocytes encapsulate wasp embryos and melanize them using a paralog of Prophenoloxidase-2 (PPO2) called PPO3. However, studies in other species of *Drosophila* have demonstrated many species do not differentiate lamellocytes nor encode *PPO3*, suggesting alternative mechanisms of parasitoid defense have evolved in these lineages. Here, we report results of a screen of >500 genomes from Drosophilidae and closely related species for PPO genes. We found few species (~13%) in the Drosophilidae encode *PPO3* (defined liberally as a *PPO2* duplication) outside of the *melanogaster* species group. Instead, we found many more species (~18% total species) encode *cytolethal distending toxin B* (*cdtB*) in their genomes. Our previous work showed that horizontally transferred CdtB toxins are nucleases that function in the humoral arm of innate immunity in *D. ananassae* and confer strong protection to diverse parasitoids when heterologously expressed in *D. melanogaster* immune tissues. The CdtB toxin is secreted into the hemolymph where it targets developing parasitoids by binding a conserved receptor. Moreover, we identified an enhancer driving the expression of a *cdtB*-encoding gene in *D. ananassae*, which retains its spatial, temporal, and parasitoid-inducible features in *D. melanogaster* despite their 21 million year divergence from a common ancestor, pointing to a conserved signaling network that responds to metazoan parasites. We propose that the humoral immune system likely plays a major and underappreciated role in anti-parasitoid immunity, and the study of toxins deployed in this branch can shed light on ancient anti-animal innate immune mechanisms.

1 Unlocking the Metabolic Mysteries of Sleep

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Why we spend ~a third of our lives sleeping and what it is that makes us sleepy are major questions about sleep that lack satisfactory answers. There is universal agreement that lack of sleep impairs performance, especially cognitive ability, during waking hours and considerable evidence supports adverse effects of sleep loss on other physiological parameters as well. Thus, sleep may be regarded as important for waking function. However, what happens during sleep to facilitate wake performance and promote health?

Driven by the successful use of Drosophila for deciphering molecular mechanisms of the circadian clock, we developed a Drosophila model to address molecular and cellular underpinnings of sleep. Through the use of forward genetic screens, we have identified genes and tissues that affect sleep amount. Coupled with our efforts to understand the fundamental basis of sleep, we are getting a handle on the regulation and function of sleep that may be broadly relevant for the brain, and perhaps even the body. We find that sleep is important for metabolic, in particular energetic, homeostasis in the brain. This includes the clearance of metabolic waste, which can be accomplished in different ways. For instance, we find that autophagy and endocytosis through the blood brain barrier (BBB), both of which facilitate clearance, are regulated by sleep. Our ongoing work implicates sleep in lipid metabolism, in particular. We find that specific lipids accumulate under conditions of high sleep need e.g. when endocytosis through the BBB is blocked. And lipids are transferred from neurons to glia in a daily cycle that helps to maintain mitochondrial homeostasis in neurons. Together this work is leading to an understanding of cellular/molecular processes that underlie sleep.

2 Invertebrate oncology: tumor-host interactions in *Drosophila*

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There is a large gap between the deep understanding of mechanisms driving oncogenic transformation and the reasons why patients ultimately succumb to cancer. We now appreciate that interactions between the tumor and surrounding non-tumor ('host') cells play critical roles in mortality as well as tumor progression, but many mechanisms remain unknown. Drosophila's high conservation of biology and genetics with humans has powered its long track record of high-impact discoveries; the reductionist fly system is particularly well-suited to probe mysteries of tumor–host interactions. I will discuss our use of Drosophila cancer models to study 'paraneoplasias' such as cachexia, coagulopathy, and fluid dysregulation, and will present unpublished work on anti-tumor immunity and immune evasion in this experimentally tractable invertebrate.

3 Lineages, Time, and Diversity: Linking Stem Cell Programs to Complex Behaviors

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During development, neural stem cells (NSCs) generate a diverse range of cell types that self-assemble to form neural circuits, regulating distinct behaviors. The specification and assembly of neural cell types into neural circuits is poorly understood.

Our research group uses a curiosity-driven approach that combines collaborative and diverse scientific and life experiences to understand the fundamental principles of neural diversity. Using our favorite model system, the fruit fly, we investigate developmental programs that generate neural cell types of the highly conserved insect brain region, the central complex. We have identified key transcription factors and RNA-binding proteins that specify neural cell types, regulating two essential behaviors: olfactory navigation and sleep. In New Mexico, we have also initiated an ambitious Pueblo Brain Science program that involves training, mentoring, and science outreach modules to train the next generation of scientists. You can read more about our research and mentoring programs here https://doi.org/10.1016/j.cub.2024.03.002

4 Cell competition and loser mutations shape the competitive landscape of tumour cells

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Cell competition is a conserved biological process through which cells of differing fitness interact, leading to the selective elimination of less fit cells from developing and adult tissues. In our laboratory, we use both *Drosophila* and mammalian model systems to investigate the mechanisms that cells use to compete and to define their relevance to normal physiology and diseases like cancer. Indeed, we and others have demonstrated that tumour cells can outcompete surrounding wild-type cells to free space that they can expand into, thus driving tumour growth.

A major model to study cell competition has been the classical *Minute* mutations in ribosomal protein genes, which render cells competitively disadvantaged, causing their elimination by wild-type neighbours. We have shown that *Minute* cells show pronounced cellular dysfunction, including reduced protein synthesis and increased oxidative and proteotoxic stress. Surprisingly, despite these defects, ribosomal protein gene mutations are unexpectedly recurrent in human cancers. This has led us to dissect how ribosome gene loss shapes tumour cell fitness and competitive behaviour and how these mutations become tolerated in oncogenic contexts with the goal of uncovering new therapeutic vulnerabilities.

5 Development of Neural Circuitry of the Lateral Accessory Lobe, a Center for Navigation and Motor Control in the Drosophila Brain

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The *Drosophila* brain is formed by approximately 100 pairs of fixed lineages, originating from neuroblasts that appear in the early embryo and divide in a characteristic, stem cell-like mode. Transcription factors expressed sequentially by a neuroblast specify the wiring properties of the neurons born during a certain time window (“sublineage”), and these cells form classes that assemble into specific modules within a circuit. Neuronal lineages and their components can be identified based on structural features in the recently constructed connectomes of the larval and adult fly brain. I will highlight the relation between lineage development, circuits and brain anatomy as exemplified by a specific compartment, the lateral accessory lobe (LAL), which plays a key role in navigation of both larval and adult flies. The LAL integrates olfactory and visual input with proprioceptive feedback information and is the site of origin of a large set of output neurons that project to the ventral nerve cord. Local neurons of the LAL fall into discrete groups that engage in dense reciprocal connectivity, forming recurrent networks capable of storing and manipulating information. I will summarize pertinent facts about the lineages and their neuroblasts of origin that give rise to the LAL; I will further focus on the relationship between larval and adult LAL circuitry. Evidence suggests that the larval (primary) LAL neuron classes persist into the adult and reconstitute their former convergence of olfactory and proprioceptive inputs, as well as its internal core circuitry. Adult specific (secondary) neuron classes of LAL lineages for the most part augment a select subset of primary classes; in addition, they generate much of the volume of the central complex, a compartment that is added to the LAL of the adult brain to provide the band width required to process the strong increase in sensory input, and to provide adequate output to a much more sophisticated motor apparatus.

6 Mechanisms of mitochondrial inheritance in the germline

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In nearly all animals, including humans, mitochondrial DNA (mtDNA) is inherited exclusively from the mother, mutates frequently, and rarely recombines. This asexual mode of transmission makes mtDNA uniquely vulnerable to the buildup of harmful mutations, a process that, if left unchecked, would eventually erode mitochondrial function and compromise species survival. To counter this, the female germline has evolved an essential yet still poorly understood purifying selection mechanism that selectively eliminates mutant mtDNA before it can be passed on to the next generation. I will discuss our recent work uncovering how this quality control system operates in the *Drosophila* female germline. I will also turn to the equally fascinating question of why mtDNA is inherited only maternally and share new insights into how paternal mtDNA is actively excluded during spermatogenesis.

7 Drosophila in context: evolution, toxins, and behaviour

Justin Crocker EMBL

Understanding how behaviours evolve requires linking genetic variation to phenotypic outcomes in the ecological contexts where selection acts. Much of our work has focused on how gene regulatory architectures encode robustness while retaining the capacity for evolutionary change. Using Drosophila as an experimentally tractable system, we have shown that enhancer organization, transcription factor binding landscapes, and regulatory redundancy shape both phenotypic stability and the release of cryptic variation.

Building on this framework, we investigate how environmental toxins act as selective and developmental perturbations that expose hidden dimensions of behavioural variation. Recent work demonstrates that larval behavioural outputs exhibit signatures of criticality, consistent with regulatory systems poised between stability and collapse. We show that toxin exposure does not merely reduce performance but reveals context-dependent genotype–phenotype relationships.

By combining synthetic perturbations of cis-regulatory networks with quantitative behavioural assays, we map how gene regulatory network structure constrains or enables behavioural evolution under stress. These results support a view of behaviour as a developmentally encoded, evolvable trait whose diversity emerges from interactions between regulatory design principles and ecological challenge. Studying Drosophila *in context* provides a powerful route to understanding how complex phenotypes evolve in variable environments.

8 Injury-induced tau pathology promotes aggressive behavior in Drosophila without neurodegeneration

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Traumatic brain injury (TBI) frequently causes acute behavioral changes and is associated with elevated levels of phosphorylated tau, yet the molecular changes that cause immediate neuronal dysfunction remains unknown. Using a *Drosophila* TBI model, we find that pan-neuronal expression of human tau coupled with injury triggers hyper-aggression in males in a competitive courtship assay. By performing a neuronal GAL4 screen and using thermogenetic assays, we demonstrate this behavior results from specific activation of Ple-GAL4-labeled dopaminergic circuits. Strikingly, this occurs in the absence of neurodegeneration. Using in vitro reconstitution assays with purified proteins, we find that human tau outcompetes fly tau on the microtubule, and upon phosphorylation, human tau maintains microtubule binding but loses its ability to stabilize microtubules, thereby enhancing microtubule dynamicity. In contrast, fly tau and unphosphorylated human tau stabilize microtubules. We hypothesize that upon overexpression, human tau initially outcompetes fly tau, but as it becomes phosphorylated post-injury, its altered properties permit increased microtubule dynamicity, stimulating neuronal activity and contributing to the manifestation of aggressive behaviors observed in male flies. Our work challenges the view of tau as a simple stabilizer, positioning it as a dynamic regulator of microtubule function and neuronal excitability. This model reveals how acute tau phosphorylation alters circuit function prior to neurodegeneration, providing insights into early tauopathy mechanisms.

9 EHMT1/G9a controls sleep maintenance in adulthood by regulating ROS homeostasis in insulin-producing cells during development

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Sleep disturbances are a prominent yet poorly characterized feature of Kleefstra syndrome (KLEFS1), a neurodevelopmental syndrome caused by rare variants in the epigenetic regulator *EHMT1*. This gap in understanding both the nature of these sleep disturbances and how *EHMT1* mutations drive them posed a major barrier for therapy development. In this cross-species study, we reveal that over 80% of individuals with KLEFS1 experience severe sleep maintenance insomnia, marked by fragmented sleep due to frequent night awakenings. Similarly, common variants *EHMT1* are associated with short sleep and insomnia symptoms in the general population. *Drosophila* mutants of the *EHMT1* orthologue *G9a* recapitulate these phenotypes, exhibiting reduced and fragmented sleep. We show that G9a is required in insulin-producing cells (IPCs) and the fat body during development to preserve adult sleep integrity. Strikingly, G9a loss in these tissues also impairs learning—a core feature of KLEFS1—highlighting epigenetic regulation of cognition beyond the nervous system.

Untargeted metabolomics revealed widespread metabolic dysregulation in *G9a* mutants, particularly within methionine metabolism. Mutants exhibited reduced methionine and elevated methionine sulfoxide (Met-SO), indicating increased reactive oxygen species (ROS). Consistently, ROS levels were elevated in IPCs during brain development but not in adulthood. Knocking down *MsrA*, the enzyme that reduces Met-SO back to methionine, reproduced sleep fragmentation when targeted to IPCs. Finally, antioxidant treatment fully rescued sleep fragmentation when administered during development, demonstrating that G9a safeguards sleep via ROS homeostasis during early life. Remarkably, we demonstrate that a translational behavioral regime based on human sleep-restriction therapy can override the developmental defects and restore sleep continuity in adulthood across multiple KLEFS1 models.

10 Pathogenic CryAB Mutations in Muscle Suggest a Novel Mechanism for Amyloid Spread via Extracellular Vesicles

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Amyloid fibrils are insoluble protein aggregates commonly linked to neurodegenerative diseases, including Alzheimer's and Parkinson's disease. While amyloid structures have been extensively observed in the brain, they are also found in other organs such as the heart, kidney, and liver, a condition known as amyloidosis. Amyloid deposits in skeletal muscle, however, remain less documented. Human mutations in the small heat shock protein αB-crystallin (CryAB) result in cataracts, cardiomyopathies, and myofibrillar myopathies, all characterized by the presence of protein aggregates. In this study, we expressed four human pathogenic alleles of CryAB in *Drosophila* muscle tissue to define how altered chaperone activity impacts protein quality control. Each of these CryAB mutations impaired autophagic flux, resulting in protein aggregates marked by ubiquitin (Ubi) and p62/Sequestosome-1 (SQSTM1). In addition, mutations located in the conserved CryAB α-crystallin domain (ACD) led to the formation of unique, CryAB-positive inclusions that colocalized with an amyloidogenic form of the human intermediate filament protein desmin. Transmission electron microscopy (TEM) and Congo red staining confirmed that these disease-causing CryAB mutations formed amyloid-like fibrillar structures. Unexpectedly, these CryAB amyloid inclusions were also detectable in the hemolymph and stained positive for extracellular vesicle (EV) markers, suggesting an EV-mediated export mechanism. Modulating proteins that affected EV secretion - either through overexpression or RNAi - altered the size and number of CryAB inclusions. The findings together expand our understanding of amyloid fibril architecture in vivo and provide a possible pathogenic mechanism for the spread of protein aggregates in disease progression.

11 Exercise rescues shortened lifespan and impaired muscle function in a new *Drosophila* model of chronic inactivity

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Prolonged inactivity due to medical conditions can cause chronic muscle disuse and lead to physical incapacity and poor quality of life. Here, we developed a *Drosophila* model of confinement inactivity (CI) to observe its effects on lifespan and muscle function. We found that, similar to mammalian models and humans, CI negatively impacted longevity and function in *Drosophila.* Confined flies had impaired mobility, shorter lifespan, and reduced muscle integrity compared to their freely mobile siblings. These findings establish a new, highly efficient platform for studying long term effects of chronic sedentary behavior and muscle disuse in the genetically tractable *Drosophila* model. In addition, we found that temporarily removing flies from CI for scheduled bouts of forced physical exercise ameliorated negative effects, in part by improving muscle homeostasis. Finally, we tested whether muscle overexpression of 3 exercise-responsive genes, *dPGC-1*α, *dFNDC5*, or *dSesn*, could prevent the negative impact of CI on fly aging, even without physical exercise. We previously established that overexpression of these factors phenocopies exercise effects in aging wild-type and disease model flies. We found that when overexpressed in muscle, *dSesn* prevented premature declines in endurance, and *dFNDC5* protected speed and endurance. This new model can be used in the future for mechanistic studies to identify preventative and therapeutic targets for diseases associated with chronic inactivity.

12 A conserved role for *Sec16/SEC16A* in the nervous system: Identification of a novel human neurodevelopmental disorder

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The Sec16 proteins are highly conserved scaffold proteins required for the organization of the endoplasmic reticulum (ER) exit sites (specialized ER subdomains where newly synthesized proteins are exported). They are required for the regulation of early steps of the secretory pathway. *Sec16* orthologs are predominantly studied in yeast and cultured cell systems largely due to the early lethality associated with their loss in multicellular organisms. We have identified individuals with bi-allelic *SEC16A* variants who present with neurodevelopmental delays, cognitive impairment, motor delay, and seizures. However, the role of *Sec16/SEC16A* in the CNS is not well characterized. From a previous forward genetic screen targeting essential genes on the Drosophila X chromosome, we identified *Sec16* as critical for neuronal function. We further generated a *Sec16T2A* loss-of-function allele that expresses GAL4 in the endogenous pattern of *Sec16* (CRIMIC), and demonstrate that *Sec16* is expressed in subsets of neurons and glia within the CNS. We show that loss of *Sec16* in flies leads to early lethality and that *Sec16* is required cell-autonomously for the developmental of numerous tissues. Particularly, a neuron-specific knockdown of *Sec16* causes reduced lifespan and neurological phenotypes including locomotor defects, seizure-like bang sensitivity, and impaired synaptic targeting as well as transmission. We also show that both loss and overexpression of *Sec16* markedly disrupt ER protein secretion, highlighting dosage sensitivity. Notably, the human ortholog *SEC16A* is also highly expressed in brain, and we have identified individuals with bi-allelic *SEC16A* variants exhibiting neurodevelopmental disorders. We show that ER stress is elevated both in fly brains with neuronal loss of *Sec16* and in patient-derived iPSC neurons. In summary, our findings establish a critical and evolutionarily conserved role for *Sec16/SEC16A* in nervous system and allowed us to identify a novel human neurodevelopmental disorder.

13 A rare recurring gain-of-function variant in *BMPR2* causes neurodevelopmental phenotypes in humans and flies

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Bone Morphogenetic Protein (BMP) signaling plays crucial roles in wide biological processes, including, but not restricted to, embryonic development, neural patterning, bone morphogenesis, and stem cell differentiation. Bone Morphogenetic Protein Receptor Type 2 (BMPR2) is an evolutionarily conserved serine/threonine kinase that phosphorylates type-1 BMP receptors to mediate intercellular communication upon ligand binding. Loss-of-function (LoF) variants in *BMPR2* are known to cause pulmonary arterial hypertension (PAH) and a related cardiovascular disorder (pulmonary venoocclusive disease 1). Here, we report a rare recurrent *de novo* missense variant, *BMPR2* p.(Glu376Lys), identified in five individuals who all present with neurodevelopmental phenotypes, including autism spectrum disorder and global developmental delay. Using *Drosophila*, we demonstrate that this variant behaves as a gain-of-function (GoF) allele, acting in a ligand-independent but type-1 receptor-dependent manner. BMPR2-specific pharmacological inhibitors can suppress the excessive BMP activation induced by this GoF variant, confirming that this variant is a hypermorphic allele. Importantly, we show that both neuronal- and glial-specific expression of this variant cause neurodevelopmental defects in the larval central nervous system. Finally, we further identified that perineurial glia is the most vulnerable neural cell type, causing lethality when *BMPR2* p.(Glu736Lys) is expressed, suggesting a role of BMP signaling pathway in the fly blood-brain barrier (BBB). Given that *BMPR2* is also expressed in various neuronal, glial and BBB cell types in the human brain, ectopic activation of BMP signaling in multiple cell types is likely to contribute to the neurodevelopmental phenotypes observed in the probands.

14 A developmental role for synaptic adhesion molecules in adult hypersomnia in flies, fish, and humans

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Idiopathic hypersomnia (IH) is characterized by excessive daytime sleepiness despite normal nighttime sleep and without any medical explanation. The pathophysiology of IH is unknown, partially impeded by the lack of animal models of the disorder. Using the fruit fly, a powerful organism for studying sleep and circadian behavior, we sought to investigate the molecular and genetic underpinnings of human hypersomnia. We conducted a reverse-genetic RNAi screen of putative sleep-propensity associated genes based on human GWAS, identifying fly orthologs whose knockdown in neurons or glia produced long-sleeping flies.

We found that neuronal knockdown of *beat-Ia*, of the *beaten path* family of synaptic adhesion molecules, recapitulates characteristics of human hypersomnia: *beat-Ia* knockdown flies exhibit increased sleep duration, heightened arousal threshold, and reduced sleep latency. Developmental knockdown of *beat-Ia* was sufficient to increase adult sleep, suggesting a role in patterning adult neural circuits. *beat-Ia* was the identified homolog of human *CADM2*, a synaptic adhesion molecule with similar functions, and *CADM2* knockout in zebrafish also increases sleep. Finally, a targeted driver screen identified a potential role for beat-Ia in cells expressing neuropeptide F, the invertebrate homolog of mammalian neuropeptide Y.

beaten path family members have a known role at the larval neuromuscular junction, but our work suggests a novel function for these adhesion molecules in development of sleep regulatory circuits. Additionally, to our knowledge this is the first time mammalian *CADM*s have been implicated mechanistically in sleep disorders, underscoring a general role for synaptic cell adhesion molecules in sleep regulation. Future directions include further investigation of *CADM2*'s role in sleep regulation and evaluation of the neuropeptide Y receptor as a potential therapeutic target. Lastly, work is currently ongoing to identify and screen additional hypersomnolence-associated genes by investigating GWAS signals of subthreshold statistical significance, as they may represent biologically meaningful pathways despite not reaching genome-wide significance.

15 Drosophila UBE3A Regulates Substrates Associated with Gliopathic Seizures Through Direct Ubiquitination of Translation Initiation and Ubiquitin Proteasome Proteins

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Epilepsy is a prevalent and debilitating condition affecting most individuals with Duplication 15q syndrome (Dup15q). Many children with Dup15q have uncontrolled seizures despite standard anti-epileptic drug (AED) treatments. Within the Dup15q locus lies the paternally imprinted gene *UBE3A*, which encodes ubiquitin protein ligase E3A (UBE3A/E6AP). Despite an established role in neurodevelopment and synaptic regulation, the full spectrum of direct UBE3A substrates remains largely unknown, limiting our understanding of how dosage imbalance, particularly in glial cells, drives neuronal hyperexcitability and seizures. To overcome this gap, we implemented an *in vivo* proteomic approach using the ubiquitin-activated interaction trap (UBAIT) *in vivo* in *Drosophila melanogaster*. The UBAIT system covalently traps proteins ubiquitinated by Dube3a, enabling their purification and identification via LC-MS/MS. From a high-confidence list of conserved candidate substrates, we conducted a targeted RNAi screen of 163 substrates and identified eight that significantly increased seizure susceptibility. Biochemical validation using the previously established GFP pulldown assays confirmed that Sap47, embargoed/exportin-1 (*emb*), Dpm1, tiptop (*tio*), and Nedd4 are direct Dube3a substrates that undergo polyubiquitination in a Dube3a-dependent manner. Notably, many of the substrates converge on pathways related to translation initiation and RNA processing, suggesting a broader role for UBE3A in regulating proteostasis and gene expression control in glial cells. Additionally, we identified a novel protein–protein interaction between Dube3a and the E3 ligase Nedd4, indicating potential cross-talk within the ubiquitin signaling network. Together, these findings represent the first systematic *in vivo* identification and validation of UBE3A substrates in a cell-type-specific context, revealing new mechanistic pathways by which *UBE3A* overexpression disrupts glial homeostasis and drives seizure pathogenesis. This study lays the groupwork for precision therapies in Dup15q and Angelman syndromes, and potentially related human epilepsies.

16 Genetic identification of miRNAs that can alter sensory organ cell fate and Notch signaling

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microRNAs (miRNAs) are ~21-24 nt RNAs that broadly regulate gene expression development, and physiology. However, a paradox remains in that individual animal miRNAs typically have hundreds of conserved targets yet many miRNA knockouts exhibit subtle or incompletely penetrant phenotypes. This suggests that miRNAs mediate "fine-tuning" or have roles that are compensated by other regulatory means to maintain biological robustness. To gain further insights, we utilize *Drosophila* genetics and comprehensive in vivo assays of miRNA activity. Here, we report genome-wide screens of UAS-miRNA transgenes for their capacity to perturb various aspects of notum development. Using several Gal4 drivers, we recovered numerous miRNAs that perturb notum fusion, epithelial polarity, cell survival or growth, and/or sensory organ patterning. Of particular interest were cell fate defects in the mechanosensory bristle organs. Each sensory bristle originates from a sensory organ precursor that undergoes a series of asymmetric divisions to yield several specialized support cells and a neuron. Overexpression of some miRNAs yields supernumerary bristles, reflecting ectopic pro-neural protein activity or reduced Notch signaling. We also found miRNAs that perturb asymmetric divisions within the bristle organ, implying modulation of Notch activity. To further probe the interaction between miRNA overexpression and Notch signaling, we found that several miRNA-induced phenotypes were modified in sensitized backgrounds with reduced or elevated Notch signaling. We used reporter tests in S2 cells to connect several miRNA hits to specific target genes. At the cellular level *in vivo*, we measured reporter expression and cellular composition in the notum to elucidate a mechanism of miRNA regulation impinging on sensory organ development. This work outlines a genetic strategy to elucidate in vivo miRNA activities and to link them to biologically relevant targets. While this may yield insights to their endogenous functions, we also suggest that miRNA dysregulation, particularly in concert with major signaling pathways, has broad potential to elicit disease and pathogenic conditions.

17 Evolutionary diversification of the gap gene network of flies

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In dipterans (true flies), symmetry breaking along the anterior-posterior (AP) axis is mediated by transcription factors known as anterior determinants (ADs), which differ between fruit flies (Drosophila), mosquitoes, and midges. The maternal AD activity regulates gap genes, which encode transcription factors that are expressed in broad, partially overlapping domains along the blastoderm's AP axis. One of those gap gene expression domains, the posterior *giant* (*gt*) domain, which is critical for proper segmentation of the abdomen in Drosophila, is missing in some dipterans, including the midge *Chironomus riparius*. This observation suggests that Chironomus differs from Drosophila not only in its AD but also in its gap-gene complement. Using chromatin accessibility (ATAC-seq data) from posteriorized (double abdomen) Chironomus embryos that were generated by AD depletion and single-nuclei RNA-seq data, we discovered gap-like, posterior expression domains of the Chironomus homologs of the transcription factors *buttonhead* (*btd*), *chronophage* (*cph*), and *shavenbaby* (*svb*/*ovo*). To elucidate the potential segmentation functions of these gap gene candidates, we are examining how their knockdown or knockout affects the segmentation process. We are also investigating the potential targets of both the novel gap gene candidates and canonical gap genes by using ATAC-seq in combination with DAP-seq, a method to identify binding sites of transcription factors in endogenous DNA sequences. Preliminary results suggest that in Chironomus, *btd* may function as an abdominal gap gene and that *cph*, *ovo*, and *knirps* (*kni*) are direct targets of Giant, which is expressed in the anterior half of the blastoderm. We therefore propose that dipteran species may differ substantially in their posterior gap gene complement.

18 Biomolecular condensates regulate myosin II dynamics during tubular organ morphogenesis

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For an organ to achieve its proper architecture, changes in cell shape and relative position must be robustly induced and precisely controlled. Salivary gland (SG) development in the Drosophila embryo provides an excellent model system to discover the molecular mechanisms underlying morphogenetic changes during tubular organ development especially since, once specified, these cells neither divide nor die during the entire process of tube formation. Generation of a 3-dimensional SG epithelial tube from a 2-dimensional sheet is driven by morphogenetic signals converging on the motor protein non-muscle myosin II (MyoII). MyoII activity is determined, in large part, by phosphorylation and dephosphorylation of critical residues in its regulatory light chain (MLC). Through a screen for genetic factors affecting SG morphogenesis, we identified *outspread* (*osp*), which encodes a potential myosin phosphatase interacting protein related to the mammalian protein M-RIP (Myosin phosphatase Rho-interacting protein)**.** We have discovered that loss of *osp* or hyperactivation of MyoII result in shorter SG tubes with wider lumens. Osp overexpression or depletion of MyoII have the opposite effect, resulting in longer thinner SG tubes with many cells completely failing to internalize. These findings suggest that Osp plays a suppressive role in MyoII activation and that MyoII drives SG cell internalization. MyoII loss (or Osp overexpression) results in fewer cells invaginating at a given time, explaining the longer thinner tubes with cells remaining on the embryo surface, whereas Myo overactivation (or *osp* loss) does the opposite – more cells invaginating at a given time, explaining the shorter wider SG tubes. Indeed, overexpressed Osp suppresses MyoII accumulation at the apical-medial plane of SG cells. Importantly, Osp directly binds the myosin phosphatase regulatory subunit Mbs through its C-terminal coiled coil domains. In both the invaginating SG and in cultured S2 cells, Osp forms a biomolecular condensate. Osp condensate formation requires the intrinsically disordered region (IDR) located in the middle of the protein. We propose that the Osp condensates serve to concentrate Mbs, providing a molecular mechanism for its negative regulation of MyoII activity. Osp localizes and concentrates MyoII phosphatase through its binding to Mbs to limit MyoII activity during SG invagination, thereby providing a novel regulatory mechanism for controlling spatial and temporal MyoII dynamics during tubular organ morphogenesis.

19 The extracellular protease *AdamTS-B* plays an important role in proper tracheal tube formation during Drosophila embryogenesis

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The Drosophila trachea is a highly branched network of tubular airways made of epithelial cells that go through elaborate migration, cell intercalation and morphogenetic processes throughout embryogenesis. Much work has been done on various aspects of the collective migration of these cells; however, the role of the extracellular environment and the interactions of these cells with this dynamically changing environment are less understood. The extracellular protease *AdamTS-B* is a member of the ADAMTS family, which is known to play an important role in cell migration. Drosophila *AdamTS-B* is expressed in the embryonic trachea from early to late stages of tracheal development. Embryos completely missing *AdamTS-B* display defects in tracheal branching and migration. Specifically, the cells at the transverse connective (TC)/lateral transverse (LT) junction are completely disorganized and misshapen, as compared to wild-type trachea. Conversely, over-expressing *AdamTS-B* throughout the trachea displayed extra-long ganglionic branches (GBs), lateral group branches (LGs), extra LT branches, as well as luminal cysts throughout the LT and GB branches. Preliminary data also shows elongated tracheal cells, and cells that do not seem to have gone through proper cell intercalation. Based on a previous study from our lab that demonstrated a link between AdamTS-B and either Dpp or EGFR signaling in the wing, we are currently examining whether *AdamTS-B* functions through one of these signaling pathways to regulate tracheal tube formation. Taken together, it is clear that this extracellular protease is required for proper tracheal tube formation. Importantly, this data sheds light on the role of the extracellular matrix and its restructuring in formation of epithelial tubes.

20 Cell-intrinsic micropeptide timer: transiency of Pri peptide defines the duration of cell fate induction

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During development, cells gradually differentiate through temporally ordered execution of fate regulatory systems. To ensure the harmonious progression, each system must be activated and subsequently inactivated at the appropriate time. In this study, we show that the duration of fate induction is defined by the transient expression of *polished rice* (*pri*), a gene encoding micropeptides. In Drosophila tracheal development, we found that *pri* is transiently expressed in prospective tracheal placodes and precedes the expression of *trachealess* (*trh*), a master transcription factor that initiates tracheal fate. *pri* induces the expression of *trh* through promoting the disappearance of the repressor form of the transcriptional factor Shavenbaby (Svb). *pri* activates *trh* expression independently of JAK-STAT, a well-known inductive signal of tracheal fate. In lack of both *pri* and JAK-STAT, *trh* expression almost completely disappears. Conversely, after placode invagination, artificially prolonging *pri* expression or constitutive loss of Svb leads to ectopic maintenance of *trh* expression in non-invaginated placode cells surrounding the properly invaginated domain. These results indicate that the rapid disappearance of *pri* properly terminates the initial fate induction system, and suggest that this termination ensures a smooth transition to the subsequent fate-regulatory program—that is, the maintenance of tracheal cell fate specifically in the invaginated cells. Together, we propose that the transiency of *pri* serves as a cell-intrinsic molecular timer that mediates the transition between sequential fate-regulatory systems, ensuring the precise coordination of cell identity during organogenesis.

21 $G\alpha_q$ controls organ size and developmental timing in *Drosophila*

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The G protein alpha subunit, Gαq, transduces extracellular signals from G-protein-coupled receptors (GPCRs) into the cell, playing essential roles in developmental processes such as organ size control, wound healing, and disease. Hyperactivating mutations in the Gαq are associated with Sturge-Weber syndrome and uveal melanoma, and thus, it serves as an important candidate drug target. However, the downstream mechanisms of Gαq signaling remain poorly defined, creating a bottleneck for designing more effective and targeted therapeutics. Here, we used *Drosophila melanogaster* wing discs to investigate the cellular and transcriptional consequences of Gαq dysregulation in a model epithelial system. We found that overexpression of Gαq in the wing discs reduces adult wing size and induces systemic developmental delay. Additional notable phenotypes include decreased apoptosis and reduced proliferation. Transcriptomic profiling reveals that the JAK/STAT signaling pathway is specifically upregulated in Gαq overexpression, but not in Gαq knockdown. Furthermore, perturbing Gαq impacts the cytoskeleton, confirmed by altered localization of phosphorylated Myosin II. Gαq overexpression in the wing disc upregulates stress-response pathways and triggers secretion of Drosophila insulin-like peptide 8 (Dilp8), a hormone that coordinates growth and developmental timing. Functional experiments confirmed that IP_3 receptor (IP_3R)-dependent calcium signaling mediates this delay and that the delay is rescued by the knockdown of Dilp8. In sum, Gαq acts as a critical regulator of epithelial growth and developmental timing via Ca^{2+}-dependent Dilp8 signaling. These findings establish mechanistic links between GPCR signaling, tissue regeneration, and systemic developmental coordination, with broader implications for understanding Gαq-related pathologies in humans.

22 Mechanistic Insights into the Role of Hedgehog Signaling in Ventral Eye Specification in Drosophila

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The Hedgehog (Hh) signaling pathway is crucial for the proper development of eye-head structures and any dysfunction in its pathway components underlines sever disease and developmental defects including Nonsyndromic holoprosencephaly (HPE). In Drosophila eye imaginal disc, Hh expressed posterior to the morphogenetic furrow (MF) and in the dorsal anterior ocellar region and is known to drive morphogenetic furrow (MF) progression anteriorly, indirctly via Decapentaplegic (Dpp). Distinct dorsal-ventral (DV) patterning and Hedgehog (Hh) signaling pathways interact to ensure precise formation of the eye and head. However, the upstream and downstream cross-talk mechanisms mediating this regulation to coordinate proper eye and head development remain poorly understood.

We found that overexpression of Hh in the Dve domain induces ventral eye enlargement while maintaining a normal dorsal eye-head boundary and this phenotype depends on the diffusion of overexpressed Hh from the dorsal to ventral region, suggesting a regulatory cross-talk between Hh and DV patterning pathways. Interaction among eye-head fate determinants including Dpp, Tsh, Hth, and Wg orchestrates cell fate decisions that guide the formation of eye versus head tissues. We investigated how these factors are selectively regulated across dorsal and ventral domains of the eye disc by modulating Hh signaling in the dorsal Dve domain. Our study reveals domain-specific responses in the regulation of key eye-head determinants, providing insights into how morphogen gradients and key molecular interactions contribute to precise eye and head patterning. Overall, these findings highlight a context-dependent mechanism by which signaling pathways integrate to shape the eye field.

23 Identifying the morphogenetic principles of tubular organogenesis in vivo

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Understanding how cells coordinate their behaviors to sculpt complex organs is a fundamental question in developmental biology. Many vital organs, such as the lungs and kidneys, are composed of tubular networks, and their correct formation is critical for proper physiological function. This project investigates the cellular mechanisms of tubular organ formation using the developing *Drosophila* renal system, the Malpighian tubules. Malpighian tubules extend from the midgut-hindgut boundary elongating four times in length, folding on themselves, and reaching their final length and form: a straight proximal region, a curved middle region, and a straight distal region. As a simple and tractable model, these tubules are a great system to uncover fundamental principles of organ morphogenesis. This project will: (1) characterize individual 3D cell shape change and how these behaviors integrate to produce tissue-level morphogenesis (2) identify region-specific differences in cell behaviors and cell packing, and (3) examine the spatiotemporal coordination between cell intercalation and cell shape along the tubule. To address these goals, I am using *in vivo* imaging, to capture and quantify individual and collective cellular changes that drive tubule elongation. To comprehensively map these dynamics, I developed a deep learning-based 3D cell segmentation and tracking pipeline to quantify cell shapes and cell intercalation along the tube. Preliminary data show that intercalating tubule cells in the curved region of the tubule undergo rapid shape deformations within tens of seconds. These cells completed intercalation in ~14 min, faster than the 24-49 min range reported at the straight, distal region (Saxena *et al*., 2014). Furthermore, this cell intercalation event initiated basally and propagates apically, demonstrating that cells show transient geometries as they rearrange. Further analysis will determine if basal initiation is preferred over apical along the tubule or if regional differences along the tubule exist. In ongoing work, I will apply this approach to disrupted tubule morphologies and identify essential cellular behaviors for proper organogenesis. Together, this work will uncover how regional tissue architecture influences cell coordination and remodeling, advancing our understanding of the general principles that govern organ shape and their disruption in disease.

24 Rapid transcriptional response to a dynamic morphogen by time integration

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During development, cells must interpret extracellular signals with speed and accuracy. While morphogen gradients pattern tissues, how cells respond to dynamic morphogens remains unclear. Here, we investigate how dorsal patterning in the *Drosophila* embryo is specified by a rapidly evolving BMP gradient. Using a live reporter of BMP pathway activity and nascent transcription reporters, we find that gene expression is best predicted by time integration of BMP signaling, rather than instantaneous levels. We show that the transcription factor Zen lowers the signaling threshold required for activation, enabling integration to drive rapid transcriptional responses even at low BMP levels. We tested our integration model using *sog* mutants, which alter BMP signaling dynamics. Within the normal expression domain, integration was still predictive of gene expression. However, higher levels of integrated signaling were needed in more lateral regions of the embryo, suggesting additional spatial patterning regulation. Together, these results suggest that cells interpret dynamic morphogen signals through the combined action of temporal integration and spatial competence, providing a framework for robust pattern formation on fast developmental timescales.

25 Deep learning models reveal separable sequence rules for chromatin accessibility and enhancer activation in *Drosophila*

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Identifying and predicting the activity of all enhancers across various cell types during development is a pivotal yet unresolved challenge. ATAC-seq stands out as a versatile assay that measures chromatin accessibility at single-cell resolution across diverse cell types. However, chromatin accessibility is not a direct measure of enhancer activity, and models of chromatin accessibility do not reliably predict enhancer activity levels derived from reporter assays such as STARR-seq performed in the same cell type. To dissect and directly compare these two assays, we trained BPNet models to accurately predict ATAC-seq data and STARR-seq data in *Drosophila* S2 cells from sequence alone. Each model learned and mapped motifs whose individual contributions correlate with the binding affinity of their corresponding transcription factors measured with ChIP-nexus, validating the quality of motifs derived from ATAC-seq and STARR-seq. However, the various transcription factor motifs exhibit different relative contributions towards chromatin accessibility and enhancer activation. While the chromatin accessibility model relies more on motifs bound by pioneer transcription factors, the enhancer activation model depends on a broader combination of transcription factors, including both pioneer and non-pioneer motifs, and more extensive motif-motif cooperativity. To validate the differences in sequence rules, we used a genetic algorithm to mutate enhancer sequences to various levels of enhancer activation while keeping the chromatin accessibility unchanged (and vice versa). We conclude that chromatin accessibility and enhancer activation are encoded by overlapping but distinct sequence rules, implying that accessibility and activation are two separable molecular processes that consecutively determine the activity of enhancers. This suggests that sequence rules derived from chromatin accessibility data could be supplemented with additional motif and syntax rules to better predict enhancer activity.

26 Beyond the Nuclear Pore: A role for Nup153 in transcriptional regulation during development

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Gene expression is tightly regulated during development to give rise to cells with unique morphologies and functions. Each cell has an identical genome but distinct gene expression profiles. A balance between gene activation and repression must be established to ensure that proper gene-regulatory networks are expressed. Conserved chromatin regulators, such as the Polycomb Repressive Complex 2 (PRC2), aid in creating this delicate equilibrium required for cell-fate specification. PRC2 promotes gene silencing through deposition of trimethylation on lysine 27 of histone 3 (H3K27me3). Inhibiting PRC2 function severely disrupts wing development. In an RNAi screen of chromatin regulators, we identified a deeply conserved component of the nuclear pore complex (NPC), Nup153, as a suppressor of these wing phenotypes. Our data indicate there must be a balance between the activities of Nup153 and PRC2 to ensure proper development, and additionally, studies in mammalian cell culture have suggested that Nup153 regulates gene expression. While the developmental function of PRC2 is well understood, the role of Nup153 in gene regulation remains unclear. To determine how Nup153 regulates developmental gene expression, we are identifying the mechanisms by which Nup153 regulates transcription and whether it exerts these functions as a part of the NPC or by redistributing to the nuclear interior. Using tissue culture, we demonstrated that Nup153 knockdown does not change the global levels of histone modifications, including the H3K27me3 mark deposited by PRC2. In contrast to the lack of changes in global histone modifications, we identified thousands of chromatin regions that change in accessibility upon Nup153 depletion and many genes that have altered expression levels. Furthermore, Nup153 binds to hundreds of promoters throughout the genome. Thus, Nup153 may affect gene expression by modulating chromatin structure at promoters. To uncover the developmental role of Nup153 *in vivo* and the relationship with PRC2, we are using a combination of live cell imaging of endogenously GFP-tagged Nup153 and genomics to determine where Nup153 localizes in different tissues and how this localization pattern changes upon PRC2 inhibition. Together, our data will clarify the regulatory balance between Nup153 and PRC2 and how their function is balanced to coordinate developmental programs.

27 Decoding regulatory genome of *Drosophila* brain

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The ~180 Mb *Drosophila* genome encodes the information required to generate an exceptionally complex nervous system comprising over 8,000 cell types and more than 50 million synaptic connections in the adult brain. Despite this compact genome size, gene expression is precisely regulated through an extensive network of non-coding regulatory elements. These elements coordinate the spatiotemporal expression of roughly 14,000 protein-coding genes during brain development. Understanding how this compact genome encodes such neuronal diversity remains a central challenge in neurogenomics. Previous work has focused on functional proteins, such as cell-adhesion molecule (CAM) genes critical for synaptic specificity, or on enhancer activity revealed by large-scale resources like FlyLight, which systematically characterized neuronal driver lines. Yet, the underlying genomic regulatory elements, including enhancers, silencers, tethering elements, and insulators, and their direct target gene relationships remain poorly mapped. Here, we employed Micro-C, a high-resolution chromatin conformation capture technique, to visualize the three-dimensional genome architecture in *Drosophila* brains. Our maps reveal a dense network of chromatin loops spanning kilobase to megabase distances, enabling direct identification of tethering elements and insulators that bridge genes to distant enhancers or insulate genes from proximal enhancers. This integrative approach allowed us to reassign tested enhancer fragments to their true targets and to uncover new enhancer–gene pairs previously hidden in “empty” genomic regions. We highlight two representative examples involving CAM paralog pairs *DIP-ε/ζ* and *hbs*/*sns*, each connected to distal enhancers through megabase-scale chromatin loops. Targeted enhancer deletions and reporter assays demonstrate that these enhancers are essential for long-range transcriptional activation. Further dissection with reporter assays shows that promoter compatibility and promoter-proximal elements modulate enhancer activity, allowing common distal enhancers to differentially activate their linked paralogs. Together, our results reveal a direct connection between 3D genome topology to transcriptional control of CAM genes in the fly brain, offering a mechanistic framework for how a compact genome encodes the vast cellular diversity and connectivity of the nervous system.

28 Relative Enhancer-Promoter Configuration Impacts Transcriptional Dynamics in *Drosophila* Embryos

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The functional role of enhancer-promoter configurations in transcription regulation remains poorly understood, despite the wide range of linear genomic distance and relative enhancer positioning observed in endogenous contexts. While canonical models suggest that enhancers operate independently of genomic position, technical limitations have obscured insights on transcriptional kinetics. Here, we employ single-cell MS2/MCP-based live imaging in *Drosophila* embryos to systematically analyze transcriptional dynamics driven by sub-10kb enhancer-promoter arrangements. Kinetic analyses reveal that while linear enhancer-promoter distance moderately tunes transcriptional output, downstream enhancer positioning reduces mRNA output by 70%. Each configuration modulates distinct transcriptional parameters: linear distance governs initiation kinetics, while relative enhancer positioning dictates transcriptional active state stability. These effects are consistent across varied enhancer and reporter sequences, revealing configuration-dependent *cis*-regulatory element arrangement as an intrinsic mechanism for transcriptional fine-tuning. This work directly correlates enhancer-promoter configuration with a distinct transcription property for the first time and establishes a framework to dissect the interplay between genome architecture and transcription regulation.

29 Balancing precision with plasticity: Redefining the roles of transcription factors in early cell fate specification

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Early embryogenesis requires precise regulation of cell fate specification immediately before gastrulation, yet the molecular mechanisms guiding this transition remain incompletely understood. Using *Drosophila* embryos, we uncover a previously uncharacterized regulatory mechanism that provides both stability and precision to early gene expression, highlighting how embryonic enhancers act as “mentors of the genome” to guide cell specification and differentiation. We focus on a conserved network centered on the pioneer factors (PFs) Odd-paired (Opa/ZIC3), GAGA factor (GAF) together with Suppressor of Hairless (Su(H)/RBPJ), integrating chromatin accessibility, histone modifications, transcription factor binding, and single-cell multiomics datasets to dissect early enhancer function.

We identify two mechanistically distinct classes of early enhancers. The first comprises distal, Opa-dependent elements that are initially inaccessible and require Opa to overcome Su(H)-mediated repression and H3K27me3 silencing. This group controls gene programs required for the transition into post-gastrulation development according to our GO analysis data.

The second class consists of proximal, feedback-primed accessible enhancers that remain accessible independently of pioneer factors due to RNAPII preloading and promoter proximity. These enhancers function as autonomous regulatory hubs, maintaining accessibility and reinforcing cell identity within Polycomb-enriched chromatin.

These feedback-primed enhancers predominantly activate dorsal ectoderm genes, ensuring robust transcription under weak or heterogeneous inputs and preventing patterning errors such as ventral fate expansion or amnioserosa loss. Our results redefine pioneer factors as stabilizers of chromatin accessibility rather than initiators and reveal a dual-mode enhancer mechanism that balances transcriptional precision with developmental plasticity. This decoupling of pioneer factor binding from chromatin accessibility suggests a regulatory strategy that leverages pre-established chromatin states, inherited accessibility, and context-specific cofactors.

This mechanism represents a conserved strategy for early developmental gene regulation, offering a framework for understanding how enhancers integrate multiple regulatory inputs to stabilize cell fate decisions and guide embryonic differentiation. This research was supported through funding from the UTA STARS program.

30 Quantitative analysis of the Notch transcriptional response using synthetic biology and *Drosophila* genetics

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Proper Notch signal strength is crucial for development as haploinsufficiency in the Notch pathway can cause syndromes that impact multiple tissues and organs. While Notch is well known to convert extracellular interactions into changes in gene expression, why some target genes are highly sensitive to gene dose, while others are not, is not well understood. Notch signaling is initiated by ligand-induced cleavage of Notch, releasing the Notch intracellular domain (NICD) to translocate into the nucleus where it activates transcription in complex with the CSL transcription factor, known as Su(H) in *Drosophila*. The Su(H)-NICD complex activates genes via two types of DNA sites: monomer sites and cooperative dimer sites. However, Su(H) can also directly antagonize Notch target gene expression by recruiting the Hairless (H) co-repressor. Unlike the Su(H)-NICD activator, the Su(H)-H repressor complex only binds DNA via a non-cooperative mechanism. While these complexes equally compete for monomer sites, Su(H)-NICD cooperativity provides a ~20-fold greater probability for dimer sites to bind the activator complex than the repressor complex. Here, we use in vivo synthetic biology and statistical mechanics modelling to quantitatively determine how the genomic architecture and relative concentrations of NICD and H control the Notch transcriptional response. We created a series of *Drosophila* GFP reporter lines that only differ in the type and number of DNA sites and use genetics to manipulate levels of Notch and the Hairless corepressor. Our combined quantitative experiments and statistical mechanics model reveal: (1) A distinct threshold of cooperative versus non-cooperative DNA binding sites is required for gene activation. (2) Reporter strength is dependent upon the relative occupancy of binding sites by NICD or H. (3) Reporters with non-cooperative sites are much more sensitive to changes in Hairless levels than reporters with cooperative sites. Ongoing studies are focused on testing new synthetic reporters of different numbers and combinations of binding site types, while also exploring the role chromatin accessibility has on our statistical mechanics’ model.

31 Hedgehog signaling coordinates stereotyped and stochastic patterns in the *Drosophila* eye

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Developmental mechanisms often produce highly stereotyped structures. Within these structures, stochastic fate decisions can diversify cell types, yielding unique and random patterns. How stereotyped and stochastic patterns arise within the same tissue remains poorly understood. Underlying the stereotyped array of photoreceptors and uniform morphology of the *Drosophila* eye is a random mosaic of two R7 photoreceptor subtypes, specified by the stochastic ON/OFF expression of the transcription factor Spineless (Ss). During development, Hedgehog (Hh), secreted by maturing photoreceptors, directs a wave of differentiation driving the stereotyped patterning of the eye. Here, we identify a second role for Hh in the random patterning of R7 subtypes. Reducing Hh pathway activity decreases the size of the eye and the proportion of SsON R7s. Hh-mediated expression of the transcription factor Atonal (Ato) in precursor cells activates expression of Ss. Thus, Hh signaling is finely tuned to coordinate stereotyped and stochastic patterning in the fly eye. Whereas intrinsic and direct cell-cell signaling mechanisms of stochastic fate specification have been well-characterized, our study shows how extrinsic signaling from a distance can randomly pattern cells within a highly stereotyped structure.

32 Splicing regulator Acn suppresses neurodegeneration by moonlighting as a cytosolic activator of autophagy in a TOR-independent pathway

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To remove cellular debris and maintain homeostasis, neurons employ basal autophagy, a pathway that is positively regulated by Acinus (Acn) protein. Under cellular stress, Acn stability is enhanced by phosphorylation of a conserved Serine^{437}, which is regulated by the balance of the activities of Cdk5-p35 kinase complex and the metal-dependent Nil phosphatase [1, 2]. Consistent with the importance of this modification, the phosphomimetic Acn^{S437D} mutation enhances basal autophagy, partially suppresses neurodegeneration and extends life span compared to wild-type flies. Acn is a primarily nuclear protein. The ASAP complex, formed by Acn together with Sap18 and RNPS1, binds to spliceosomes and regulates a subset of alternative splicing, suggesting that Acn may stimulate autophagy through its function in alternative splicing. To test this, we generated the Acn^{RBS} mutant that interrupts its binding interface with Sap18 and RNPS1 and, as predicted, interferes with ASAP-mediated alternative splicing. However, the Acn^{RBS} protein is primarily cytosolic and enhances autophagy indicating that this mutant separates the regulatory functions of Acn in splicing and autophagy. Further support for a splicing-independent, non-nuclear role of Acn came from expression of a membrane-enriched myristoylated Acn that robustly enhanced autophagy. Moreover, an Acn^{NES} mutant that enhanced nuclear occupancy reduced autophagy, whereas an Acn^{NLS} mutant with reduced nuclear occupancy enhanced autophagy. These results are consistent with a model that, despite its primarily nuclear localization, Acn must exit the nucleus to induce basal autophagy which does not require inhibition of TOR. A BioID approach to define the proteome preferentially associated with cytoplasmic Acn identified Rab32. The Rab32 GTPase has been linked to autophagy and binds the LRRK kinase. Loss of Rab32 or LRRK function suppresses autophagy induction in genetically activated Acinus. By contrast, LRRK is not necessary for starvation-induced autophagy. This is consistent with cellular stress inducing autophagy through an Acinus-Rab32-LRRK axis, independent of inhibition of TOR.

1. Nandi, N. et al. (2017) eLife 6:e30760

2. Nandi, N. et al. (2022). eLife 11:e72169

33 Proteostasis & Therapeutic Target Discovery: Mapping VCP Cofactor Specificity Under Glycosylation Stress in Rare Disease Models of DPAGT1-Congenital Disorder of Glycosylation

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Glycosylation is an essential biological pathway that involves the co- and post-translational modification of proteins, determining their structure, functionality, and folding. Mutations in glycosylation-related genes lead to improper protein folding, resulting in rare conditions known as Congenital Disorders of Glycosylation (CDGs). DPAGT1-CDG is an autosomal recessive disorder caused by mutations in the *DPAGT1* gene, which encodes the initial enzyme in *N*-glycosylation. This induces protein misfolding and accumulation within the ER, thereby eliciting cellular stress responses. Patients with DPAGT1-CDG experience severe symptoms, including neurodevelopmental delays, gastrointestinal complications, seizures, among others. Given the high mortality rate and the absence of curative treatments, there is a pressing medical need to identify effective therapeutic options promptly. Our laboratory utilizes *Drosophila melanogaster* and RPE-1 cell culture to develop disease models of DPAGT1-CDG. Previously, we identified key pathways that enhance the survival of *D. melanogaster* following *DPAGT1* inhibition, notably two quality control systems: endoplasmic reticulum-associated degradation (ERAD) and the unfolded protein response (UPR). Our objective is to elucidate how ERAD modulates substrate recognition under glycosylation stress and to identify a therapeutic target for an FDA-approved drug. The UPR is activated by ER sensor proteins—*IRE1*, *PERK*, and *ATF6*—to initiate downstream signaling pathways and promote transcription of ERAD, autophagy, and apoptosis. We find that RNAi knockdown of ERAD components (*HRD1*, *SEL1L*, *EDEM1/2*, *VCP*) rescues the disease phenotype *in vivo*. We hypothesize that under glycosylation stress, ERAD is overburdened and unable to process proteins as quickly as they are synthesized. Notably, *VCP*, a component of ERAD, demonstrated improvement when knocked down and when overexpressed in the disease model. *VCP* participates in two distinct ERAD complexes: ERAD-lumen, involved in degrading proteins, and ERAD-cytosol, which regulates autophagy. Furthermore, knockdown of the autophagy regulator *BECLIN-1*, along with ubiquitin-proteasome cofactors *NPLOC4*, *YOD1*, and *PLAA*, rescued the disease phenotype. This research will provide insights into the interplay between glycosylation, ERAD, and autophagy, thereby enhancing our understanding of the underlying mechanisms and guiding the development of novel therapeutic strategies.

34 The Chemical Fly Cell Atlas: A Single-Nucleus Map of Systemic Xenobiotic Responses in Drosophila

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Evaluating the toxicological impact of xenobiotics requires a systemic, whole organism understanding of their effects across diverse cell types. However, a comprehensive, high-resolution map of chemical-response pathways is lacking. Using Drosophila, we generated a Chemical Fly Cell Atlas (Chem-FCA) based on whole-body single-nucleus transcriptomes, profiling 487,201 nuclei from 76 cell types. This atlas maps the response to 18 distinct chemical compounds, including drugs, pollutants, and synthetic chemicals, creating a rich resource for systems toxicology. Our integrative analysis identified a core universal response signature. It consists of a set of genes commonly upregulated in different cell types across the 18-chemical treatment, representing a general xenobiotic stress response. Meanwhile, we also defined highly distinct gene signatures unique to specific chemicals in specific cell types, such as a strong metabolic response localized to fat body and oenocyte. Furthermore, by analyzing the data by sex, we uncovered the differences in the sex-specific response to chemical stress. The Chem-FCA offers new insights into the systemic impact of chemical exposure and provides a valuable resource for defining universal biomarkers for future toxicological screening. This work also demonstrates that Drosophila, when combined with whole-body single-cell sequencing, serves as a cost-effective testing model that enables tracing the evolutionary origins of toxicity pathways across species and predicting potential health risks to humans

35 A Ubiquitous Sphere of Influence: Defining the Roles, Partners and Targets of E3 Ligases in Synaptonemal Complex Function during Meiosis

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The synaptonemal complex (SC) is a ribbon-like protein structure that forms during early meiosis and facilitates meiotic recombination. Work in *Drosophila* and other species have shown that post-translational modifications like ubiquitination and SUMOylation guide SC assembly, maintenance and disassembly, while the enzymes controlling modifications use the SC as a platform for signal transmission. However, the enzymatic targets or mechanisms that ensure SC function remains poorly understood.

Our lab is studying how E3 ligases regulate the assembly, stability and function of the SC. E3 ligases catalyze the final step of ubiquitin or Small Ubiquitin-like Modifiers (SUMO) conjugation onto targets, marking them for degradation, scaffolding, or further modification. Recent work shows that a single amino-acid mutation in E3 ligase seven in abstentia (Sina) interrupts the normal dynamics of SC assembly, while reduction of deubiquitinase Usp7 expression leads to early collapse of the SC, indicating important but unexplored roles for ubiquitin in SC dynamics. In addition, orthologous to mechanisms seen in other organisms, the RING-containing E3 ligases Vilya, Narya and Nenya localize first to the SC, then to sites of double-strand breaks that resolve as crossovers. In order to define how ubiquitin and SUMO regulate SC dynamics and crossover resolution in *Drosophila* female meiosis, we are using proximity-based labeling techniques and mass spectrometry to uncover the targets of E3 ligase activity, as well as the co-factors and adaptor proteins that convey target specificity. We are also seeking to define the mechanisms that regulate how E3 ligases facilitate meiotic events such as SC maintenance and crossing over by targeting E3 ligases to specific locations using a LacO/LacI tethering system. Determining the targets, regulation, and functions of E3 ligases during meiosis is highly relevant to understanding the mechanisms to prevent meiotic errors that lead to infertility and miscarriage.

36 Neighboring cells support dividing cells to preserve epithelial architecture in the *Drosophila* wing imaginal disc

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Epithelial tissues must maintain their integrity while undergoing continuous cell divisions. Columnar epithelial cells, such as those in the *Drosophila* wing imaginal disc, face a particular challenge: their elongated geometry and tight packing restrict space for mitotic rounding. How these cells overcome crowding to move apically and preserve tissue architecture during division remains unclear. Using live imaging of wing imaginal discs, we show that dividing cells first lose basal attachment to the extracellular matrix and then generate basal-to-apical F-actin flows that elevate the cell body toward the apical surface, allowing it to adopt a spherical shape. Meanwhile, neighboring cells assemble F-actin beneath the dividing cell, effectively “zipping up” the basal space to maintain epithelial continuity. Disruption of F-actin polymerization in neighboring cells leads to downward displacement of dividing cell nuclei, resulting in an altered orientation of the cell division plane. These findings reveal a previously unrecognized cooperative mechanism between dividing and neighboring cells that ensures mechanical integrity and proper architecture of proliferating epithelia.

37 Yorkie Activation Restores Epithelial Integrity in Neoplastic Tumors of *Drosophila* Wing Discs

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Our recent work has established a link between mitosis and tumor progression in epithelial tissues. Using the Drosophila wing disc epithelium as a model, we have found that enhancing cell division by overactivation of the cell cycle regulators E2F1 or Cdk1 not only causes an increase in tissue size, but also exerts morphogenetic stress on the epithelium resulting in permanent loss of epithelial integrity, leading to neoplasia. We have also shown that decreasing cell proliferation in neoplastic models such as *scrib* knockdown or Crb overexpression rescues restores epithelial integrity, further implicating enhanced cell proliferation as a driving force for tissue growth and neoplastic tumor progression. In contrast, accelerated cell proliferation mediated by Yorkie (Yki) activation does not result in loss of epithelial integrity, causing hyperplasia rather than neoplasia. Yki is a downstream effector of the Hippo pathway and promotes tissue growth via transcription of pro-proliferative and anti-apoptotic genes such as CyclinE and Diap1. Dysregulation of the Hippo pathway and increased activity of Yki or its mammalian homologs YAP/TAZ have been implicated in numerous epithelial cancers. In the *Drosophila* wing disc, overexpression of Yki (Yki-WT), or its constitutively active form (Yki-3A) results in dramatic hyperplastic tissue overgrowth and extensive epithelial folding.

To explain this discrepancy between neoplastic tumors and Yki-mediated hyperplastic tumors, both driven by excessive cell proliferation, we hypothesize that Yki has target genes that can stabilize or restore epithelial polarity, counteracting proliferation-induces morphogenetic stress that causes a loss of epithelial polarity. To test this hypothesis, we have expressed Yki-WT and Yki-3A in various neoplastic backgrounds and examined both epithelial integrity and levels of JNK signaling, a signature of neoplasia. Preliminary observations indicate that Yki activation can strongly suppress neoplastic development and rescue epithelial polarity in neoplastic tumor mutants. A significant reduction of JNK signaling is also observed. These findings suggest that Yki plays a dual role, enhancing proliferation and protecting epithelial integrity at the same time.

38 A fast-evolving C-terminus underlies species-specific function of an orphan gene required for sperm nuclear shaping

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Orphan genes are lineage-restricted and encode proteins without detectable homology. In *Drosophila melanogaster*, the orphan gene *saturn* is required for male fertility and encodes an adaptively evolving protein, but its cellular function and the consequences of its rapid evolution have remained mysterious. Here, we used a CRISPR-generated null allele, tagged rescue constructs and immunofluorescence to demonstrate that *saturn* is required for spermatid nuclear shape changes. Null males are near-sterile, show defective spermatid nuclear shape, and fail to complete sperm individualization, while immunofluorescence in wild-type males shows that Saturn protein localizes to the nuclear membrane precisely during this transition. To understand the functional consequences of Saturn's rapid, adaptive evolution, we performed gene swap experiments in which we assayed the ability of *saturn* orthologs from other *Drosophila* species to restore fertility in *D. melanogaster* males lacking endogenous copies of the gene. The degree of fertility rescue declined with evolutionary distance, culminating in the complete inability of the *D. ananassae* ortholog to restore fertility, despite its robust expression and proper localization to spermatid nuclei. By constructing chimeric proteins containing portions of the *melanogaster* and *ananassae* orthologs, we mapped this cross-species incompatibility to Saturn's rapidly evolving C terminus, suggesting that this region may coevolve with other spermatogenesis components. Finally, using an antibody raised to the *ananassae* Saturn ortholog, we found that this protein's subcellular localization is conserved in its native species, suggesting that Saturn evolved this localization pattern ancestrally. These results reveal an essential role for an orphan gene in spermiogenesis and demonstrate concrete functional consequences to its rapid, adaptive evolution.

39 Uncovering the mechanism behind female meiotic drive of B chromosomes in *D. melanogaster*

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Recently discovered supernumerary B chromosomes in *D. melanogaster* exhibit higher-than-Mendelian transmission (>50%), or drive, during female meiosis, ensuring their inheritance into the next generation. Foundational work identified that this drive is dependent on a genetic reduction of *matrimony* (*mtrm*), which normally regulates Polo kinase (Polo) in a 1:1 genetic ratio during female meiosis to ensure proper segregation of non-crossover chromosomes. We genetically manipulated this ratio using null alleles and found that B chromosome transmission decreases as the levels of *mtrm* increase relative to *polo*, suggesting that an excess of unregulated Polo promotes B chromosome drive. The direct interaction of Polo and Mtrm is necessary to suppress this drive because the expression of a *mtrm* allele that cannot bind Polo ($mtrm^{T40A}$) is unable to restore Mendelian B chromosome segregation. To determine if Mtrm-independent regulation of Polo can also influence the drive of the B chromosomes, we measured B chromosome transmission in the presence of a hypermorphic allele of *greatwall* (gwl^{Scant}), a meiotic and mitotic kinase that antagonizes Polo activity. Our data show a significant reduction in B chromosome transmission in gwl^{Scant} females, leading us to conclude that unchecked Polo activity during meiosis promotes drive of the B chromosomes. To begin connecting how a reduction in Mtrm – and thus misregulated Polo - may promote B chromosome drive, we are directly investigating the segregation behavior of the B chromosomes during the female meiotic divisions in both wild-type and Mtrm-reduced genetic backgrounds. Preliminary findings reveal the presence of multiple meiotic spindles and lagging chromosomes during anaphase when Mtrm is reduced. Overall, our work demonstrates that the regulation of Polo kinase activity, such as through adequate levels of Mtrm, is necessary to suppress meiotic drive of B chromosomes. Furthermore, our work reveals that factors known to prevent chromosome missegregation during meiosis may also have a novel function to prevent biased chromosome segregation, which may protect the genome from the accumulation of selfish supernumerary genetic elements.

40 Morphological innovation without gene co-option: the *Drosophila* sex comb evolved via changes in developmental tempo and energy metabolism

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The individualization of serially repeated homologs is one route through which novel traits are thought to evolve. Under this model, a repeated character—like a limb, digit, or sensory bristle—is individualized from its homologs by changes in the regulatory apparatus ('character identity network', ChIN) that specifies its development. Individualization then enables downstream gene networks that build the repeated character to diverge from one another in different parts of the body, ultimately allowing new phenotypic endpoints to be reached. Despite this model's intuitive appeal, the genetic mechanisms through which new ChINs rewire trait-building gene networks remain largely uncharacterized. A promising system in which to study this process is the *Drosophila* sex comb. Found in a sublineage of *Drosophila* species, the sex comb is a recently evolved, male-specific innovation that evolved from a more evolutionarily ancient precursor—the mechanosensory (MS) bristle—following the gain of a novel ChIN centered on the sex determination gene *dsx* and HOX gene *Scr*. Here, we use time-series single-cell RNA-seq to show that rather than co-opting new genes, this new ChIN orchestrates quantitative and heterochronic changes in the ancestral MS bristle transcriptome. These changes affect gene modules that control energy metabolism, endoreplication, and actin dynamics. The net effect of these changes is an organ-specific shift in developmental rate, leading to accelerated growth in sex comb teeth. Collectively, our work suggests that morphological innovation can proceed without the co-option of new genes into downstream trait-building networks and instead through metabolically driven differences in developmental rate between serial homologs.

41 Distinct ancestral origins and limited repeatability of clinal variation between South America and other continents

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The colonization of habitats worldwide by Drosophila melanogaster makes it an excellent model to study adaptive processes on recent timescales. Latitudinal clines in phenotypic and genetic markers observed in North America and Australia support the species' recent adaptation to different climates. However, whether similar patterns occur in South America remains unknown. Here, we investigate clinal genetic variation in South America by asking: (1) what are the geographical origins of South American populations, and (2) do South American clinal patterns parallel those from other continents? We used Pool-Seq data from six populations spanning 35 latitudinal degrees (45° S to 7° S). To infer ancestral origins, we used published data from Europe and Africa. To assess the repeatability of clinal evolution we compared our results to published data from Australia and to North American data from two time points (1997 and 2009/2010). Our results reveal admixture between European and African ancestry in most of the Brazilian populations, whereas Argentinean populations show exclusively European ancestry, highlighting a population structure within South America. We found that 2% of the South American SNPs are clinal. These clinal SNPs are enriched for functional categories (e.g. nonsynonymous coding and UTR) on the X chromosome but not the autosomes. We used population branch excess (PBE) to identify signatures of selection between clinal extremes, highlighting SNPs that may underlie climate adaptation in South America, including regions without functional enrichment. Comparisons with North America and Australia revealed limited overlap among clinal SNPs, indicating that clinal evolution has a heterogeneous molecular basis. Only ten neighboring SNPs are clinal across all continents and show signatures of selection, seven of these occur within the lethal (3) 80Fg gene, an orthologue of the human Hsp40. Our study fills a gap in the species' out-of-Africa history and advances our understanding of the genetic basis of repeated clinal adaptation.

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42 Replication-Enhanced Detection of Quantitative Traits under Adaptation (REDQuanTA): an improved statistical framework to detect locally adaptive traits

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Trait differentiation can be due to genetic drift, mutation, migration, and selection. Q_{ST}–F_{ST} comparisons, which evaluate trait differentiation to neutral genetic differentiation, are a classic tool to infer locally adapted phenotypes, but its current application is hampered by demanding breeding designs, environmental variance that can confound Q_{ST} estimation if not well controlled, and biases in Q_{ST} estimators. We present AdaptiveQ_{ST}, a statistical framework and experimental design that leverages replicated measurements within genotypes to separate environmental from genetic variance, enabling less biased estimation of Q_{ST} and detection of local adaptation without formidable experimental demands. For a given trait, such as the mRNA expression of a gene, AdaptiveQ_{ST} estimates Q_{ST} using a combination of nested ANOVA and approximate bayesian computation (ABC) approach, compares the estimated query Q_{ST} to a null distribution of Q_{ST} estimated from trait data simulated under empirical F_{ST} distribution of neutral loci, then tests if the query trait Q_{ST} is larger than the 95th percentile of the Q_{ST} distribution of neutral traits. By accounting for environmental variance of each trait during the simulation of neutral trait data, this dynamic thresholding strategy offers robust power and consistent false positive rates across different types of traits that have varying levels of environmental variance. By comparing query Q_{ST} to the Q_{ST} distribution of neutral traits, instead of F_{ST} distribution as in traditional practices, our method also avoids biases introduced by the Q_{ST} estimator. By standardizing how Q_{ST} is estimated and tested, AdaptiveQ_{ST} also makes detection of adaptation comparable across diverse organismal and molecular traits, such as chromatin accessibility, mRNA expression, and protein abundance. We illustrate the utility of this framework using demographic models of natural *Drosophila melanogaster* populations, and offer practical guidelines for experimental design that can maximize the power of detection of locally adaptive traits with even limited numbers of samples. AdaptiveQ_{ST} will facilitate the identification and cross-level comparison of locally adapted traits in *Drosophila* and other species. Beyond the ability to analyze organismal phenotypes, it will also provide a better toolkit to study adaptive evolution on unexplored molecular trait types.

43 The coevolutionary dynamics of transposable elements and their hosts in Drosophila

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Transposons are mobile pieces of DNA that replicate selfishly within animal genomes, and they are widespread across eukaryotic life. The co-evolution between transposons and their hosts is a major source of selective pressure. Hosts can suppress transposon movement, which in turn puts pressure on the transposon to resist host defenses or find a novel genome to invade. In *Drosophila* the advent of long read sequencing has allowed scientists an unprecedented ability to document the movement of transposons within and between genomes. We have found evidence of extensive recent horizontal transfer in insect genomes, far beyond that expected given the known number of transposon families. In *D. melanogaster* alone there have been twelve transposon invasions in the last two hundred years, compared to an expected rate of about one every 100,000 years. We posit that because D.melanogaster is a humna commensal, this may be due to human introduction of dipterans to new territories and thus, new congeners. However, we have also found that the rate of TE invasion in other drosophilids is quite high, suggesting this phenomenon may be quite widespread. For example, we have evidence of a single TE transferring recently between species as many as 16 times. The implications of this genomic instability induced by anthropogenic changes to the environment is unknown, but is a potentially important selective factor on species genomes.

44 Investigating the early damage response in *Drosophila* wing imaginal disc regeneration

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Organisms experience tissue damage, and their ability to regenerate varies widely across species. Regeneration is a coordinated process involving wound repair and tissue regrowth. One of the earliest responses to injury is wound closure, marked by restoration of epithelial continuity, clearance of cellular debris, and dynamic changes in epithelial architecture. How these early damage responses progress to successfully initiate regeneration remains poorly understood. We use a genetic damage-induction system in *Drosophila* wing imaginal discs, which are the larval tissues that give rise to adult wings. Following damage, epithelial cells in the affected region exhibit changes in cell architecture, including flattening, elongation, and loss of their columnar morphology. They regain their columnar structure within 24 hours post-damage. To uncover the regulators of these epithelial transitions during wound closure, our lab previously screened actin-associated genes for a role in regeneration and identified *Btk29A*, the *Drosophila* ortholog of Bruton's tyrosine kinase, as crucial for regeneration. *Btk29A* heterozygous animals failed to regenerate fully, with cellular debris persisting at the basal surface, suggesting impaired debris clearance, possibly resulting from or trapping by extracellular matrix (ECM). To identify additional regulators of early disc regeneration, we screened known *Btk29A* activators and effectors and identified *WASH* and *Src42A* as candidates involved in the regenerative response. WASH is an actin nucleation-promoting factor that regulates Arp2/3-dependent branched actin assembly, and *Src42A* is one of the two Drosophila homologs of the non-receptor tyrosine kinase Src. Notably, *Btk29A*, *Src42A,* and *WASH* mutants all exhibit irregular and compacted wing disc morphology immediately after tissue ablation, indicating early defects in epithelial organization and tension following damage. *WASH* heterozygous mutant wing imaginal discs failed to regenerate, and epithelial cells did not flatten or elongate, retaining their columnar shape. By contrast, *Src42A* heterozygotes resembled *Btk29A* mutants, with poor regeneration and cellular debris trapped basally. These findings suggest that *WASH* is required for epithelial reorganization following damage, while *Btk29A* and *Src42A* may be required for debris clearance or extracellular matrix remodeling after damage. We hypothesize that these changes in cytoskeletal dynamics and ECM remodeling affect mechanical tension, vesicle trafficking, and growth signaling during tissue regeneration. Thus, we are currently investigating cytoskeletal changes, ECM remodeling, tension, vesicle trafficking, and growth signaling in these mutants during wound closure and the early stages of regeneration.

45 Netrins are involved in the epithelial wound response in *Drosophila* and zebrafish

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The Netrin family of proteins are secreted chemotropic guidance molecules with well-established roles in axon guidance. Recent findings have shown that Netrins also regulate the developmental morphogenesis of epithelial sheets in *Drosophila*, zebrafish and humans, and can aid repair in kidneys and corneas.

We hypothesised a role for Netrins in controlling the morphology and response of epithelial sheets during repair. Netrins may act as diffusible ligands that drive epithelial plasticity through its known receptors, such as Frazzled, and this system may be used in epithelial remodelling and closure in both development and repair.

We found a conserved response in both zebrafish and *Drosophila* where Netrins are rapidly upregulated in response to mechanical wounds of fin and wing disc tissue respectively. In zebrafish, immunostaining revealed marked Netrin upregulation within 30 minutes of wounding tail fins. Furthermore, combined knockdown of *Netrin1a* and *Netrin1b* resulted in impaired tissue regeneration in the tail fin. In Drosophila, live imaging of punch-wounded wing imaginal discs undergoing closure similarly showed upregulation of an endogenous NetrinA-GFP reporter specifically in cells surrounding the wound edge. Using the DUAL control genetic ablation system (Harris et al., 2020, *eLife* **9**:e58305), we found that both overexpression of NetrinA, and either RNAi knockdown or overexpression of NetrinB improved wound repair in the disc proper of the wing disc.

To investigate the upstream regulators of NetrinA in *Drosophila*, we utilised the GAL4-UAS system to overexpress transgenes in a band down the midline of the disc using patched-GAL4, along with the long-lived marker H2A-RFP. This allowed for within-tissue comparisons and detection of non-cell-autonomous effects. Expression of activated forms of the Jun-N-terminal-Kinase Kinase, Hemipterous, and of the Src Family Kinase, Src42A, a known upstream regulator of the JNK pathway, both resulted in upregulation of NetrinA-GFP and disruption of epithelial integrity. Interestingly, expression of Snail, a key transcription factor driving epithelial-mesenchymal transitions, also led to NetrinA-GFP upregulation, suggesting that epithelial dissociation may itself activate Netrin expression.

In summary, Netrins are upregulated rapidly around the edges of epithelial wounds and modulate repair outcomes. The conservation of this response in both *Drosophila* and zebrafish raises the possibility of a similar function in humans, with potential therapeutic use.

46 Damage recognition by intestinal stem cells via Draper-Src-Shark-STAT signalling promotes adult *Drosophila* midgut regeneration

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Regeneration after injury is crucial for restoring epithelial structure and function. Upon damage, a regenerative microenvironment forms that provides cues that stimulate stem cell proliferation to replace damaged or lost cells. While this process is well understood, how stem cells themselves sense damage, translate this into their proliferation and shape the regenerative microenvironment remains unclear. Here we show that Draper-Src-Shark signalling in ISCs recognises midgut damage by sensing phosphatidylserine on dying midgut epithelial cells and is required for STAT activation in ISCs to promote their proliferation. Unlike its known role in phagocytosis, Draper in ISCs does not promote the clearance of apoptotic enterocytes but rather is required to boost ISC proliferation in the presence of damaged enterocytes. Moreover, Draper-Src-Shark signalling in progenitors regulates STAT activity in the visceral muscle, indicating that progenitors can shape the regenerative microenvironment. As Src and Stat are activated in the mammalian intestinal epithelium after damage and tumour formation, these findings may help develop therapies for tissue regeneration, inflammatory diseases and cancer.

47 Grainyhead regulates wound-induced polyploidization in *Drosophila*

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Wound repair is critical for organism survival, and while many organisms replace lost cells through cell division, wounds can also heal by cell growth when cells become polyploid. Polyploidy is the more than doubling of a cell's diploid genome and can arise by endoreplication or cell fusion. The adult fruit fly epithelium relies on both mechanisms to repair puncture wounds. Using this model of wound-induced polyploidization, the molecular mechanisms that regulate the endocycle and cell fusion can be elucidated. Grainyhead (Grh), a conserved pioneer and transcription factor, was previously reported to regulate DNA replication, cell adhesion, and extracellular matrix genes which facilitate epithelial differentiation and wound healing during embryonic development. However, Grh's role in adult epithelium and polyploid cell growth has not been tested to date. Using the Gal4/UAS system to conditionally regulate Grh expression, we found that Grh is required, but not sufficient for entry into the endocycle post injury. Wound-induced endocycling is also dependent on the transcriptional coactivator Yorkie (Yki), and knockdown of Grh is sufficient to block Yki-induced gene expression (bantam reporter). Likewise, knockdown of Grh reduces the endocycle even when Yki is overexpressed. We suspect that the genetic interaction between Grh and Yki is dependent on Grh's ability to regulate chromatin state as a pioneer factor. In addition to its effect on the endocycle, we found that Grh regulates epithelial cell fusion post injury. Epithelial specific knockdown or knockout of Grh significantly reduces syncytium size, which appears to be due to a reduction in cell adhesion (FasIII). Therefore, we have discovered that Grh is a master regulator of wound-induced polyploidization as it regulates both cell fusion and the endocycle. This is of particular importance as Grh is also a key regulator of epithelial cancers. Thus, our findings may help to elucidate the link between polyploidization and cancer through Grh's regulation of epithelial cell polyploidization.

48 The Role of Hemocytes in Regeneration Following Necrotic Ablation

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Necrosis is an unregulated form of cell death characterized by membrane swelling and rupture, resulting in the leakage of intracellular contents. It is highly prevalent in a number of significant human medical conditions, including ischemia, cancer, and physical trauma. Despite this, relatively little is known about how necrotic cells influence surrounding healthy tissues and stimulate regenerative responses. The Harris lab has advanced the study of necrosis in *Drosophila* by developing two novel necrotic ablation systems within the wing imaginal disc. Recently, we have discovered non-apoptotic functions of the initiator caspase, Dronc, which are crucial to sustaining proliferation following necrotic ablation.

Importantly, Dronc has previously been shown to be vital for promoting tumor-like growth in the "undead" cell model, where cells overexpress pro-apoptotic factors but are unable to die due to p35-mediated inhibition of effector caspases. This model has been invaluable for deciphering apoptosis induced proliferation (AiP), the phenomenon in which apoptotic cells promote cell proliferation and survival. While "genuine" and "undead" AiP share many similarities, including a complex, poorly understood interplay between JNK signaling and Dronc, several important differences are evident. Namely, the basement membrane (BM) becomes disrupted in the undead model, leading to the recruitment of hemocytes which amplify JNK signaling, a conserved feature in various cancer models.

Given the similarity we see with the undead cell model, as well as the highly immunogenic and inflammatory nature of necrosis, we wanted to investigate if BM disruptions and hemocyte signaling occur following genetically-based necrotic ablation. To do so, we used several validated fluorescent reporter lines for hemocytes and the BM. Surprisingly, we found that hemocytes are not recruited to multiple forms of genetic necrotic ablation and that the BM remains continuous. Interestingly, overexpression of Mmp2, which degrades the BM, during ablation causes the attraction of hemocytes and dramatically improves clearance of apoptotic cells from the wing disc. However, our preliminary data suggest this largely does not improve regenerative outcomes, as assayed by adult wings. Overall, we conclude that regeneration following necrosis in the wing imaginal disc likely occurs without immune cell signaling.

49 The *Drosophila* proventriculus lacks stem cells but compensates for age-related cell loss via endoreplication-mediated cell growth

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The *Drosophila* proventriculus is a bulb-shaped structure at the juncture of the foregut and the midgut, which plays important roles in ingestion, peritrophic membrane synthesis, and the immune response to oral pathogens. A previous study identified a population of cells in the proventriculus which incorporate bromodeoxyuridine (BrdU), a marker of DNA synthesis, and proposed that these cycling cells are multipotent stem cells that replace dying cells elsewhere in the tissue. Here, we re-investigate these cycling cells and find that they do not undergo mitosis, do not generate clonal lineages, and do not proliferate in response to tissue damage, and are therefore not stem cells. Instead, we find that these cells continually endoreplicate throughout the fly's life, increasing their ploidy and cell volume, while at the same time nearby cells are lost into the gut lumen in an age-dependent process. These cells play a critical role in the synthesis of peritrophic membrane components, and we show that when endocycling in these cells is experimentally increased or decreased, nuclear and cellular volume as well as peritrophic membrane synthesis is concomitantly enhanced or reduced. Further, we show that blocking endocycling in these cells leads to functional defects in the peritrophic membrane, ultimately leading to increased susceptibility to orally infectious bacteria. Altogether, we show that continual endocycling of these cells is critical for maintaining tissue size and function over the course of the adult lifespan.

50 Hippo signaling regulates cuticle pigmentation and dopamine metabolism in *Drosophila*

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Pigmentation plays multiple important roles in development, physiology and evolution. Melanization of the insect cuticle requires dopamine as a precursor of melanin and involves key enzymes in dopamine biosynthesis including Tyrosine hydroxylase (TH) and Dopa decarboxylase (Ddc). Some studies have hinted that disruption of the evolutionarily conserved Hippo signaling pathway, which has been primarily studied in the context of tissue growth, may lead to changes in cuticle pigmentation in the fruit fly *Drosophila melanogaster*. However, there have not been any systematic investigations into their potential mechanistic links. In this study, we identified that all genes that comprise the canonical Hippo signaling pathway [*hippo* (*hpo*), *salvador* (*sav*), *mats*, *warts* (*wts*), *yorkie* (*yki*) and *scalloped* (*sd*)] are involved in cuticle pigmentation in the fly notum based on tissue-specific gene knock-down/out experiments and epistatic analysis. Despite the notable divergence of pigmentation mechanisms between invertebrates and vertebrates, these phenotypes can often be rescued by the human orthologs of corresponding fly genes. While we find that manipulation of Hippo signaling in dopaminergic neurons does not affect global dopamine levels in the fly brain, developmental inhibition of this pathway can increase dopamine levels in the fly head, indicating that the mechanism by which dopamine levels are regulated in the nervous system is distinct from that in epithelial cells. Through single nuclei RNA sequencing of the developing fly nota and subsequent functional studies of differentially expressed genes that are altered upon inhibition of Hippo signaling, we found four genes [*ciboulot* (*cib*), *transaldolase* (*taldo*), *yellow* (*y*) and *Insulin-like receptor* (*InR*)] that contribute to cuticle pigmentation downstream of *wts*. Finally, we provide multiple pieces of evidence that indicate that the function of Hippo signaling in cuticle pigmentation and tissue growth can be genetically uncoupled and also identify *beaten path Vc* (*beat-Vc*) as a novel tissue growth regulator that acts downstream of *wts* but is not required for cuticle pigmentation. We conclude that regulation of cuticle pigmentation by canonical Hippo signaling acts through multiple downstream genes rather than directly through *TH* and *Ddc*. We also propose that the *Drosophila melanogaster* cuticle may serve as a useful platform to identify previously uncharacterized mediators of Hippo signaling as well as an *in vivo* experimental system to test the functionality of rare genetic variants found in human Hippo signaling orthologs associated with a variety of diseases.

51 3D Chiral Morphogenesis of the Embryonic Midgut

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Breaking left-right (LR) symmetry is fundamental to the positioning and function of visceral organs, including the looped structure of the digestive tract. Asymmetric looping allows for compact elongation of digestive organs inside of the confined body cavity. While amniotes are known to break LR symmetry through early genetic patterning of growth factors, no LR asymmetric gene expression patterns have been identified in the developing fly embryo to date. Instead, *Drosophila* LR symmetry-breaking is dependent on the unconventional class I myosins, Myo1D and Myo1C. The gut is the first to break LR symmetry, forming a coiled loop. Myo1C activity reliably inverts both the hindgut and midgut when driven in tissues expressing the transcription factor Brachyenteron (Byn), which includes the hindgut and the longitudinal visceral muscles surrounding the midgut. How, where, and when does the activity of Myo1C inform cell and tissue-scale behavior to determine the chirality of the midgut? Because Myo1C overexpression in the *byn* domain is sufficient to invert coiling, we investigated *byn* mutants, which lack the hindgut and longitudinal muscles. We found that their midguts nonetheless coil, but show randomized chirality; therefore, the *byn* domain controls the initial asymmetry, but is not required for coiling mechanics. In further experiments, we found that genetically ablating the longitudinal muscle is likewise sufficient to randomize chirality. An overexpression screen suggests the *byn* domain is unique in its control, and we isolate the developmental timing of Myo1C's chirality-inverting activity using heat-shock induced Myo1C expression. Altogether, our data suggest a muscle-dependent, post-translational mechanism for controlling organ chirality across tissue layers.

52 Puratrophin-1–like RhoGEF controls epithelial morphogenesis during *Drosophila* development

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Epithelial morphogenesis requires precise regulation of Rho-family GTPases to coordinate cytoskeletal remodeling, ensuring proper cell shape changes and rearrangements. Here, we identify the *Drosophila* RhoGEF Puratrophin-1-like (Pura) as a key regulator of Rho1 and Rac1 activities during epithelial development. We show that *pura* is expressed in all epithelial cells during embryogenesis, with strong upregulation in the trachea throughout morphogenesis and transient expression in the salivary gland. An endogenously tagged Pura:mKate2 transgenic line confirms ubiquitous expression with marked upregulation in the trachea, consistent with the mRNA pattern. *pura* null mutants generated via CRISPR/Cas9 are viable and fertile but exhibit developmental delay and reduced viability. In *pura*$^{m/z}$ mutant embryos, tracheal branches display discontinuities accompanied by mislocalized E-Cadherin, disrupted apical F-actin, and altered Rho1 and Rac1 activity distributions. These phenotypes are rescued by tracheal-specific Pura overexpression, indicating a cell-autonomous requirement. In the salivary gland, *pura*$^{m/z}$ mutants exhibit defective apical constriction during invagination, while salivary gland-specific *pura* knockdown suppresses hyperconstriction induced by excessive Fog–Rho kinase signaling, indicating that Pura modulates Rho1 activity output. Isoform-specific analysis reveals that the long N-terminal region unique to Pura-PA mediates apical targeting via vesicular trafficking, and overexpression of apically localized Pura induces excessive membrane growth and apoptosis. Finally, *pura*$^{m/z}$ mutants exhibit germband retraction defects, characterized by increased apical areas and impaired cell shape changes in germband and amnioserosa cells, along with disorganized myosin cables at parasegment boundaries. Together, these findings establish Pura as a critical spatiotemporal regulator of Rho-family GTPases that orchestrates epithelial tube formation and tissue morphogenesis in *Drosophila*.

53 Developmentally regulated changes in basement membrane composition affect matrix viscoelasticity and tissue morphogenesis

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Organs are formed, in part, by an imbalance of physical forces that drive changes in tissue shape. One such force is constraint from the extracellular matrix (ECM). We are using the *Drosophila* egg chamber (ovarian follicle), which is surrounded by a basement membrane (BM) ECM and elongates >2-fold along its anterior-posterior (A-P) axis, to study how differential patterning of BM mechanics can lengthen an organ.

The egg chamber consists of a central germ cell cluster enclosed by follicular epithelial cells, with a BM encasing the whole tissue. Early in development, the follicle cells mechanically pattern the BM, creating a softer BM at the poles of the egg chamber and a stiffer BM in the central region. This stiffness gradient is thought to channel germ cell growth along the A-P axis to lengthen the tissue. While the field has long assumed that this mechanical patterning controls tissue lengthening throughout development, published data indicate that the symmetric A-P stiffness gradient later transforms into a sharp division between a soft anterior and stiff posterior BM. Our data show that the change in mechanical patterning coincides with increased Perlecan levels and decreased Laminin levels in the anterior BM region. We hypothesize that this compositional change drives the shift in BM mechanics to accommodate a change in the pattern of germ cell growth, from being largely symmetric during early developmental stages to being posteriorly focused during later stages. Using the sophisticated genetic tools and *ex vivo* culture system available for the egg chamber, we have also developed methods to study how BM composition affects its viscoelastic properties, revealing region-specific variations in the BM's response to stress over different timescales.

These studies are beginning to reveal how the mechanical patterning of a BM can be altered over time to meet the changing demands of a developing tissue. Furthermore, they highlight the utility of the egg chamber as a platform to study the fundamental viscoelastic properties of the BM in a living organ.

54 Innexins orchestrate border-cell specification and delamination via channel-dependent and channel-independent mechanisms

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During embryonic development and cancer metastasis, cohesive cell groups must detach from an epithelium to initiate migration. We establish the *Drosophila* border cell cluster as a tractable in vivo model for this *delamination* step. In oogenesis, border cells are specified from the follicular epithelium, delaminate, and migrate toward the oocyte. Here, we identify gap-junction–forming Innexins (Inx1, Inx2, Inx3) as critical regulators acting through two separable mechanisms. First, channel-dependent Innexin activity is required for border cell specification. Second, channel-independent functions of Inx1/2/3 regulate integrin-mediated adhesion to drive delamination. Our findings position Innexins as bifunctional coordinators that link cell–cell communication to adhesion remodeling, providing a mechanistic framework for how epithelial collectives start migration—an organizational principle relevant to normal development and disease.

55 Activation versus repression: evolutionary rewiring of the gene regulatory network to establish head-to-tail polarity in flies

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The remodeling of developmental programs is essential for animals to maintain their homeostasis while adapting to new environments or mutational pressure. As a result, gene regulatory network underlying critical phenotypes can vary even between closely related species. Embryos of the fruit fly Drosophila and the midge Chironomus specify the anterior-posterior (head-to-tail) body axis using unrelated, structurally distinct transcription factors. In Drosophila, this process is governed by the homeodomain protein Bicoid, which functions as anterior determinant in the syncytial early embryo. Bicoid activates numerous target genes as a concentration-dependent "morphogen" by promoting chromatin accessibility. In Chironomus, which lacks the *bicoid* gene, the C-clamp transcription factor Panish fulfills the same role, but through a fundamentally different mechanism. Combining genetic perturbations with genomic approaches, we found that Panish functions as a transcriptional repressor that breaks symmetry along the anterior-posterior body axis by directly repressing a zygotically expressed posterior determinant, encoded by the Chironomus ortholog of *tailless* (*Cri-tll*). In turn, *Cri-tll* expression is sufficient to suppress anterior development in the posterior embryo and to induce posterior development ectopically. Cri-Tll does this by directly inhibiting chromatin accessibility and expression of multiple genes required for anterior patterning. These anterior genes include *orthodenticle* and *sloppy-paired*, the earliest two targets of Cri-Tll, which are required for the suppression of ectopic abdomen specification in the anterior embryo. Thus, a simple repressor cascade can substitute for a morphogen, such as Bicoid.

56 Drop regulates cell shape change and arrangement to coordinate tissue internalization in early salivary gland development

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Epithelial tube development is a complex dynamic process that tubular organ precursors undergo to achieve their final shape and proper function. Many mechanisms such as cell shape change, cell rearrangement, and cell migration have been shown to coordinate tube morphogenesis; however, how these complex processes are orchestrated in time and space to generate tubular organs of correct dimensions and placement is not well understood. Importantly, defects in this process have been associated with numerous developmental disorders. To gain insight into the coordination of epithelial tube development, we use the Drosophila salivary gland (SG) as our model. We aim to discover and characterize the network of transcription factors controlling cell fate, morphogenesis, and secretory capacity in the salivary gland. So far, we have discovered several transcription factors that control development and specialization of the SG during embryogenesis. Recently, single cell RNA sequencing (scRNA-seq) data from our lab revealed expression of Drop (Dr) mRNA in a subset of cells of the SG during the earliest stages of its development. Dr encodes an NK-like homeodomain transcription factor that is homologous to the human Msx1 and Msx2 proteins. Previous work has shown that Dr function is required for specification of myoblasts and neuroblasts, development of muscle and neuronal cells, regulation of glucose metabolism, and patterning of the neuroectoderm and wing disc; Dr function, however, had not yet been described in the SG. Here, we identify a role for Dr in SG tube morphogenesis. We show that Drop loss and overexpression affects the levels of non-muscle myosin II and Crumbs at the apical surface to coordinate tissue invagination. Perturbation of Dr activity–either loss-of-function or overexpression–results in enlarged invagination pits and a wider SG lumen compared to wild type. Strikingly, ectopic expression of Dr in the entire SG placode can promote the formation of additional invagination pits, leading to SG branching. These findings implicate another level of regulation in the dynamic and complex process of tissue internalization. Moreover, our data may shed light on the roles of the human orthologs Msx1 and Msx2 in developmental diseases and in specific cancers characterized by changes in expression of these proteins.

57 Rap1 Activity during Cellularization is Essential for Ventral Furrow Formation

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Many morphogenetic events involve a continuous epithelium that undergoes changes in response to contractile forces. In *Drosophila* embryos, the embryonic epithelium is established during cellularization, prior to morphogenesis, when the syncytium is transformed into an epithelial sheet complete with adherens junctions coupled to the actomyosin cytoskeleton. The small GTPase Rap1 regulates cadherin- and integrin-mediated adhesion in multiple contexts, acting through effectors such as Canoe/Afadin to promote junction assembly, link junctions to the cytoskeleton, and maintain apical polarity. Ventral furrow formation, the first morphogenetic event in the *Drosophila* embryo, requires both Rap1 and its activator, Dizzy. However, it remains unclear where Rap1 is active and when its function is required for successful formation of the ventral furrow.

To dissect the spatiotemporal behavior and requirements of Rap1 during early development, we developed and validated two complementary tools—a fluorescent biosensor that reports on Rap1 activity dynamics *in vivo* and an optogenetic system to perturb Rap1 activity with high spatiotemporal control. The biosensor reveals high cortical Rap1 activity throughout early development and a ventral specific, transient decrease preceding and during gastrulation. Temporally precise inhibition of Rap1 using a light-inducible dominant-negative construct (optoRap1^{DN}) demonstrates that Rap1 function is critically required during early cellularization. Inhibiting Rap1 during this period—but not later—disrupts adherens junction assembly, slows cellularization front ingression, and prevents proper ventral furrow formation. Spatially patterned inhibition further reveals that uniform Rap1 activity across the ventral epithelium is necessary for coordinated invagination and sealing of the furrow.

These results identify a critical early developmental requirement for Rap1 in establishing epithelial integrity, with major consequences later during development. Furthermore, these studies illustrate the importance of spatiotemporal readouts and perturbations to define the mechanisms of development.

58 The lean that lasts: Investigating the mechanisms driving TRE-induced fat loss

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Today, we live in an age of unprecedented food accessibility. One consequence of overeating is obesity, which is the accumulation of fat stores and is linked to diseases like cardiovascular disease and type 2 diabetes. One way to decrease fat stores is to lower caloric intake; GLP-1 agonists are a modern therapy that effectively reduces fat stores but can also cause muscle loss. Time-restricted feeding (TRF) diets are known to decrease fat stores, protect against high-fat diets, and prevent obesity in mice. However, the molecular mechanisms underlying these metabolic benefits of TRF remain unclear. Using *Drosophila*, our lab developed a TRF diet that significantly extends lifespan and delays molecular markers of aging. While lifespan benefits are of interest to aging researchers, most people practicing time-restricted eating do so to lose weight. We found that after just 10 days of TRF, flies became highly sensitive to starvation compared to *ad libitum*-fed flies and had significantly less fat, confirmed by thin-layer chromatography (TLC). TRF flies did not eat less than *ad lib*-fed flies; in fact, they ate more overall. The rate of fat loss during fasting was similar between flies on *ad lib* or TRF diets; instead, fat stores were not fully replenished during TRF refeeding periods. Unexpectedly, unlike in humans, TRF-induced fat loss in Drosophila was permanent: 10 days of TRF followed by 20 days of *ad lib* feeding did not restore fat levels to control levels. Both males and females experienced fat loss during TRF, and this reduction was independent of lifespan-extending mechanisms like autophagy and the circadian clock. We also found that TRF protects flies against fat accumulation, whether caused by genetic defects in lipolysis genes or by a high-sugar diet. To investigate how TRF flies lose fat stores, we performed bulk RNA sequencing during fasting and feeding phases after 10 days on the diet. Gene-set enrichment analysis (GSEA) and KEGG pathway mapping revealed that oxidative phosphorylation is upregulated during the first 12 hours of refeeding, including various subunits of the mitochondrial electron transport chain. We confirmed increased levels of Complex I and II through Western blot analysis and are currently investigating the role of altered mitochondrial function in iTRF-induced fat loss.

59 Dgat2 modulates lipid dysmetabolism and neuroinflammation associated with Alzheimer's Disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive impairment, amyloid-β (Aβ) accumulation, lipid dysregulation, and chronic neuroinflammation. Although mutations in the Amyloid precursor protein (APP) and *Aβ42* aggregation are well-established drivers of AD, the molecular link between amyloid toxicity, lipid metabolism, and inflammation remains poorly defined. Using *Drosophila* models, we demonstrate that neuronal expression of *App*^{NLG} *or Aβ42* leads to locomotor and memory deficits, abnormal sleep patterns, lipid accumulation, synaptic degeneration, and neuroinflammatory activation. Similar lipid and inflammatory alterations were observed in the *App* $^{NLG-F}$ knock-in mouse model, supporting conserved mechanisms underlying amyloid pathology across species. We identified diacylglycerol O-acyltransferase 2 (*Dgat2*), a critical enzyme in the terminal step of triglyceride synthesis, as a key regulator of these AD-like phenotypes. Pan neuronal knockdown of *Dgat2* in *Drosophila* significantly reduced lipid accumulation and restored neuronal lipid homeostasis. This intervention preserved synaptic integrity, improved locomotor performance, enhanced cognitive function, and reduced neuroinflammatory markers. Notably, *Dgat2* modulation also normalized sleep behavior and circadian rhythmicity, suggesting broader benefits on neuronal function and metabolic balance. Together, our findings reveal that *Dgat2* plays a pivotal role at the intersection of amyloid toxicity, lipid metabolism, and neuroinflammation. These results establish *Drosophila* as a powerful model to dissect lipid–neuroimmune interactions and provide evidence that targeting *Dgat2*-mediated lipid pathways could serve as a promising therapeutic approach for mitigating neurodegenerative processes in Alzheimer's disease.

60 Microbiome influence on brain transcription programs during aging revealed by single-cell analysis in *Drosophila*

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The gut microbiome plays a critical role in brain function and the brain-gut axis, yet its cellular and molecular mechanisms remain unclear. Here, using *Drosophila melanogaster*, we present the first comprehensive single-cell transcriptomic atlas of brain cells from adult flies raised under axenic and microbiome-associated conditions, spanning young and old ages. Profiling 34,427 cells across 101 clusters, we annotated 56 cell types and identified cell type-specific gene signatures influenced by the microbiome. Transcriptional shifts were most pronounced in old flies, with glial cells and dopaminergic neurons among the most microbiome-responsive cell types. Differentially expressed genes (DEGs) were enriched in pathways related to mitochondrial activity, energy metabolism, and Notch signaling. We also quantified age-associated changes in the gut microbiome, observing reduced *Acetobacter* dominance and increased microbial diversity that corresponded with heightened brain transcriptional responses. These findings illuminate the cell type-specific impacts of the microbiome on brain gene expression and lay the groundwork for understanding the molecular underpinnings of the microbiome-gut-brain axis.

61 Single-cell transcriptomics of the renal tubule reveals cell type-specific ferroptosis in aging

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Background

Aging markedly impairs kidney function and is a major risk factor for chronic kidney disease (CKD). Ferroptosis, an iron-dependent form of programmed cell death characterized by iron accumulation and lipid peroxidation, has recently emerged as a key mechanism underlying aging and cellular senescence. However, it remains elusive how ferroptosis drives kidney aging and what renal cell types are most vulnerable to it.

Methods

This study used *Drosophila* as a model organism to construct a single-cell atlas of the renal tubules (Malpighian tubules) at young and old ages. We assessed the role of ferroptosis in renal aging by measuring intracellular and mitochondrial iron levels and lipid peroxidation. Furthermore, we examined the iron transporter Malvolio (Mvl), the fly homolog of divalent metal transporter 1 (DMT1), in ferroptosis through renal cell-specific gene knockdown experiments.

Results

We annotated 8 cell clusters corresponding to distinct renal cell types and functions. Notably, ferroptosis-related genes were up-regulated during kidney aging. Confocal imaging analysis revealed elevated intracellular and mitochondrial iron markers and increased lipid peroxidation. Our analysis also revealed an unexpected cell-specific signature of aging-induced ferroptosis. Ferroptosis was strongly induced in the aged main segment of the renal tubules, the homolog of the mammalian proximal tubule. This difference may be due to segment-specific variations in ion transport and lipid metabolism. The iron transporter Mvl expressed highly in main segment, suggesting a central role in ferroptosis. Intriguingly, Mvl knockdown reduced ferroptosis by preventing mitochondrial labile iron accumulation, but not intracellular iron.

Conclusion

These findings highlight cell-specific induction of ferroptosis in aging kidney tubules. Targeting mitochondrial ferroptosis in proximal tubule-like cells may represent a novel therapeutic strategy to combat kidney aging and CKD.

62 Widespread Cellular Atrophy Emerges Early in the Aging Drosophila Brain.

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Aging related brain atrophy has been observed across organisms including humans. We examined whether atrophy could be measured at the single cell level in the *Drosophila* brain to identify where and when aging-related atrophy begins. We used flow cytometry combined with imaging cytometry to quantitatively assess cell size and DNA content in the *Drosophila* brain, examining region-specific differences between the optic lobes (OLs) and central brain (CB) during aging. Our results reveal pronounced heterogeneity in brain cell sizes that persist with age, with a significant shift toward smaller cell sizes emerging as early as day 21. This shift towards smaller cell sizes occurs uniformly in both OLs and CBs, however OLs consistently contained smaller cell somas relative to the CB. In parallel, we observed a significant age-related accumulation of polyploid cells (>2C DNA content), primarily in the OLs. Notably, although polyploid cells are generally larger than diploid counterparts, they also decrease in size with age, and their increased prevalence does not offset the overall reduction in average cell size.

Our findings suggest widespread cellular atrophy occurs early in the aging process affecting both diploid and polyploid populations throughout the aging fly brain. Importantly, we detect cellular atrophy as early as day 21, suggesting this is an early hallmark of brain aging. This work establishes a critical framework for future work dissecting mechanisms and functional consequences of age-related cellular atrophy.

63 Elevated Wnt, Ca^{2+}, and JNK signaling hyperactivates migration of intestinal stem cells during aging

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Somatic stem cell migration is critical for positioning newly-divided cells near sites of injury during tissue repair. The effects of aging on stem cell migration and the consequences of migratory dysfunction on regenerative capacity, however, are poorly understood. We demonstrate that migration of intestinal stem cells (ISCs) in the adult *Drosophila* intestine becomes hyperactivated during aging. Chronic migration is caused by elevated non-canonical Wnt signaling in parallel with increased Ca^{2+} and Jun-N-terminal kinase (JNK) signaling. Interestingly, we find interplay between these three signaling pathways. Elevated JNK activity in aging enteroendocrine cells promotes metalloprotease activity and the cleavage of Otk, the *Drosophila* homolog of the senescence-associated secretory phenotype (SASP) factor Ptk7. Extracellular release of Otk then non-autonomously activates non-canonical Wnt in aging ISCs. Non-canonical Wnt signaling increases cytosolic Ca^{2+} through IP_3, which in turn, activates JNK within ISCs and promotes ISC migration. Critically, chronically stimulating migration causes ISCs to lose directionality during tissue injury while interfering with chronic ISC migration is sufficient to improve intestinal organization and increase lifespan. Altogether, our findings provide insight into how SC migration becomes dysregulated during aging, the interplay between aging-associated signaling pathways, and the mechanisms preserving long-term tissue homeostasis.

64 Hunger induces a starvation-like state in the gut, fat body, and oenocytes that extends lifespan

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Aging underlies many diseases so pharmaceutical and behavioral interventions that slow aging will drastically improve public health. *Drosophila melanogaster* is an ideal model for examining the mechanisms underlying aging, as it has a relatively brief lifespan and exhibits relationships between aging nutrition that are deeply conserved across taxa. More specifically, limiting calories or protein slows aging. Recent work reveals that hunger, a subjective neural state that can be induced by activation of hunger neurons or isoleucine restriction, also slows aging, despite stimulating flies to consume more calories and protein. However, the effect of hunger on tissues that regulate nutrient homeostasis, namely the gut, fat body, and oenocytes, remains largely unexplored. We tested how hunger affects these peripheral tissue by inducing hunger with optogenetic activation of R50H05 neurons and performing transcriptomic sequencing on both these tissues and fly heads. We analyzed the transcriptomes of the head, gut, and a combined sample of fat body, oenocytes, and body wall after 1 day or 2 weeks of hunger. We found that optogenetic hunger causes small transcriptomic changes in the gut, fat body, and oenocytes after only one day, but that these changes cascade into larger changes after 2 weeks of hunger. Notably, GO term analysis detected an enrichment of genes that contribute to (i) lipid metabolism, (ii) amino acid transport, and (iii) hormone production and reception in the differentially expressed transcripts. We genetically manipulated the expression of a subset of these differentially expressed transcripts and found that their tissue specific expression can extend lifespan, suggesting that the response of peripheral tissues can drive the lifespan extending effects of hunger. In contrast, the heads of hungry flies displayed more differentially expressed genes after 1 day of hunger than after two weeks of hunger. Overall, our results suggest that communication between tissues and a metabolic remodeling of the periphery is critical for the lifespan extending effect of hunger.

65 Loss of DOR drives aging and cellular senescence in *Drosophila melanogaster*

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Late-life mortality reflects aging dynamics in populations. Understanding the genetic mechanisms underlying late-life mortality is essential for developing interventions that promote healthspan and extend lifespan. To identify genetic determinants of late-life mortality, lifespan data from 160 *Drosophila melanogaster* Genetic Reference Panel strains were analyzed. A genome-wide association study identified Diabetes and Obesity-Regulated (DOR) as a significant candidate gene, whose human ortholog, TP53INP1, encodes a transcriptional regulator involved in stress response modulation via p53 activity and autophagy.

To validate these findings, DOR expression was ubiquitously suppressed in female flies using *UAS-DOR-RNAi* driven by *Da-GeneSwitch-GAL4*. Lifespan assays revealed a pronounced reduction in survival beginning around day 19 compared with controls. To assess temporal specificity, DOR inhibition was induced after day 19, resulting in a delayed onset of mortality, suggesting that DOR plays a time-dependent role in lifespan regulation. Functional assays demonstrated that DOR downregulation impaired organismal health, as evidenced by decreased climbing ability, increased gut permeability in the SMURF assay, and reduced starvation resistance.

Tissue-specific knockdown experiments showed that DOR inhibition only within ovarian somatic stem cells can recapitulate the systemic lifespan reduction observed with whole-body knockdown. Somatic ovarian DOR depletion also led to reduced fecundity, impaired climbing ability, and compromised gut integrity, indicating a central role of ovarian DOR in maintaining systemic homeostasis. RNA sequencing of ovaries with DOR inhibition revealed differentially expressed genes enriched for lysosomal, cellular senescence pathways, including genes associated with the senescence-associated secretory phenotype (SASP). Consistently, ovarian DOR knockdown elevated the expression of senescence and DNA damage markers, SA-β-Gal and γH2AX, respectively.

Cross-analysis of human orthologs of the upregulated *Drosophila* genes with the UK Biobank plasma proteome database identified overlapping targets linked to age-related outcomes, notably earlier menopause and shortened lifespan.

Collectively, these findings identify DOR as a key regulator of systemic aging in *Drosophila*, acting through ovarian-derived senescence-associated signaling that influences organismal longevity.

66 Investigating somatic cyst cell dynamics under homeostasis and following germline stem cell loss

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Soma–germline interactions are highly conserved and play essential roles in regulating differentiation, tissue regeneration, and homeostasis. In the *Drosophila* testis, differentiating germ cells are enclosed by two somatic cyst cells that create an isolated microenvironment throughout spermatogenesis. However, the mechanism by which the cyst complex forms after a germ cell (gonialblast, Gb) is released from the niche remains poorly understood. To investigate this process, we established a live-imaging system that visualizes both somatic and germline lineages, allowing direct observation of cyst formation dynamics. Contrary to the previous assumption that the Gb leaves the niche as a preformed soma–germline complex, we found that somatic cyst cells actively encapsulate two-cell cysts outside the niche. This observation suggests an active role for somatic cyst cells in coordinating and initiating germ cell encapsulation. Beyond homeostatic conditions, somatic cyst cells are also crucial for maintaining spermatogenesis following stress. Loss of germline stem cells (GSCs) is a hallmark of nutrient-deficiency stress, leading to spermatogonial dedifferentiation during recovery after stress removal. The dedifferentiation process involves the fragmentation of fully formed cysts, allowing individual germ cells to exit the cyst complex and re-establish stable contact with the niche. However, the contribution of somatic cyst cells to this dedifferentiation process remains unclear. The soma–soma septate junction, present in all fully formed cysts, is essential for maintaining an isolated cyst microenvironment and supporting germline differentiation. To investigate the dynamics of septate junction integrity, we used Neurexin (Nrx), a marker of septate junctions, as a reporter. Our results revealed that septate junction components are depleted during the GSC recovery phase following stress removal. Furthermore, permeability assays demonstrated that the previously isolated cyst microenvironment becomes transiently accessible due to modulation of septate junctions. Together, these findings tell us that somatic cyst cells transiently compromise cyst integrity to release germ cells, thereby facilitating spermatogonial dedifferentiation during tissue recovery.

67 Investigating how Smooth represses *cdc25/twine* translation to delay the meiotic divisions in the *Drosophila* male germline

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The *Drosophila* male germline is a powerful model system for investigating how developmental cues can influence expression of core cell cycle components to direct a cell-type-specific cell cycle. In *Drosophila* males, an extended meiotic G2 prophase occurs in the spermatocyte stage, following premeiotic S phase. This unusually long G2 phase lasts 3.5 days, during which the cells grow 25-fold in volume and turn on a major transcriptional program that activates expression of about 3000 transcripts needed for meiotic progression and post-meiotic differentiation. Once the spermatocytes mature, they enter M phase, divide twice in quick succession, and undergo another 3-4 days of spermatid differentiation. The long G2 prophase relies on delaying expression of specific cell cycle components that are crucial for entry into the meiotic divisions. Twine, the meiotic Cdc25 phosphatase needed to drive cells into meiotic divisions, is subject to delayed translation in spermatocytes. The *twine* RNA is present starting in young spermatocytes, but expression of a Twine-LacZ reporter appears only in mature spermatocytes, and activation of *twine* translation requires function of conserved RNA-binding protein Boule (Maines & Wasserman, 1999). The requirement for Boule to activate *twine* translation hints at a potential translational repressor acting in opposition to Boule. We found that Smooth, a highly conserved RNA-binding protein and fly homolog of human hnRNPL, represses *twine* translation in immature spermatocytes. The testis-specific isoform of Smooth is required for meiotic division and normal post-meiotic differentiation. In a *smooth* mutant, meiotic divisions fail to occur, the mitochondrial derivative does not form, and the round spermatids have disorganized microtubule arrays and only partially elongate. Immature spermatocytes mutant for Smooth prematurely express Twine-LacZ protein. Intriguingly, epistasis tests showed that although Twine-LacZ is not expressed in the *boule* mutant, it is still prematurely expressed in the *smooth boule* double mutant. These findings suggest that Boule counteracts Smooth activity in late-stage spermatocytes to activate *twine* translation. Additionally, attempted spermatid elongation and aberrant microtubule overgrowth are present in *smooth* and *smooth boule* but absent in *boule* alone, suggesting that there may be other RNAs that are repressed by Smooth and activated late by Boule antagonism of Smooth activity.

68 ILP8 serves as a mature follicle sensor to prevent excessive accumulation of mature follicles in Drosophila ovaries and oocyte aging

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Excessive mature follicle accumulation in ovaries harms oocyte health and offspring viability while also wasting maternal energy. Therefore, the number of mature follicles in the ovary is tightly controlled. In Drosophila, each ovary is comprised of ~16 ovarioles, each typically containing 1-2 mature follicles, regardless of the female's mating status. However, the mechanism by which females count mature follicles in their ovaries remains a mystery. Recent work demonstrated that Drosophila insulin-like peptide 8 (ILP8) is expressed in mature follicle cells but not in younger follicles. Here, we found that ILP8 is not essential for mating-induced ovulation but plays an essential role in inducing ovulation and preventing excessive accumulation of mature follicles in virgin females. Excessive accumulation of mature follicles in ilp8-knockdown females leads to reduced oocyte quality. Additionally, knockdown of Lgr3 (Leucine-rich repeat-containing G protein-coupled receptor 3), encoding a previously identified ILP8 receptor, globally or in all neurons, resulted in similar ovulation and egg laying defects, accumulation of mature follicles, and poor oocyte quality in virgin females. In contrast, Lgr3 knockdown specifically in octopaminergic neurons that innervate the ovaries and reproductive tract did not impair ovulation/egg laying, indicating that ILP8-Lgr3 signaling acts through non-octopaminergic neurons to regulate virgin ovulation. Using an enhancer screening, I will demonstrate the specific Lgr3+ neurons involved in this process. Together, our findings indicate that ILP8 functions as a mature follicle sensor to prevent excessive accumulation of mature follicles and maintain oocyte quality likely through neuronal the Lgr3 receptor in virgin females. Our findings suggest that ILP8/Lgr3 is likely essential for maintaining the optimal reproductive fitness of virgin females and for species survival in the wild, where mating success is not guaranteed.

69 An *in silico* screen identifies the correct ortholog of the meiotic double-strand break protein TOPOIVBL in Drosophila

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Traditional mutagenesis screens have identified numerous genes required for meiosis in Drosophila, including the key enzyme that catalyzes meiotic double-strand breaks, MEI-W68 (the *spo11* ortholog). Previous published work based on primary sequence alignments predicted that Drosophila *mei-P22* is the ortholog of the Spo-11 binding partner TOPOVIBL. However, close inspection of the primary sequence alignment shows that MEI-P22 does not have the critical and highly conserved protein domains of other TOPOVIBL genes. Using Alphafold to predict protein-protein interactions, we found that MEI-P22 protein does not interact with the predicted TOPOVIBL:Spo11 interaction interface; rather a strong interaction with MEI-W68 is predicted in a different location. We next used Alphafold to perform an *in silico* search for structural homologs of TOPOVIBL in Drosophila and we identified CG14410 as having a highly similar structure. Importantly, CG14410 is predicted to interact with MEI-68 at the same location as the TOPOVIBL:Spo11 interaction. To provide *in vivo* support for our observations, we made a CRISPR deletion of CG14410. We found that X-chromosome nondisjunction increases to 28% and that very few meiotic crossovers occur. We analyzed meiotic double-strand break formation by immunostaining for phosphorylated H2AV and found that no double-strand breaks form. We will also present the results of on-going yeast two hybrid experiments to detect direct interactions between CG14410 and other meiotic double-strand break proteins. Overall, we conclude that CG14410 is required for meiotic double-strand break formation and is most likely the functional ortholog of TOPOVIBL.

70 Harnessing CRISPR/Cas13 for Temporal Control of Gene Expression in Late-Stage Spermatogenesis

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Prior to individualization, elongating spermatids are stripped of mitochondrial DNA nucleoids, cytoplasmic material, and organelles by actin-based individualization complexes (ICs). Many of the genes implicated in this integral process exist under translational repression during the latter stages of spermatogenesis due to DNA condensation affected by the histone-to-protamine transition of post-meiotic germ cells (Lim et al., 2012). The transcriptional patterning observed during late spermatogenesis precludes genetic knockdown via traditional strategies (UAS/Gal4, FLP/FRT) due to the inability to efficiently express the respective molecular factors necessary to drive gene silencing. We seek to employ the RNA-targeted CRISPR/Cas13 system to obviate these aforementioned challenges for specific, efficient, and temporally inducible gene knockdown in late-stage spermatogenesis. A genetic screen of late stage spermatogenesis genes in D. melanogaster testes revealed POLDIP2 as an essential gene for removal of mtDNA nucleoids during individualization (Chen et al., 2024). Knock-in of a Cas13 nuclease variant B (PspCas13b) expression cassette into the POLDIP2 locus has provided us with a platform for temporally specific expression of PspCas13b, independent of global transcription levels. A genetic cross of flies expressing POLDIP2-Cas13 with a separate fly line containing a guide RNA (gRNA) with sequence homology to a given gene of interest induces catalytic activation of CRISPR/Cas13 and targeted mRNA transcript cleavage in resulting progeny. Preliminary data testing the POLDIP2-Cas13 expression cassette with a gRNA complementary to the ATP synthase gene ATPsynCF6 demonstrated a marked reduction in ATPsynCF6 transcripts following post-meiotic POLDIP2-Cas13 induction when comparing the experimental condition with a negative control. The efficacy of this system will continue to be evaluated with a variety of genes to confirm these results, evaluate potential collateral effects, and ultimately prove the versatility of this novel tool for the *Drosophila* research community.

71 Investigating the contribution of male- and female-derived proteins to sperm reproductive success

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Unlike most cells that remain within a single organism, sperm embark on a unique journey, spending a crucial period of their life in the female reproductive tract (FRT) after being produced in the male. Sperm are transferred in seminal fluid, which contains male-derived proteins, RNAs, and metabolites that may assist sperm in the FRT. Drosophila seminal fluid proteins (SFPs) influence many aspects of the female post-mating response, including sperm storage and release, ovulation, and changes to female behavior. However, SFPs are insufficient for complete sperm maturation, and sperm persist long after most SFPs are depleted from the FRT. Some SFPs bind to sperm in the mated female, and McCullough et al. 2022 showed that female-derived proteins also associate with sperm. We hypothesize that both male- and female-derived proteins must associate with sperm for long-term fertility and fecundity. We are using genetic tools to determine the reproductive functions of sperm-associated SFPs and female-derived metabolic enzymes as novel contributors to reproductive success. We assay fecundity, fertility, hatch rate, and various aspects of sperm function to identify defects in reproduction upon depletion of each protein. Thus far, we have identified a novel, putative member of the network of SFPs that bind the Sex Peptide to sperm, as well as three female-derived metabolic enzymes that are necessary for long-term fertility. We are determining the nature of their effects on sperm viability and function, as well as the ways in which they associate with sperm to illuminate how male- and female-derived molecules jointly contribute to reproductive success.

72 Fusome regeneration and asymmetry in female germline stem cells relies on importin-dependent microtubule reorganization

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Many tissue-resident stem cells are retained through asymmetric cell division, ensuring self-renewal while simultaneously producing differentiated progeny. The asymmetric distribution of organelles is one mechanism by which stem cells are marked for long-term retention; however, it remains unclear whether biased organelle localization is a cause or effect of asymmetric division. In the ovary, an endoplasmic reticulum-like organelle termed the fusome is continually regenerated, asymmetrically distributed in germline stem cells (GSCs) and associated with GSC division. Here, we report that β-importin, Tnpo-SR, is essential for the asymmetric distribution of the fusome. While complete loss of *Tnpo-SR* abolishes fusome regeneration, depletion of *Tnpo-SR* disrupts cytoskeletal organization during interphase and localization of microtubule associated protein Asp. Tnpo-SR does not localize to microtubules or the fusome, suggesting an indirect role in maintaining fusome regeneration. Despite this, we find that restoring fusome area in *Tnpo-SR*-depleted GSCs is sufficient to restore the maintenance of GSCs. We conclude that Tnpo-SR functionally links fusome regeneration to cell cycle progression, supporting the model that the coordination and stability of microtubules maintains fusome asymmetry and promotes GSC identity.

73 Proximity Biotinylation identifies novel interactors of male germline promoting factor Tdrd5l in testes

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In order to ensure proper gametogenesis and the successful continuation of a species, germline sex determination is tightly regulated by a combination of paracrine signals from the somatic gonad and autonomous signals within the germline. The RNA-binding protein Sex-lethal (Sxl) serves as a master regulator of these pathways, promoting the correct sexual identity of the gonad. Our lab previously identified Tudor-domain containing protein 5-like (Tdrd5l) as a Sxl target through a combination of bulk RNA-sequencing and validation of Sxl binding sites in the 3′-UTR and 3rd intron of *Tdrd5l* transcripts. We have begun to characterize *Tdrd5l* in the male gonad as an important regulator of male identity. Under normal conditions, Tdrd5l exhibits male-biased expression in germline stem cells (GSCs) and early germ cells, forming bright, hollow foci that are evenly distributed between the cytoplasm and perinuclear regions of germ cells. In *Tdrd5l* mutant flies, we observe fertility defects, loss of germline tissue, and displacement of the GSC niche, known as the hub. However, which protein factors Tdrd5l interacts with in the male gonad to promote proper germline development and identity, and how Tdrd5l regulates these targets, remains unknown.

To identify novel interactors of Tdrd5l in the testis, we employed proximity biotinylation followed by mass spectrometry using a transgene containing *Tdrd5l* with a C-terminal APEX tag. Strep-purification of putative interactors revealed protein factors involved in RNA metabolism, glucose metabolism, and ubiquitin-mediated protein degradation. RNAi knockdown of these candidate interactors resulted in altered Tdrd5l foci stability and morphology in germ cells. One such interactor is Upf1, a nonsense-mediated RNA decay (NMD) factor whose role in protecting cells from proteotoxic stress has been expanded into a pathway independent of NMD. Immunofluorescent imaging of Tdrd5l in the background of a transgene expressing Upf1-GFP under UAS control revealed tight co-localization of Upf1-GFP with Tdrd5l foci. Surprisingly, in *Tdrd5l*-mutants, we observed a loss of Upf1-GFP foci in early germ cells, suggesting that Tdrd5l is important for Upf1 stability and/or localization. Further studies aim to determine how Tdrd5l impacts Upf1 stability in the cytoplasm and how this interaction contributes to proper development and identity of the male gonad. Likewise, we hope to understand how other interactors of Tdrd5l identified in our proximity biotinylation impact Tdrd5l function.

74 Asymmetric centromere and gene locus positioning in *Drosophila* neural stem cells

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Neural stem cells (neuroblasts, NBs) in larval Drosophila brains maintain prominent asymmetries along their apical-basal polarity axis throughout the cell cycle. In interphase, the most recognized feature of this asymmetry is the single, active microtubule organizing center (apical MTOC). Our lab demonstrated that centromeres are also polarized throughout interphase, positioned at the nuclear periphery near the apical MTOC, and that this positioning is dependent on the apical MTOC. Here, we use live cell imaging of brain explants and detailed quantitative image analysis to broaden our mechanistic insight into this non-random centromere positioning. We show that more differentiated progeny lose the stereotypical centromere positioning seen in NBs. We provide evidence that in NBs, microtubules are linked to centromeres through the nuclear pore complex (NPC) via the minus end-directed motor protein Dynein and its binding partner Mushroom body defect (Mud). We also show that Lamin is necessary for asymmetric centromere positioning, either by anchoring NPCs and/or by facilitating centromere – NPC binding. We also reveal that two developmentally regulated genes, *hunchback* (*hb*) and *pendulin* (*pen*), occupy stereotypic angular positions relative to the apical-basal polarity axis and that positioning of these gene loci is disrupted when centromere polarity is lost. We thus propose that fly neural stem cells translate their inherent polarity into stereotypical chromatin organization, potentially influencing cell fate decisions and stem cell behavior.

75 Uncoordinated-76, essential for neurite outgrowth, is a regulator of microtubule–microtubule sliding by kinesin-1

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Kinesin-1, a major plus-end-directed microtubule motor, is essential for transporting many cellular cargoes along microtubules. Past findings from our lab have shown that, in addition to cargo transport, sliding of cytoplasmic microtubules against each other by kinesin-1 is crucial for neuronal development. However, the kinesin-1 interactions that regulate its multiple activities remain incompletely understood. Uncoordinated-76 (UNC-76) is a conserved cytosolic protein important for axonal outgrowth and transport across species. It was initially proposed that UNC-76 functions as a kinesin-1 cargo adaptor. However, our new data demonstrate that UNC-76 regulates microtubule–microtubule sliding by kinesin-1, rather than organelle transport, in neurons and other cell types. Depletion of UNC-76 leads to a dramatic increase in microtubule sliding. Remarkably, axonal outgrowth is stunted, and the defects can be rescued by the human ortholog of UNC-76, fasciculation and elongation protein zeta-1 (FEZ1), demonstrating its conserved role in regulating neuronal development.

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76 Elucidating the Structural and Functional Roles of Sarcalumenin, a Key Component of the Muscle Fiber Sarcoplasmic Reticulum

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Proper structural organization of muscle fibers is crucial for their function. This is particularly evident at the sarcomere level, where alternating bands of thin (actin) and thick (myosin) filaments mediate muscle contraction. A second example is the sarcoplasmic reticulum (SR), a muscle-specific subdomain of the endoplasmic reticulum and a critical mediator of the process by which neuronal excitation is translated into muscle fiber contractility. The SR, which serves as a major store of Ca2+ ions- key mediators of excitation-contraction coupling- aligns with the sarcomeres along its longitudinal domain, while its junctional domain interfaces and interacts with the t-tubule indentations of the muscle fiber membrane.

Sarcalumenin (SRL), a relatively uncharacterized SR and muscle-specific protein, is recognized as an abundantly-expressed luminal Ca2+-buffering protein of the mammalian longitudinal SR. Intriguingly, SRL possesses a dynamin-like domain in addition to its Ca2+-binding domain, suggesting a parallel functional role in SR membrane remodeling. In fact, our evolutionary sequence analysis suggests that SRL proteins constitute an ancient and distinct branch of the dynamin protein family.

We have begun to characterize the single *Drosophila* homolog of SRL (CG9297), in order to complement and extend our ongoing studies of mouse SRL. Similar to its mammalian counterpart, *Drosophila srl* is expressed specifically in muscle fibers. We have generated a GFP-tagged variant of the *Drosophila* SRL protein, and find that it localizes, as expected, to the SR of larval body-wall muscles. Both homozygous disruption of the *srl* locus and muscle-specific RNAi-based knockdown result in larval lethality. Characterization of the *srl* mutant muscle fiber phenotype via both light and electron microscopy approaches reveals significant defects in SR organization, as well as impaired Ca2+ homeostasis in larval body-wall muscles, while overall sarcomeric morphology appears intact. Knockdown of *srl* at post-larval stages identifies a second essential phase of *srl* function during adult muscle formation. Intriguingly, *srl* is expressed and required in larval and adult tubular muscles, but appears to be dispensible for the function of the fibrillar indirect flight muscles, which power *Drosophila* flight, yet are SR-poor and contract in an asynchronous manner. All told, our findings imply a critical involvement of SRL in proper formation of the SR reticular network and in mediating muscle SR function.

77 The Autophagy Protein Blue Cheese Affects Nuclear Spacing in Muscle Cells

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Disrupted nuclear spacing in muscle cells is a hallmark of muscle disease. Thus, it is crucial to understand the mechanisms that underlie proper nuclear spacing in healthy muscles, and how these processes are compromised in muscle disease. Recently, defects in lysosome acidification and endosome maturation have also emerged as a phenotype shared amongst several muscle diseases. Based on the prevalence of these distinct cellular defects across several diseases, we speculated that the nuclear spacing and endolysosomal pathways in muscle may be connected. We hypothesized that autophagy may contribute to myonuclear spacing. To test this idea, we generated muscle-specific knockdowns of blue cheese (bchs), a highly conserved BEACH-domain protein known to regulate autophagy. Specifically, bchs has been shown to localize to endolysosomes and to regulate endolysosome transport and endosome maturation. The gene encoding bchs has also been shown to genetically interact with a number of autophagy-related (Atg) genes. Our data show that muscle-specific knockdown of bchs strongly disrupts myonuclear spacing in *Drosophila* larvae. Strikingly, myonuclear spacing defects are observed both when bchs is knocked down only during embryonic development, and when bchs is knocked down only during larval development. These findings suggests that bchs is required both for the movement of myonuclei to their proper position, and for the maintenance of these positions once they are reached. Furthermore, knockdown of bchs in muscles caused strong disorganization of the microtubule network and Golgi bodies. While control muscles showed even distributions of microtubules and Golgi, there were immense clumps and large gaps in the distribution of both microtubules and Golgi bodies when bchs was depleted. These data suggest a widespread and dramatic disruption of cellular structure in response to bchs knockdown, and a previously unknown role for bchs in muscle development. More broadly, these data suggest that autophagy and myonuclear spacing are mechanistically linked, and that the intersection of these two processes may be crucial for muscle function.

78 Investigate the role of condensate formation in actin organization during myoblast fusion

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Cell surface receptors often form clusters to mediate signal transduction. At sites of *Drosophila* myoblast fusion, known as fusogenic synapses, immunoglobulin domain-containing cell adhesion molecules (CAMs)—Dumbfounded and Sticks and stones (Sns)—form clusters. Sns recruits over a dozen additional proteins into a «focus» that promotes actin polymerization and bundling to drive cell-cell fusion. Here, we demonstrate that the largely disordered cytoplasmic domain of Sns (SnsCT) forms liquid condensates both *in vitro* and in cells. These SnsCT condensates specifically recruit the SH2 and SH3 domain-containing adaptor protein Crk to the fusogenic synapse, which subsequently recruits actin bundling proteins, dynamin, and WASP-interacting protein (WIP) to facilitate cell-cell fusion. Through a series of internal deletions, we narrowed the region in SnsCT required for condensate formation to a small domain of 46 amino acids. Mutational analysis within this domain identified multiple tyrosine residues critical for condensate formation. Surprisingly, mutating these tyrosine residues to phenylalanine, which cannot be further phosphorylated, did not abolish Crk partitioning into SnsCT condensates, suggesting that SnsCT recruits Crk through non-canonical mechanisms rather than conventional phosphotyrosine-SH2 interactions. This is consistent with our observation that SnsCT lacks tyrosine phosphorylation. In contrast, mutating tyrosine residues to alanine blocked both SnsCT condensate formation and Crk recruitment, indicating that SnsCT-SnsCT and SnsCT-Crk interactions are mediated by the aromatic side chains of tyrosine residues. Functional analysis of Sns mutants deficient in condensate formation revealed a strong correlation between condensate-forming ability and fusion-promoting activity. Taken together, we propose that Sns condensate formation, mediated by tyrosine-based interactions, increases the local concentration of Crk and actin bundling proteins at the fusogenic synapse, thereby assembling mechanically stiff actin protrusions that promote cell-cell fusion.

79 Tissue geometry and mechanochemical feedback initiate rotational collective migration in *Drosophila*

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Collective migration of epithelial cells drives diverse tissue remodeling processes. In many cases, external asymmetries such as a free tissue edge can polarize epithelia for directed migration. In contrast, edge-free epithelia, such as cultured epithelial spheres, and those grown on circular micropatterns or other curved substrates, spontaneously break symmetry and undergo persistent rotational migration. The ability to self-organize for rotational migration appears to be a fundamental property of epithelia that adopt distinct geometries, but the mechanisms that initiate and orient epithelial rotation are unknown. To determine how symmetry is broken in these tissues, we study the rotational collective migration of follicular epithelial cells in the egg chamber. Follicle cells form a topologically-closed epithelium that surrounds a central cluster of germ cells. During early development, follicle cells migrate collectively along a basement membrane (BM) that encapsulates the egg chamber. This drives rotation of the entire egg chamber around its anterior-posterior (AP) axis within the stationary BM. The onset of rotation depends on the atypical cadherin Fat2 which functions to align lamellipodial protrusions made by each cell across the tissue. How Fat2 mediates the initial symmetry breaking event that drives rotation onset is unclear. Additionally, the mechanisms that orient rotation around the AP axis remain unknown. By employing new methods for long-term live imaging and for delaying the onset of rotation, we reveal the dynamic behaviors of the follicle cells as rotation initiates and show that symmetry breaking is a self-organized process. Combining experiments and theoretical modeling, we identify a positive feedback loop in which Fat2 stabilizes and aligns individual cell protrusions, and the resulting tissue motion polarizes Fat2 to cells' trailing edges. The coupling between the mechanosensitive behavior of Fat2 and rigid-body dynamics of the egg chamber breaks chiral symmetry leading to sustained tissue rotations. We also identify mechanical constraints arising from inter-tissue interactions and tissue geometry that may ensure rotation is oriented around the AP axis across developmental time. Our findings reveal a biophysical mechanism — combining Fat2-mediated velocity-polarity alignment, rigid-body dynamics, and tissue geometry — by which a closed epithelial tissue self-organizes into persistent, large-scale rotational migration *in vivo*.

80 To Be or Not to Be an Oocyte: Msps/XMAP215 Controls Oocyte Cell Fate in the *Drosophila* Ovary

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Cell fate determination, the process by which cells commit to specific identities, is fundamental to development. Oocyte specification in *Drosophila* provides an excellent model to dissect these molecular mechanisms. In the *Drosophila* ovary, 16 interconnected germline cells arise through four mitotic divisions with incomplete cytokinesis. Among them, the two oldest cells become pro-oocytes, and one is selected to develop into the oocyte while maintaining diploidy; the remaining 15 differentiate into nurse cells and enter endoreplication. Previously, we showed that Mini spindles (Msps), a microtubule polymerase and homolog of XMAP215, is essential for oocyte maintenance. Here, we report that Msps/XMAP215 is expressed early in development, with both mRNA and protein enriched in the pro-oocytes prior to oocyte specification. Knockdown of Msps prevents oocyte specification, leading to egg chambers with 16 nurse cells. Loss of Msps also disrupts the accumulation of the oocyte marker Orb and the microtubule minus-end binding protein Patronin/CAMSAP, both critical for oocyte specification. Remarkably, optogenetic recruitment of Msps is sufficient to increase microtubule polymerization and promote Orb accumulation in nurse cells, suggesting that Msps activity is sufficient to drive oocyte fate determination. Furthermore, we show that Msps associates with the germline-specific organelles, spectrosome and fusome, and becomes asymmetrically distributed among sister cells, allowing the two pro-oocytes to inherit higher levels of Msps than their siblings. Together, we propose a model in which Msps-mediated microtubule polymerization provides the pro-oocyte with a competitive advantage, initiating a positive feedback loop that involves dynein-dependent transport of *msps* mRNA to reinforce oocyte specification.

81 A new microtubule population critical for sperm development

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Infertility impacts ~15% of couples and is due to male infertility in ~50% of these cases. However, most infertility treatment targets females, highlighting a gap in knowledge in male reproduction and sperm development. Sperm are comprised of a head containing the genetic material and a tail acting as a motor. A strong connection between the head and tail is required for successful sperm motility and fertilization. The sperm head and tail are connected by the Head-Tail Coupling Apparatus (HTCA), which is mediated by the basal body, or centriole. Failure of the HTCA leads to partial or complete detachment of the head from its tail, resulting in immotile sperm and male infertility. Through a localization and loss-of-function screen, we found that loss of Togaram results in abnormal alignment between the sperm head and tail, suggesting a failure in the HTCA. Togaram is a TOG domain containing protein in the Crescerin1 family which canonically regulate microtubules in cilia. To understand the role of a microtubule associated protein in spermatogenesis, we used Expansion Microscopy (ExM) to visualize sperm microtubules, which revealed a novel microtubule population we call “The Sperm Microtubule Cage (SMC)”. Chemical disruption of the SMC disrupts the HTCA, suggesting that the SMC is critical for maintaining the connection. We found that Togaram localizes to the SMC, and that disruption of Togaram causes destabilization of the cage microtubules as shown by FRAP analysis. We investigated the stabilization of the SMC by looking at acetylated tubulin, a hallmark of stable microtubules, and found SMC acetylation to require Togaram. To better understand how Tog functions to regulate microtubule acetylation and head-tail stability, we focused on two known molecular partners of Tog - Cep104 and CCDC66. In Mammalian cells, this complex tog-cep-ccdc66 is known to slowly polymerize microtubules. Our work with the *Drosophila* orthologs shows the complex is conserved, and disruption of these proteins replicates the HTCA defect. I hypothesize that this complex slowly polymerizes the SMC, regulating microtubule dynamics and stability, thereby promoting tubulin acetylase activity. We are testing this through acetylase localization and loss-of-function experiments, seeking to unravel the tog-cep-ccdc66 mechanism imperative to spermatogenesis and male fertility.

82 The use of Drosophila models of rare muscular dystrophy identified drug repurposing candidates for personalized medicine

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The *LMNA* gene encodes A-type lamins, intermediate filaments that form a meshwork lining the inner nuclear membrane. Three rare types of muscular dystrophies are caused by mutations in the *LMNA* gene, with most resulting in single amino acid substitutions in the coding region of the gene. Treatment development has been challenging because mechanisms of pathogenesis are not well understood and different lamin mutants exhibit different properties, suggesting a need for personalized medicine. The overall goal of our research is to identify drug repurposing candidates for the treatment of *LMNA*-associated muscular dystrophy. Expression of Drosophila *Lamin C* possessing human disease-causing mutations reduces larval motility relative to controls and specific mutants cause 100% lethality at the pupal stage. We took a dual approach to identify drug candidates. First, we performed an unbiased screen of 1,520 FDA/EMA approved drugs where we raised larvae expressing mutant lamins on food laced with drugs and assayed for rescue to adulthood. We identified multiple L-type calcium channel antagonists as capable of partially restoring adult viability for flies expressing *Lamin C* R237P, R264Q, R264W, but not other lamin mutants. Second, we tested drugs that inhibit the TGFβ/SMAD pathway, as activation of this pathway is known to be deleterious to muscle function. In addition, we have shown that specific mutant lamins activate SMAD signaling in fly and human muscles. Furthermore, over-expression of *Dad (*a repressor of SMAD signaling) in fly muscles possessing mutant lamins improves muscle function. Thus, we treated larvae expressing *Lamin C* R564P with small molecule inhibitors of TGFβ and identified three of five tested that increase larval motility relative to controls. Collectively, our findings suggest that mutation-specific disease mechanisms underlie the pathogenesis caused by mutant lamins. Furthermore, L-type calcium channel antagonists and TGFβ inhibitors are drug repurposing candidates that could serve as personalized medicine for individuals with *LMNA*-MD.

83 An Innovative Drosophila Model of Lipid Storage Myopathy Recapitulates Critical Disease Hallmarks and Reveals Therapeutic Potential of Moderate Exercise

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Lipid storage myopathy (**LSM**) is a rare metabolic disorder with pathophysiology still elusive and therapeutic strategies largely unexplored. Multiple Acyl-CoA Dehydrogenase Deficiency (**MADD**), major LSM subtype caused by **ETFDH** mutations, presents with widely heterogenous and challenging clinical manifestations such as lipid accumulation, cardiomyopathy and multisystem dysfunction. However, current models fail to capture the complexity of this disease or enable systematic testing of treatment interventions. To address this gap, we developed CRISPR knock-in *Drosophila* lines carrying patient-relevant **ETF-QO** mutations, the fly ortholog of human **ETFDH**. Our models recapitulate hallmark features of **MADD**, including age-dependent locomotor decline (Flight Index (**FI**) reduced by ~65%), ectopic lipid deposition, and cardiac impairments such as fractional shortening (**FS**) decreased by 30% and arrhythmia index elevated two-fold. Mechanistic analyses revealed mitochondrial dysfunction, oxidative stress, and activation of compensatory mechanisms aimed at maintaining proteostasis and lipidomic balance. Strikingly, a 15-minute daily exercise regimen for 2.5 weeks improved locomotor and cardiac function, increasing **FI** by ~40% and **FS** by ~15%. These benefits were accompanied by reduced lipid burden (~35%), highlighting moderate exercise as a promising non-pharmacological intervention. Our findings establish an innovative Drosophila model for modeling human metabolic myopathies and testing lifestyle-based therapies. This work provides mechanistic insights into mitochondrial lipid disorders and opens avenues for precision strategies integrating exercise and metabolic modulation to mitigate disease progression.

84 Regulation of ETS Signaling Governs Developmental Remodeling and Hypertrophy in the *Drosophila* Heart

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Hypertrophic cardiomyopathy (HCM) is a genetically heterogeneous cardiac disorder marked by left ventricular hypertrophy and interstitial fibrosis, which can lead to heart failure and sudden cardiac death. Among the known genetic causes of HCM are pathogenic variants in the RAS/MAPK signaling cascade, a pathway crucial for cellular growth, differentiation, and survival. Constitutively active RAF1 variants have been identified in individuals with Noonan syndrome and related RASopathies, where they contribute to early-onset HCM.

To model RAF1-associated HCM in vivo, we utilized *Drosophila,* an established genetic model with conserved cardiac signaling pathways. Cardiac-specific over expression of Raf^{L613V}, Raf^{S257L}, and $PTPN11^{Q510E}$, encoding constitutively activated forms of RAF, produced phenotypes resembling human HCM. These included increased heart wall thickness, luminal dilation, and excessive extracellular matrix deposition. To dissect the downstream transcriptional mediators of Raf-induced hypertrophy, we examined the potential role of Pointed (Pnt), the *Drosophila* ortholog of mammalian ETS transcription factors. Cardiac overexpression of both *Pointed P1* and *P2* isoforms resulted in similar cardiac hypertrophy and fibrosis phenotypes. Further, *pnt OE* caused a significant increase in the expression of *myosin heavy chain* (*Mhc*), a marker for cardiac hypertrophy. These results suggested that Pointed acts downstream of Raf to drive pathological cardiac remodeling.

We tested and identified Rigosertib, a Ras-mimetic inhibitor that disrupts Ras–Raf and Ras–PI3K interactions, from a small-molecule drug screen. Adult-specific treatment with Rigosertib normalized heart dimensions, reduced collagen accumulation, and restored functional output in Raf^{L613V}, Raf^{S257L}, $PTPN11^{Q510E}$. Significantly, Rigosertib also rescued the cardiac phenotypes in response to *pnt P1* and *pnt P2* overexpression suggesting that Ets1/Pnt is a downstream mediator of Raf associated cardiac hypertrophy.

These results demonstrate a role for Ets/pnt in adult cardiac hypertrophy and provide a model for dissecting the downstream signaling pathways activated in Ras – Raf mediated cardiac hypertrophy.

85 Functional analysis of O-GlcNAc in sleep and circadian rhythm in *Drosophila*

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Introduction: Sleep disturbances are a major health concern. Impaired sleep increases the risk of short-term deficits in learning and memory, as well as the development of long-term chronic conditions such as cardiovascular disease, diabetes, obesity, stroke, and dementia. Post-translational modifications (PTMs) are essential for maintaining circadian clock and sleep homeostasis. O-linked N-acetylglucosamine (O-GlcNAc) is a dynamic PTM involved in the regulation of protein activity, signaling, and stability. O-GlcNAc is maintained by the writer O-GlcNAc transferase (OGT) and eraser O-GlcNAcase (OGA). Dysregulation of O-GlcNAc is linked to many disorders associated with disruption of sleep and circadian rhythm, including neurodegenerative disorders. However, the molecular contributions of O-GlcNAc in sleep and neurodegeneration remain unclear.

Methods: We first undertook phenotypic studies of *Drosophila* with neuronal knockdown of *OGT* or *OGA*. Second, we performed glycoproteomic analysis to map O-GlcNAc sites in fly neurons. Then, to assess the role of specific glycoproteins in regulating sleep, we developed an approach using OGT/OGA fused-nanobodies for targeted modulation of O-GlcNAcylation of specific proteins.

Results: We show that upon knockdown of *OGT* or *OGA*, flies experience a shortened circadian period or an increase in arrhythmic behavior. Glycoproteomic analysis identified candidate proteins with O-GlcNAc-dependent roles in circadian rhythm and sleep regulation. Using our OGT/OGA fused-nanobody approach, we are investigating the role of O-GlcNAcylation to influence neurobehavior by targeting key circadian and sleep-related proteins. Additionally, we are investigating neurobehavioral effects resulting from changes in the O-GlcNAc status of novel glycoproteins identified through our comprehensive glycoproteomic analysis.

Conclusion: Overall, this work will identify candidate glycoproteins for investigation of sleep and circadian rhythm in disease. These results will provide novel targets for pre-clinical studies to ameliorate sleep disorders and protect against the development or progression of neurodegenerative disease.

86 Intellectual disability disease modeling in flies uncovers new regulators of synaptic function and plasticity

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To identify the underlying causes of rare genetic diseases, we collaborate with local pediatricians to associate variants of unknown significance with neurological disorders. The patient variants we are studying affect genes that are not presently associated with congenital disease, so we used tissue-specific knockdown of the orthologous genes in flies to identify potential disease "phenologs", or phenotypes conserved across species. We then assessed genes with disease relevant phenologs at the base-pair level using genomic knock-ins of the patient variants. A variant in AGAP3 (AGAP3.F607C) was identified in a patient with neonatal seizures and severe autism. Neuronal knockdown of the homologous gene in flies caused anxiety-like behaviors and increased seizure susceptibility. Remarkably these phenotypes were also present in AGAP3.F607C knock-in flies. AGAP3 mRNA is a direct target of Fragile X messenger ribonucleoprotein (FMR1), and FMR1 variants cause Fragile X Syndrome (FXS), characterized by autism-like behaviors and intellectual disability. Genetic interaction studies showed an FMR1 amorphic allele dominantly suppressed AGAP3.F607C behavioral phenotypes. In mammals, we found AGAP3 knockdown suppressed hyperactive glutamate release in FMR1 knockout neurons. FMR1 regulates synaptic plasticity, and our results argue FMR1 controls synaptic function in part through an AGAP3-dependent mechanism. Our study identified disease-associated phenologs and provides essential gene-phenotype associations for patients with idiopathic genetic disorders. In addition, disease variants in FMR1 targets such as AGAP3 are revealing modular components of the FXS clinical phenotype, providing the missing platform for understanding the complexities of FXS disease pathology.

87 Tumor-Host Fly Cell Atlas: Mapping Tumor-Induced Systemic Remodeling at Single-Cell Resolution

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Tumors can trigger widespread physiological disturbances, yet organ-specific transcriptional responses to established tumors remain poorly characterized. To systematically investigate these effects, we developed the Tumor-Host Fly Cell Atlas (TH-FCA), a Drosophila tumor-host model in which Notch-driven tumor tissues are injected into the adult abdomen and allowed to grow for ten days, enabling analysis of tumor-induced systemic effects independent of host genetics. Using whole-body single-nucleus RNA sequencing, TH-FCA captures transcriptional changes across multiple organs, providing a comprehensive view of cell-type-specific responses to tumor presence.

Tumor-bearing flies exhibited pronounced systemic alterations, including ovarian atrophy, extensive muscle loss, and abdominal bloating. Although adipocyte numbers remained largely unchanged, both cell size and transcriptional programs were extensively remodeled, with prominent alterations in metabolic pathways—including insulin signaling, fatty acid biosynthesis, and oxidative phosphorylation—reflecting widespread systemic metabolic reprogramming. Across multiple organs, pathways related to stress response, immune activation, and tissue remodeling were consistently upregulated, indicating coordinated, organ-specific responses to tumor growth. Notably, the metabolic rewiring observed in adipocytes highlights the ability of tumors to remotely reshape systemic energy homeostasis.

This study demonstrates that allograft tumors induce complex, organ-specific transcriptional remodeling in adult Drosophila. TH-FCA provides a comprehensive whole-body atlas of tumor-induced transcriptional changes and serves as a valuable resource for dissecting the mechanisms underlying tumor-host communication and systemic organ responses at single-cell resolution.

88 Investigating neurodevelopmental disorder variants in *HNRNPUL2* using *Drosophila* models

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Heterogeneous nuclear ribonucleoproteins (hnRNPs) are highly conserved between flies and humans, and *Drosophila* studies modeling neurodevelopmental disorder (NDD)-associated variants in *HNRNP* genes offer an opportunity to explore evolutionarily conserved features of this gene family and better understand mechanisms of human disease. CG30122 is orthologous to the mammalian hnRNP-U family—which includes hnRNPU, hnRNPUL1, and hnRNPUL2—and shares about 25% identity and 40% similarity with each. RNAi knockdown (KD) of *CG30122* with ubiquitous or early pan-neuronal (*elav*) drivers causes lethality, whereas KD with a late pan-neuronal driver (*nsyb*) does not, suggesting that CG30122 has essential roles in a critical period of neurodevelopment. We did not observe lethality with glial KD (*repo*), though studies using a *CG30122-T2A-GAL4* line to drive *mCherry* expression revealed that *CG30122* is expressed in a subset of neurons and glia in the larval and adult brain. To investigate variants-of-unknown-significance (VUS) for *HNRNPUL2*-NDD, we generated *UAS* lines for human *HNRNPUL2*-Reference (Ref) and -Variant (Var), including six missense variants: p.R212Q, p.G352R, p.W569C, p.N641I, p.D669Y, and p.E707A. Similar to *CG30122* KD, we observed lethality with ubiquitous or early pan-neuronal expression of all *HNRNPUL2* constructs, suggesting that *HNRNP-U* family members may have dosage-sensitive effects. With late pan-neuronal expression of *HNRNPUL2*, we saw a range of wing inflation defects that differed between Ref and some variants. Defects were less severe for the p.R212Q and p.W569C variants, located within putative DNA-binding or NTPase domains respectively, suggesting a loss-of-function mechanism. In contrast, defects were more severe for the p.E707A variant, located in a low-complexity domain. This variant is predicted to alter the prion-like properties of hnRNPUL2 and may affect its protein-protein interactions in a gain-of-function or dominant-negative manner. Because hnRNPUL2 participates in double-stranded DNA (dsDNA) break repair, we also investigated whether this may be a conserved function of CG30122. We raised flies on 6% H_2O_2, which induces dsDNA breaks, and found that those with *CG30122* KD or heterozygous deficiency died significantly earlier than controls. Our results establish *Drosophila* as a tractable model for investigating *HNRNPUL2* variants and highlight conserved roles of this essential RNA-binding protein in neurodevelopment and dsDNA repair.

89 Genetic Drivers *PSMC3, SEC16B, and HTR2A* Underlie Obesity-Induced Dysregulation of Cardiac-Circadian Synchrony

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Obesity is a multifactorial disorder shaped by genetic and environmental influences, and it is strongly associated with cardiovascular dysfunction and disruption of circadian rhythms. Disruption of coordination between the central circadian clock and peripheral oscillators such as the heart is increasingly recognized as a driver of systemic physiological dysfunction, yet the molecular mechanisms underlying this bidirectional communication remain insufficiently defined. Genomewide association studies have identified obesityrisk genes *including PSMC3, SEC16B, and HTR2A*, which regulate proteostasis, endoplasmic reticulum function, and serotonin signaling. To investigate their roles, we used *Drosophila melanogaster* and performed tissuespecific knockdowns of the conserved orthologs *Rpt5, Sec16, and 5HT2B*. Functional analyses revealed that cardiac suppression of these genes impaired heart performance and disrupted sleep-wake architecture, while neuronal knockdowns altered circadian behavior and compromised cardiac function. Notably, cardiacspecific knockdown also triggered inflammatory responses in the head, underscoring systemic consequences of disrupted *heart-brain* signaling. These findings demonstrate that obesitylinked genes act as genetic drivers coupling cardiac physiology with circadian control through both cell autonomous and non-cell autonomous mechanisms. Building on these observations, future studies will employ transcriptomic profiling of cardiac and neural tissues together with cytological analyses to define stress and inflammation pathways that mediate multiorgan desynchrony. Together, this work introduces the first genetic *Drosophila* models of cardiocircadian disruption and establishes an innovative framework for dissecting crossorgan communication. By integrating mechanistic discovery with translational relevance, these models provide a novel platform to uncover conserved pathways and accelerate strategies for mitigating obesityassociated cardiovascular risk.

90 Serine/Threonine protein phosphatase 1(PP1) is a regulator of notch signalling

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Notch signaling is a conserved intercellular communication pathway between adjacent cells crucial for development, regulating cell fate, proliferation, and differentiation. Being a critical pathway during development, notch activity is tightly regulated at multiple levels of its components. In this study, we identified Protein phosphatase 1(PP1) as a novel post-translational regulator of Notch signalling pathway. Using *Drosophila* larval CNS as model, we show that PP1 modulates notch signaling activity by regulating the phosphorylation state of Su(H) at S269 position, known to critical for its DNA binding ability. We also identified PNUTS as the primary PP1 interacting partner (PIP), which regulate notch activity in a PP1 dependent manner. Our results also show that this regulation of Notch signaling by PP1/PNUTS complex is extendable to other non-neuronal tissues and conserved across species. Thus, our results reveal a novel post-translational mechanism of Notch activity regulation during development specifically during neurogenesis.

91 Hemilineage-specific deployment of the pro-apoptotic RHG genes *reaper* and *grim* during neurogenesis sculpts segment and sex-specific neural network composition in *Drosophila*

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During development, populations of neuronal stem cells generate neurons in a modular fashion to produce a striking diversity of subtypes. Within the *Drosophila* central nervous system, a stereotyped, segmentally repeated array of stem cells, called neuroblasts, generate identifiable lineages of neurons, each comprising two hemilineages. Each hemilineage manifests a distinct transcriptional identity that underpins whether its neurons project locally or to distant targets and which neurotransmitter repertoire it utilises. A key part of early fate determination within a hemilineage is selective neuronal programmed cell death (PCD). This precise deletion of neurons occurs throughout the nervous system, removing neurons of every transmitter type in a segment-specific fashion. Using hybridisation chain reaction in situ (HCR) we reveal the proapoptotic RHG genes *reaper* and *grim*, but not *hid*, are transcribed within doomed neurons in the developing ventral nerve cord (VNC). Novel T2A-GAL4 knock-in reporters for *reaper* and *grim* reveal complex but repeatable expression patterns within hemilineages. These data support functional analysis with null mutants showing that *reaper* and *grim* play a complex role in sculpting lineage populations. Using the genetically encoded timer *TransTimer* (He *et al.,* 2019) we show that segmental and sex-specific differences in neuronal subtypes is mediated by a temporal switching to death within identified hemilineages during neurogenesis, and that this works to shape adult VNC circuitry. In females this temporal switching eliminates a critical male-specific population of interneurons via a mechanism in part mediated by the sex-determination gene *Transformer* and its regulation of *reaper* transcription. These data strongly support the growing idea that changes in the patterns of developmental death within hemilineages is a critical mechanism for generating circuit differences within the CNS and contextualises comparative studies we have carried out investigating species specific patterns of PCD within wingless dipterans.

92 Tweek promotes ER–plasma membrane contact formation to support astrocyte phagocytosis during neuronal remodeling

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During developmental neuronal remodeling, large amounts of neuronal debris must be cleared by glial phagocytosis to maintain tissue homeostasis and ensure proper circuit refinement. In *Drosophila*, astrocytes transform into highly phagocytic cells during metamorphosis, engulfing synaptic, axonal, and dendritic remnants. We identify Tweek, a Bridge-like Lipid Transfer Protein (BLTP), as an essential regulator of this process. Tweek localizes to endoplasmic reticulum–plasma membrane (ER–PM) contact sites, where it promotes lipid transfer required for phagosome membrane expansion. Loss of Tweek disrupts lipid organization and ER–PM contacts, leading to defective astrocyte phagocytosis. Notably, disease-associated mutations in the mammalian homolog BLTP1 (linked to Alkuraya–Kucinskas syndrome) produce similar phenotypes. Structural analysis of the *C. eleg*ans homolog *lpd-3* reveals an extended lipid-conducting tunnel coordinated by ionizable residues, and identifies TMEM170 and Spigot as potential regulators of lipid entry. Together, these findings uncover a conserved role for the Tweek/LPD-3/BLTP1 family in lipid transfer at membrane contact sites and establish a mechanistic link between lipid homeostasis and glial phagocytosis.

93 A Roundabout guidance receptor code is regulated by early temporal transcription factors to coordinate the circuit wiring of sensory processing interneurons

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Functional nervous systems require that neurons wire together during development to form neuronal circuits. To establish circuits, neurons from different developmental origins must grow to the same space within the central nervous system to build specific connections. However, it was unknown how spatial pathfinding and circuit wiring are coordinated across synaptic partners. Investigations at the tissue and single neuron levels identified multiple classes of ligands and receptors, or guidance cues, that regulate neuronal growth and pathfinding. For example, along the medial-lateral axis the repellant ligand Slit and its three Roundabout (Robo) receptors regulate neuronal morphology and midline crossing. Therefore, we investigated the extent to which a code of guidance receptors is shared across neuronal partners to coordinate circuit wiring. We first determined that while *robo 1* is ubiquitously expressed across the *Drosophila melanogaster* ventral nerve cord (VNC), *robo 2* and *3* are differentially expressed associated with the developmental birth order of each neuron. With this pattern in mind, we assayed a well characterized neural lineage that establishes connections with two distinct somatosensory circuits. Neuroblast 3-3 (NB 3-3) produces two cohorts of **E**ven-skipped (+) **L**ateral (EL) sensory processing interneurons depending on the birth order of the neuron. Early-born ELs receive input from Chordotonal (CHO) sensory neurons, and late-born ELs receive input from Dorsal Bipolar Dendrite (DBD) sensory neurons. Previous work established that CHO sensory neurons express and require *robo 3* to position their axon terminals (Zlatic et al. 2003). We show that the early-born ELs express *robo 3* like their CHO inputs, while late-born ELs are *robo 3* negative, similar to their DBD inputs. Therefore, *robo 3* is differentially expressed at the level of neural lineages to potentially coordinate unique circuit wiring outcomes. We find through analyzing both a genetic knockout MARCM assay and an early-born EL specific knockdown that Robo 3 is necessary for early-born EL morphology, specifically their dendritic arborization. Additionally, we show that Robo 3 is necessary for the sensory processing function of early-born ELs. Finally, we demonstrate that *robo 3* differential expression across EL interneurons and the VNC is regulated by early but not late temporal transcription factor expression. Our findings reveal that neuronal partners share a guidance receptor code for circuit wiring. Altogether, our research establishes a new mechanism for neuronal circuit wiring in which guidance receptors are differentially regulated during development to coordinate the wiring of synaptic partners and functional circuits.

94 Expanding the role of *Dscam1* in regulating dendrite tiling and cross-class repulsion

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A neuron's dendritic branching pattern is critical for the neuronal connectivity and information processing. To pattern dendritic arbors, neurons utilize contact-based repulsion to maximize dendrite coverage while minimizing overlaps. This contact-based repulsion gives rise to self-avoidance among dendrites of the same neuron, dendritic tiling of neighboring neurons of the same type, and is believed to be absent among different neuronal types to allow cross-type coexistence. Although self-avoidance is known to be mediated by *Down Syndrome Cell Adhesion Molecule 1 (Dscam1)*, due to its potential to be alternatively spliced into 19,008 unique extracellular domains, the molecules responsible for repulsion during dendritic tiling remains elusive. To systematically investigate dendrite interactions, we used dendritic arborization (da) neurons in the *Drosophila* peripheral nervous system as a model, given that these neurons exhibit all three types of dendrite interactions. By visualizing different classes of neurons simultaneously, we discovered previously unknown cross-class dendrite repulsion between class I (C1da) and class IV (C4da) neurons. Surprisingly, through CRISPR-mediated knockout of *Dscam1* in da neurons, we observed defects in dendritic tiling and cross-class repulsion, in addition to the failure of dendrite self-avoidance. By progressively reducing *Dscam1* expression to weaken Dscam1-mediated repulsion, we found that self-avoidance is more sensitive to *Dscam1* reduction than tiling and cross-class repulsion, suggesting that different adhesions within and among neurons may be an additional factor modulating dendrite repulsion. Furthermore, by tagging endogenous *Dscam1* with mNeonGreen, we found that Dscam1 dynamically forms protein clusters on dendrites, which seem to correlate with dendrite contacts. Finally, using OptoTrap, a light-inducible protein clustering tool developed in our lab, we discovered that acute Dscam1 clustering disrupts dendrite repulsion, phenocopying *Dscam1* loss-of-function. Altogether, these findings reveal shared and distinct mechanisms among different types of dendrite repulsion and clustering-dependent regulation of Dscam1 activity. This work opens a new avenue for understanding the detailed molecular mechanisms governing dendrite-dendrite interactions and morphogenesis.

95 Identification of a novel transcription factor *giraffe* that regulates midline crossing and wiring specificity

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Decades of research have identified conserved guidance cues and receptors that mediate axon attraction and repulsion, forming the universal logic of axon pathfinding. However, how these guidance programs are transcriptionally regulated to generate precise neural circuits remains a major unresolved question in the field of neural development.

Using a single-cell RNA sequencing-guided genetic screen in *Drosophila*, we identified a previously uncharacterized gene, *CG43689*, which we named *giraffe* for its distinctive "long-neck" miswiring phenotype. *giraffe* encodes a putative transcription factor whose human ortholog, *MYT1L*, is implicated in intellectual disability and autism spectrum disorder, yet its molecular role in neural wiring has not been investigated. In wild-type flies, olfactory receptor neurons targeting the ventral glomerulus (V-ORNs) project only to the ipsilateral side. Upon *giraffe* knockdown (KD), however, this specificity is disrupted, leading to two miswiring phenotypes: midline crossing and ipsilateral mistargeting. Single-nucleus RNA-seq of *giraffe*-KD ORNs, combined with a secondary genetic screen, identified several downstream targets, among which *espinas* (*esn*) showed a strong functional link. *esn* encodes a LIM-domain cytoplasmic protein, and its knockdown in V-ORNs phenocopies the midline crossing phenotype of *giraffe*-KD without inducing ipsilateral mistargeting. This indicates that *esn* functions downstream of *giraffe* to specifically inhibit midline crossing. Notably, overexpression of *esn* is sufficient to block commissural ORNs from crossing, reinforcing its role as a key effector that suppresses midline crossing. In contrast, knockdown of other *giraffe*-regulated genes causes ipsilateral mistargeting without midline crossing. This suggests that accurate targeting requires the combinatorial action of multiple downstream factors under *giraffe* control.

Together, our findings identify *giraffe* as a novel transcription factor that regulates multiple downstream effectors to coordinate midline crossing and targeting specificity. This work reveals a previously unrecognized transcriptional layer controlling neural wiring, with possible implications for neurodevelopmental disorders.

96 Fat2 and Sidekick regulate photoreceptor pathway choice during development

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The establishment of functional neural circuits requires neurons to extend axons into the correct brain region, and to make connections with specific synaptic partners. The molecular cues that define how photoreceptors select the appropriate synaptic partners within the medulla are well characterized, but how photoreceptors choose a path between neuropil regions remains unclear. In wild-type flies, early-born photoreceptors extend axons through the posterior lamina and project into the anterior-most distal face of the medulla while axons of later-born photoreceptors extend from the anterior lamina into the posterior-most distal medulla face. A population of glial cells separates axon tracts in the lamina from parallel tracts of medulla neurons and expresses the secreted ligand Slit; loss of the Slit receptor Robo3 causes posterior photoreceptors to extend projections aberrantly, entering the medulla from the proximal face. We found that loss of the atypical cadherin Fat2 or the tricellular adherens junction protein Sidekick causes a subset of posterior photoreceptors to follow a similarly aberrant path into the medulla, extending projections through the medulla before correctly reinserting along the distal face. We show that these defects occur during late larval stages and persist throughout pupal development but are limited to a subset of photoreceptors; anterior photoreceptors that extend projections correctly between neuropils do not display targeting defects. Upon reentry into the medulla, axons of misdirected photoreceptors appear to terminate in the correct synaptic layer. Fat2 has been shown to interact with the WAVE regulatory complex to orient the actin-rich protrusions that direct collective cell migration in the follicular epithelium. We found that loss of the intracellular WAVE regulatory complex interacting motifs is dispensable for directing photoreceptor targeting, but complete loss of the intracellular domain is not. Loss of Sidekick enhances the *fat2* phenotype, increasing the number of photoreceptor axons that take an abnormal path into the medulla. Similar photoreceptor phenotypes have been observed when the Ig adhesion protein Roughest or the transcription factor Bunched are lost. These data suggest that multiple parallel pathways may regulate photoreceptor pathway choice between synaptic regions.

97 A novel role of a visual signaling complex in the development of light-sensing compartments

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Drosophila photoreceptor neurons have highly specialized light-sensing compartments (rhabdomeres) that house millions of light-sensing pigments and the associated phototransduction proteins. The phototransduction proteins are assembled into a signaling complex by the PDZ-domain containing scaffolding protein INAD (Inactivation No Afterpotential D). The resulting spatial organization reduces diffusional delays, promotes protein-protein interactions, and thus increases visual signaling speed. While this *physiological* role of the INAD complex for phototransduction is well understood, we found that INAD and some of its binding partners are expressed during early rhabdomere development, long before the onset of phototransduction. Therefore, we hypothesized that the scaffolding protein INAD and its binding partners play an additional *structural* role during the development of the light-sensing compartments.

We study the INAD scaffold in *Drosophila* rhabdomeres as a model for the mechanisms that mediate the establishment and maintenance of specialized neuronal compartments. We discovered that the PDZ domain protein INAD and its binding partners - the phototransduction proteins INAC, TRP, and NINAC - are expressed during early rhabdomere development. In addition, we identified a novel interactor of INAD during rhabdomere development, the transmembrane protein Mps (Major photoreceptor stabilizer). Remarkably, we found that Mps is required for the rhabdomeric localization of both INAD and the light-sensitive cation channel TRP. Consistent with the developmental expression of these proteins, we unraveled that INAD and its binding partner Mps are required for the establishment of wild type rhabdomere morphology. Furthermore, our data suggest that INAC (INAD's PDZ2 domain interactor) and NINAC (INAD's PDZ1 domain interactor) are not essential for rhabdomere development but are important for maintaining the proper rhabdomere morphology in adults.

Taken together, in addition to the well-known role of INAD and its binding partners in phototransduction, our findings elucidated a novel role of the INAD complex in the development of the light-sensing rhabdomeres. These studies will provide conceptual insights into the role of molecular scaffolds in the establishment and maintenance of specialized neuronal compartments.

98 Confinement limits the oscillatory migration of *Drosophila* cardiac progenitors

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Heart development begins with the formation of a tube. Improper heart tube formation can cause congenital heart disorders. Heart tube formation occurs as two groups of cardiac progenitors (the cardioblasts) move from opposite sides of the embryo, meet, and fuse. Using *Drosophila melanogaster* embryos, we showed that cardioblasts take periodic forward and backward steps as they migrate. Cardioblast steps are associated with waves of the molecular motor non-muscle myosin II cycling between the leading and trailing edges of the cells in a process controlled by Cdc42 signalling. Myosin waves induce cardioblast shape oscillations, which are rectified into directional movement by a supracellular actin cable at the trailing edge of the cells. These results reveal a novel mechanism of rectified, collective cell movement *in vivo*. However, the reason that cardioblasts move in an oscillatory manner is unclear. Cells cultured in confinement can display oscillatory, directional migration. Cardioblasts move through a dense extracellular matrix, in the narrow space between the yolk and an extraembryonic epithelium, suggesting that confinement may induce cardioblast oscillations. To test this possibility, we increased cardioblast confinement by placing a 5-mm-thick, 2% agar block on embryos glued to a coverslip. Using *in vivo* quantitative time-lapse microscopy, we found that confinement decreased the speed of cardioblast migration by 20%. The reduced speed of cardioblast movement was due to a reduction in the amplitude of both forward and backward steps, with the forward steps experiencing a 20% greater reduction. Thus, our results suggest that *in vivo*, confinement limits oscillatory cell movements. Confinement affects cell movement *in vitro* through stretch activated ion channels (SAICs). To determine if SAICs control the dynamics of cardioblast migration, we treated embryos with 50 mM gadolinium chloride, a SAIC inhibitor. SAIC inhibition slowed cardioblast migration by 57% with respect to controls, and the amplitude of cell shape change decreased by 72%. Notably, myosin oscillations stopped when SAICs were inhibited. Our results indicate the SAICs are necessary for cardioblast oscillations and directional movement during *Drosophila* heart tube formation. Together, our findings contribute to our understanding of the mechanisms of collective cell movement in living animals, and may reveal the forces that drive oscillatory cell migration.

99 Molecular genetic and cell biological anaysis of Ccm3-dependent signaling in morphogenesis.

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Morphogenesis of epithelial tubes requires coordination between membrane trafficking and signaling, but the mechanisms linking these processes remain poorly defined. Here, we identify a conserved Hippo-like signaling module that directs Rab11-dependent apical recycling to shape tissue architecture. In *Drosophila*, maternal and zygotic loss of *Ccm3* disrupts tracheal tube morphogenesis, abolishes activation of its Hippo-like partner kinase, GckIII, and prevents phosphorylation of the NDR kinase Tricornered (Trc). We show that Mo25 acts as an obligate cofactor for GckIII, and that Ccm3/GckIII-dependent signaling regulates apical trafficking and junctional remodeling. Importantly, genetic suppression demonstrates that Rab11 activity is a critical downstream effector driving tube dilations. The pathway extends beyond tracheal morphogenesis, controlling planar polarity readout in wing epithelia and motor neuron morphology. These findings define a fundamental mechanism by which scaffolds couple Hippo-related kinases to Rab11 trafficking.

100 The novel adapter protein Bbo regulates targeting of Hobbit to ER-PM contact sites

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The bridge-like lipid transfer proteins (BLTPs) are an emerging family of intracellular lipid transporters that drive bulk movement of lipids between membranes at organelle membrane contact sites. Dysfunction of BLTPs is associated with neurodevelopmental and neurodegenerative diseases in humans; thus, understanding the mechanisms that regulate the function of these proteins is essential to uncover their role in disease. Previously, we found that BLTP2 (Hobbit in *Drosophila*) localizes to endoplasmic reticulum-plasma membrane (ER-PM) contact sites, with the N-terminus anchored in the ER membrane via a hydrophobic helix and the C-terminus interacting with the PM through an unknown mechanism. Here we show that a previously uncharacterized and highly conserved protein, which we name *bilbobaggins* (*bbo*), acts as an adapter protein that mediates interaction between the C-terminus of Hobbit and the PM. Our genetic and cell biology analyses demonstrate that *bbo* is required for *hobbit* localization and function, and we identify critical, conserved protein sequences that mediate Hobbit-Bbo binding. Altogether, our work highlights adapter protein binding as a novel regulatory paradigm that governs targeting of BLTPs to membrane contact sites.

101 PDZD8 regulates synaptic growth by promoting autophagy at ER-Lysosome contact sites through WNT signaling

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Building synaptic connections requires coordinating a host of cellular activities, from cell signaling to protein turnover - placing a high demand on intracellular communication. Membrane contact sites (MCSs) formed between organelles have emerged as key signaling hubs for coordinating diverse cellular activities, yet their roles in the developing nervous system remain poorly understood. Through an *in vivo* CRISPR screen in *Drosophila*, we identified the conserved endoplasmic reticulum (ER) MCS tether and lipid-transfer protein PDZD8 as a regulator of activity-dependent synapse development. PDZD8 was recently linked to intellectual disability, underscoring its conserved role in the nervous system. We have found that PDZD8 functions at ER-late endosome/lysosome MCSs to promote lysosomal maturation and increase autophagic flux during periods of high demand, such as prolonged neuronal activity. To identify PDZD8's synaptogenic target, we screened growth factor signaling pathways and found that PDZD8-dependent autophagy promotes synaptic growth through the WNT/Wg pathway. We hypothesize that PDZD8 targets a negative regulator of the pathway and conducted co-immunoprecipitation-mass spectrometry to identify potential direct targets. We identified Carrier of Wg (Cow), a heparan sulfate proteoglycan, as a prominent PDZD8 interactor and find that PDZD8-dependent autophagy modulates Cow levels. Together, our findings support the model that PDZD8-dependent autophagy modulates WNT signaling to promote synaptogenesis, revealing a crucial role for lipid transfer at MCSs in dynamically regulating neuronal signaling.

102 Mutual dependence of Osbp and PI4KII in secretory granule maturation

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Secretory granules (SGs) store and secrete biologically active molecules important for animal physiology. SGs originate at the *trans*-Golgi Network (TGN) as small, immature granules that contain cargo proteins and undergo homotypic fusion to form larger, mature SGs. Successful SG biogenesis requires a convergence of post-Golgi trafficking and endosomal transport pathways. Of the many facets of regulation these pathways are subjected to, the role of signalling phospholipids and sterols is particularly intriguing. SG membranes are enriched in phosphatidylinositol 4-phosphate (PI4P). However, little is known about how PI4P on SG membranes is regulated, nor how PI4P affects SG biogenesis. Here, we identify Oxysterol binding protein (Osbp) as a novel regulator of SG biogenesis in Drosophila larval salivary glands. Loss of Osbp leads to smaller granules. Genetic analysis reveals that Osbp requires type II phosphatidylinositol 4-kinase (PI4KII), which synthesizes PI4P, to increase SG size. Reciprocally, Osbp is needed for proper localization of PI4KII and PI4P, as loss of Osbp results in aberrant distribution of PI4P and loss of cholesterol-rich endosomal tubules. In addition, crinophagy, an SG-specific autophagic modality, is upregulated in both Osbp overexpression and null mutants. Feeding larvae with food supplemented with sterol partially suppresses the SG size and PI4P distribution defects in Osbp mutants. We propose an SG maturation model in which reciprocal regulation of Osbp and PI4KII drives the formation of cholesterol-rich membrane tubules from endosomes. These tubules, in turn, elevate PI4P in the granule membranes and aid in their maturation.

103 An RNAi-based screen of cadherin proximal proteins reveals candidate regulators of differential cadherin function

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Collective cell migration is critical for morphogenesis, homeostasis, and wound healing. Classic cadherins mediate cell-cell adhesion in many of the body's organs, with E-cadherin predominant in most epithelial tissues. During development, migrating mesenchymal cells linked by N-cadherin form tissues that shape some of the body's organs. We are interested in exploring how these two cadherins mediate different tissue architectures and cell behaviors. We use the *Drosophila* testis nascent myotubes as our N-cadherin mediated mesenchymal model. During development, precursor muscle cells migrate from the genital disc onto the testis, ensheathing the testis in muscle and creating a characteristic spiral shape. Both classic cadherins link to the actin cytoskeleton via multi-protein complexes, and we hypothesize that there might be differences in the proteins used by each cadherin. To test this hypothesis, we performed a proximity proteomics screen with the protein Afadin, the homolog of *Drosophila* Canoe, one of the proteins linking E-cadherin's cytoplasmic domain to actin. This provided a list of proteins that might work with E-cadherin, N-cadherin, or both. We identified fly homologs of the Afadin proximal proteins and set out to knock them down in our mesenchymal model. We use RNA interference knockdown under the control of the muscle-specific Mef2-GAL4 driver to assess how knockdown of individual candidate proteins affects myotube adhesion, migratory behavior, and the morphogenesis of the testis. So far, we have knocked down 74 genes using over 168 different RNAi lines. Intriguingly, while knocking down all components of the core cadherin-catenin complex affects testis morphogenesis, knocking down several other known cadherin partners, like Canoe and Bazooka/Par-3, does not. This supports our hypothesis that different proteins act in epithelial and mesenchymal cells. We have a number of other interesting hits, including the Rab5 GAP RN-tre. We are currently live cell imaging RN-tre knockdown myoblasts during migration. We are visualizing E and N-cadherin staining in multiple stages of fixed pupal testes to understand where these cadherins localize during myotube development, and to explore the potential differential localization of known cadherin partners like Canoe and Bazooka/Par-3. Together, our dataset will provide an important resource for our lab and the broader community to define genes regulating N-cadherin based migration and adhesion.

104 Crag couples Calmodulin-mediated polarized cortical targeting and Rab10/Rab8 dual activation to enforce basal deposition of basement membrane in epithelial cells.

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Apical-basolateral polarity (ABP) is essential for the proper functioning and structural integrity of epithelial cells, which are crucial to organismal development. The basement membrane (BM), a specialized extracellular matrix underlying the epithelial cells on their basal side, contributes to the establishment and maintenance of ABP by providing vital environmental cues. Despite its importance, the mechanisms that confine the BM to the basal side of epithelia are poorly understood. To study BM deposition, we use the follicular epithelium (FE) of *Drosophila melanogaster* ovary as our model system. Using the FE, the GEF/RabGTPase complex, Crag/Rab10, has been shown to be a key regulator of the biological pathway specifically dedicated to the basal restriction of BM components. However, the exact mechanism governing Crag's polarized intracellular localization and its function in BM polarity remains yet to be elucidated. Crag is a multidomain protein that contains the N-terminal DENN domains responsible for its GEF activity, a Calmodulin binding site (CBS) responsible for its Calmodulin-binding activity, and a conserved C-terminus domain with yet unknown function. Our structure-function analysis of Crag reveals that CBS, particularly the binding of CBS to Calmodulin, has a critical role in Crag's apico-lateral cortical localization, while the DENN domains are not required in this process. However, the DENN domains are necessary, but not sufficient, for Crag's activity in restricting the BM components basally. Additionally, we also show that Crag not only controls the activity of Rab10, but also Rab8 – another key regulator of BM polarity – of whose simultaneous activation results in proper deposition of the basement membrane via parallel routes governed by Rab10 and Rab8. This suggests a more central role of Crag in the pathway regulating BM polarity than previously thought. Altogether, our findings enhance our understanding of Crag's role in BM polarity and provide insights into the molecular mechanisms underlying the restriction of the BM.

105 Rcp, a regulator of G-protein-coupled receptor signaling, controls the polarized deposition of basement membrane proteins in epithelial cells.

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Epithelia are organized in sheets of tightly adherent cells that are polarized along an apical basal axis. Epithelial cells rely on their cellular organization for their structure and functions. The apical-basal polarity is characterized by the cytoplasmic and surface organization of individual cells in apical, basal, and lateral domains. The establishment and maintenance of these different domains rely on the transport and sorting of newly synthesized or recycled proteins to their correct locations. A key component of this organization is the basement membrane (BM), a specialized sheet within the extracellular matrix. The BM lies along the basal side of epithelial cells and is essential for the establishment and maintenance of epithelial cell structure and functions, tissue organization, and organ morphogenesis. The loss of integrity and misregulation of the BM leads to epithelial defects which can cause changes in signaling and cellular communication and has been associated with carcinomas and tumor metastasis. The BM components are produced within the epithelial cells and are secreted to the basal side through a dedicated pathway. Despite the BM's important roles in epithelial organization, the process of the secretion of BM proteins is poorly understood. To study BM protein deposition, we use the follicular epithelium (FE) of the Drosophila ovary as a model system. In a genetic screen looking for new genes involved in the proper placement of BM proteins, we identified a new gene, Rcp (Receptor component protein), which has been shown to be involved in G-protein coupled receptor (GPCR) signaling. Our data show that the loss of Rcp leads to an apical mislocalization of BM proteins without primarily affecting the general polarity, indicating that Rcp specifically controls the proper placement of BM proteins. Interestingly, Rcp is the first component of a signaling pathway implicated in BM polarity. In the FE, Rcp assumes cytoplasmic and nuclear localizations, this data paired with Rcp's protein domain suggests a role of Rcp in gene regulation. Finally, we also showed that Rcp genetically interacts with other key members of the pathway dedicated to the polarized deposition of BM proteins such as the GEFs Crag and Stratum and the phospholipid PIP2. Altogether, our data indicate a specific role for Rcp in epithelial organization by regulating the polarized deposition of BM proteins, a process that is critical for the establishment and maintenance of epithelial cell polarity.

106 The functional organization of chromosome territories in single nuclei during zygotic genome activation

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Chromosome territories (CTs) are intricately organized and regulated within the nucleus. Despite remarkable advances in our understanding of genome packaging and gene expression, the interplay among CTs, pairing of parental homologous chromosomes, and genome function during development remains elusive. Here, we employ an Oligopaints-based high-resolution imaging approach to examine variable CT organization in single nuclei during the developmental process of zygotic genome activation. We reveal large-scale chromosome changes in the genome as the embryonic genome awakens. To understand the impact of homolog pairing in the diploid genome, we eliminate *trans*-homolog interactions entirely using haploid embryos. Our data shows the dynamics of CT compaction and RNA polymerase II recruitment are supported by transcriptional changes in haploid embryos. Finally, global inhibition of transcription results in decreased CT opening and no significant impact on CT pairing levels. These findings enhance our understanding of parental genome folding and regulation, which may inform strategies for chromosome-based diseases.

107 Inferring Chromatin Architecture at a Single Locus through Probabilistic In Situ DNA Localization

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While chromatin conformation is likely critical for enhancer function, its dynamic and nanoscale organization remains challenging to study directly. Here, we present PLOTTED (Probabilistic Localization of Oligopaint Tagged Target Element Distances), an integrated imaging, computational, and conceptual framework for inferring chromatin architecture from targeted high-resolution imaging of cis-regulatory modules (CRMs). PLOTTED constructs probabilistic spatial distance distributions between DNA loci, enabling quantitative modeling of chromatin configurations across time, space, and genotype. PLOTTED focuses on visualizing and assaying local DNA interactions associated with a single gene locus using a widely accessible DNA FISH protocol, an imaging approach that is compatible with protein staining, and a super-resolution confocal microscope. The technique also provided a novel conceptual framework for looking at how three DNA regulatory elements interact in probabilistic space to regulate gene expression. To demonstrate its utility, we measured spatial distances between three CRMs at the brinker locus in Drosophila embryos, using chromatin conformation as a proxy for regulatory activity. We analyzed two mutations that delay brinker expression: an 800 bp deletion near the promoter (Δ800PPE), and a MiMIC transposon insertion that moves an enhancer 7 kb farther from the promoter. In wild-type embryos, CRM geometry shifts dynamically at nuclear cycle 13; while these shifts are delayed in both mutants and vary along the dorsal-ventral and anterior–posterior axes. Importantly, the conformational changes correlate with altered gene expression. In summary, PLOTTED enables interpretation of mutant phenotypes and offers a probabilistic, single-locus approach for inferring chromatin conformation from high-resolution nuclear images, advancing our understanding of spatial and temporal gene regulation. PLOTTED is a useful tool that can expand the functionality of preexisting labeling techniques, and provides insight into the interplay between chromatin conformation and gene expression. This technique can be used in any system that has DNA, can be fixed, and can be imaged on a superresolution confocal microscope.

108 Quantitative analysis of the impact of local chromatin organization on transcriptional dynamics

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Transcription, the conversion of DNA to RNA, is a precisely-controlled process that is essential for proper development and maintenance of an organism. Gene promoters and their regulatory enhancers must interact in spatial proximity to initiate transcription. However, in the eukaryotic genome, enhancers and their target promoters are often separated by large linear genomic distances containing numerous non-target genes, calling into question how these elements reliably find each other in the crowded nuclear environment. Insulator elements serve a prominent role in this long-range enhancer-promoter communication by organizing local chromatin into loop-like domains, facilitating interaction within the loop and silencing interaction with elements outside of it. Despite their importance, systematic analysis on the properties of insulators and their effect on transcription is lacking. This study employs single-cell live imaging techniques to elucidate the dynamics of insulator-mediated chromatin looping and its role in transcriptional regulation. Our system consists of a single enhancer that regulates two equidistant promoters in cis. We show that a single enhancer can co-activate two target promoters in cis, such that transcriptional kinetics from the two reporter genes are coordinated. Insulators are then introduced in various positions and orientations to analyze their effect on transcription. Insulator-mediated looping of the system further synchronizes gene activity and increases transcriptional output. Interestingly, bidirectional insulator conformations produce a more pronounced effect than unidirectional ones, indicating an orientation-dependent stability in insulator pairing. Insertion of an insulator between enhancer and promoter results in a sharp decrease in transcriptional output of the blocked reporter gene. Surprisingly, the blocked promoter is able to escape silencing and often exhibits coordinated transcriptional activity with the unblocked promoter. These results challenge the traditional view that insulator-mediated chromatin loops are largely static and suggest that the dynamic nature of insulator pairing contributes to transcriptional regulation to varying degrees, based upon their relative positions and orientations. Taken together, this work enhances our understanding of the relationship between local 3D chromatin architecture and transcription, providing new insights into the complex mechanisms governing gene expression.

109 Lower-order methylation states preserve parent-of-origin Polycomb modification asymmetries in *Drosophila* embryogenesis

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To begin the development of a multicellular organism, a single, undifferentiated cell undergoes a period of intense proliferation. During this time, maternally supplied proteins interact with the chromatin of the zygote to determine its epigenetic landscape. How maternal proteins establish and maintain histone modifications over early development remains an open question, given the rapid pace of the cell cycle creates unfavorable conditions: with every round of genome replication, the incorporation of unmodified histones to chromatin dilutes modification levels. Here, we consider the embryogenesis of *Drosophila melanogaster* to investigate how Polycomb Group (PcG) proteins regulate the methylation of histone H3 Lysine 27 (H3K27), a modification associated with silenced gene expression states. By live-imaging GFP-tagged PcG proteins, we find that the H3K27 methyltransferase Enhancer of Zeste (E(z)) occupies nuclei from the beginning of development, while factors involved in nucleating E(z) activity on chromatin are excluded from nuclei until later stages. With genome-wide sequencing we reveal that, despite H3K27me3 decorating maternal chromosomes at the onset of development, the mark is lost over the cleavage stage and not re-established until cell cycle lengthening. To interpret these measurements, we developed a computational Monte Carlo model for predicting the H3K27 methylation composition of a nucleosome array across cell divisions. This model accounts for the nuclear concentration dynamics of E(z) and the nucleating factor Pleiohomeotic (Pho), as well as allosteric enhancement of E(z) activity by existing H3K27me2/3. When calibrated to our measurements of H3K27me3 later in development, the model predicts that allosteric regulation of E(z) allows for the maintenance of lower-order H3K27 methylation states on maternal chromosomes over the cleavage stage. In contrast, the model predicts that paternal chromosomes lack all forms of H3K27 methylation until Pho enters nuclei near the end of the cleavage divisions. We confirm these predictions experimentally with *in vivo* imaging experiments using wild-type and mutant embryos. Our work provides a mechanistic explanation for the inheritance of Polycomb states over rapid cell cycles.

110 Scm recognition of H4K20me1 in de novo Polycomb domain formation

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Mitotically stable Polycomb chromatin domains enable the robust maintenance of cell fate required during development, but how these domains are initially constructed remains poorly understood. We sought to understand how the chromatin landscape that precedes the establishment of Polycomb chromatin domains might influence the de novo formation of these domains during the Maternal-to-Zygotic Transition.

Using genomics, biochemistry, quantitative imaging, and classical genetics, we examined the Polycomb Group protein Scm (sex combs on midleg) during this pivotal developmental window.

Given prior reports that Scm can bind mono- and di-methyl lysines on diverse histone tails, we measured its affinity for these modifications on recombinant nucleosomes, reasoning that the broader nucleosome context might reveal Scm's specificity for particular histone modifications. We found that, in this context, Scm's MBT domain specifically binds to the mono-methylated lysine 20 on the tail of histone H4 (H4K20me1), and that a nearby Zinc Finger domain contributes to this binding.

To investigate in vivo settings in which binding of Scm to H4K20me1 might contribute to Polycomb target gene regulation, we performed immunofluorescence experiments and discovered that H4K20me1 is present in chromatin during early embryonic development, in stark contrast to many other histone modifications.

Additionally, live imaging of Scm revealed that it remains bound to chromatin during early mitotic divisions, indicating that it may function as a mitotic bookmark.

To determine the consequence of Scm-H4K20me1 interactions on de novo Polycomb domain formation, we are using the JabbaTrap system to transiently remove maternally deposited Scm while leaving behind engineered binding-deficient variants. Ongoing genomic profiling of Scm and H4K20me1's localization patterns in both wild-type and binding-deficient embryos will provide additional insight into how these interactions influence early Polycomb domain formation.

Taken together, our findings raise the possibility that maternally deposited Scm, potentially through its interaction with H4K20me1, contributes to the emergence of the de novo Polycomb landscape post-fertilization. However, the extent and mechanistic basis of this contribution remain to be clarified, and ongoing in vivo perturbation experiments will be essential for refining this model.

111 Transposable elements induce trans-allelic epigenetic effects through interaction with pericentromeric heterochromatin

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Transposable elements (TEs) are "jumping genes" that can copy and move to other genomic locations at the host's expense. To silence TEs' selfish replication, eukaryotic hosts epigenetically silence euchromatic TEs by enriching them with repressive H3K9me2/3 epigenetic marks. However, this mechanism is imperfect, as the H3K9me2/3 can spread into adjacent genomic regions, resulting in cis-silencing. Beyond these local cis effects, previous observations of trans-regulation through somatic pairing suggest that TE-mediated silencing may act across homologous chromosomes. For example, transvection in Drosophila involves gene activation in trans by an enhancer on the homologous chromosome. Similarly, in alleles exhibiting paramutation, the epigenetic state from one homologous chromosome can be transferred to the other. These examples demonstrate that spatial interactions across homologs and genomes can facilitate regulatory interactions. Interestingly, euchromatic TEs enriched with H3K9me2/3 have been observed to spatially contact pericentromeric heterochromatin (PCH) via phase separation. We investigate whether TEs can be paramutagenic, where TE-induced enrichment of H3K9me2/3 can spread in trans to the homologous TE-absent allele and subsequently cause trans silencing. We hypothesize that PCH interactions with the TE-present allele drag the homologous TE-absent allele into proximity to the suppressive PCH domain, and that somatic pairing of TE-present and absent alleles will lead to epigenetic changes of both homologs (trans-epigenetic effects). To test this, we crossed two DSPR founder lines with distinct TE insertion profiles to generate F1 progeny with heterozygous TE insertion sites. We performed CUTnTAG on the embryo of the trio to assay genome-wide H3K9me3 enrichment levels. Our analysis has identified examples where the TE-present allele leads to the enrichment of H3K9me3 on the homologous TE-absent allele, thereby silencing adjacent genes. To systematically characterize these trans effects, we are developing a genome-wide statistical framework to quantify the prevalence of TE-mediated trans-epigenetic silencing, identify the biological features that promote these effects, and assess the associated functional impact on adjacent gene expression. Results from this study will provide novel insights into the functional consequences of TEs, demonstrating that TEs can influence the adjacent genome in both cis and trans manners through 3D nuclear organization.

112 Sex-specific transposable element and heterochromatin dynamics in the germline of species in the *Drosophila nasuta* group

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Transposable elements (TEs) comprise a substantial portion of eukaryotic genomes and influence genome evolution through their mobility and regulation. In germline tissues, TE activity must be tightly silenced to preserve genome integrity, yet the extent and mechanisms of this repression differ between sexes. While TE regulation in ovaries is well characterized, the dynamics in testes remain poorly understood.

To investigate sex-specific differences in TE control, we generated transcriptome, small-RNA, and H3K9me3 profiles from testes and ovaries across multiple species of the *Drosophila nasuta* group. We find that TE expression is consistently higher in testes than in ovaries. This elevated activity coincides with reduced heterochromatin enrichment at repetitive regions, lower piRNA abundance, and fewer ping-pong signatures targeting TEs in testes. Integrative modeling of these datasets suggests that weaker transcriptional and post-transcriptional silencing underlies the pervasive derepression of TEs in the male germline. Our results reveal a conserved bias in germline TE regulation, pointing to fundamental sex-specific constraints on genome defense. Such asymmetry may influence how new TE insertions arise and spread through populations, revealing how sex-specific genome defense shapes the evolutionary trajectory of eukaryotic genomes.

113 Suboptimal ribosomal RNA variants are a 'genomic fuse' that triggers germline ribosomal DNA magnification

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Ribosomal DNA (rDNA) loci contain the hundreds of tandemly duplicated copies of ribosomal RNA (rRNA) genes needed to transcribe sufficient rRNA for ribosome biogenesis. The repetitive structure of rDNA loci makes them inherently unstable, yet the consistent inheritance of functionally sufficient rDNA copies at each generation implies the existence of germline mechanisms to monitor and maintain rDNA copy number. These mechanisms are best understood in the Drosophila male germline, where rapid expansion of rDNA copies in germline stem cells (GSCs) restores rDNA lost over time – a phenomenon called 'rDNA magnification.' This activity is tightly regulated to only function when rDNA copy number is reduced, though it is unclear how genomic rDNA copy number is sensed or what severity of rDNA deficiency is needed to elicit rDNA magnification. Here we find that ribosome biogenesis serves as a cellular sensor to monitor rDNA copy number status within GSCs and regulate rDNA magnification. Combined transcriptomic and cellular analyses reveal that ribosome biogenesis is disrupted in low rDNA GSCs, leading to reduced overall translation. We further find that perturbing ribosome biogenesis in normal rDNA conditions induces rDNA magnification. Intriguingly, we do not find any difference in rRNA transcription in the GSCs of low rDNA animals, indicating that ribosome biogenesis is disrupted despite there still being sufficient rDNA to meet rRNA transcriptional demand. Instead, we find that ribosome biogenesis in low rDNA GSCs is disrupted by the aberrant expression an rRNA variant containing sequence polymorphisms that impedes ribosome biogenesis. rDNA copies containing this 'suboptimal' rRNA variant are normally silenced but become expressed in low rDNA conditions, likely out of necessity to meet the demand for rRNA transcription. Strikingly, low rDNA conditions lacking this suboptimal rRNA variant do not induce rDNA magnification, and conversely, normal rDNA conditions forced to express this variant ectopically activate rDNA magnification. Together, these findings reveal that Drosophila rDNA copy number is not directly monitored, but that it is the disruption of rDNA function that triggers rDNA expansion. We propose that genomes retain suboptimal rRNA variants whose consequential expression upon rDNA reduction acts as a 'fuse' to disrupt ribosome biogenesis prior to rDNA copy number being reduced below functionally insufficient – and potentially unrecoverable – levels.

114 Neuronal circuitry downregulating aggression across sexes

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Regulating aggression is particularly critical for survival across species, as engagement alters group dynamics and can lead to injury or even death. While *Drosophila* display aggression across sexes, features of female and male aggression differ, including the environmental contexts and behavioral components. Previous work by our lab and others has implicated several isomorphic and sexually dimorphic cell types in guiding the approach, engagement, and continuation of female aggression (Palavicino-Maggio et al., 2019; Schretter et al., 2020; Deutsch et al., 2020; Chiu et al., 2021; Chiu et al., 2025; Schretter et al., 2025). However, little is known about the circuitry downregulating aggression across sexes due to the lack of a complete map of the central nervous system, or connectome, in a male fly.

In the present study, we used the female connectomes alongside the recently completed male dataset to map a sexually dimorphic aggression-promoting cell type, aIPg, across sexes. We uncovered a novel glutamatergic cell type that preferentially targets the aIPg circuit. Further quantitative behavioral analysis uncovered that this cell type downregulates components of aggressive behavior across sexes. In addition to divergent inputs, common visual and auditory pathways innervating this isomorphic cell type suggest specific circuit mechanisms for both conserved and variable regulation of aggression. Beyond aggression, our study has important implications for how the brain flexibly regulates social behaviors across sexes and in changing environmental contexts.

115 From perception to valence: a pair of interneurons that assign positive valence to sweet sensation in *Drosophila*

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Assigning valence—appeal or aversion—to sensory stimuli and relaying it to higherorder brain regions to guide flexible behaviors is crucial to survival. Yet the neural circuit that transforms sensory input into motivationally relevant signals remains poorly defined in any model system. In *Drosophila melanogaster*, substantial progress has been made in mapping the sensorimotor pathway for feeding and the architecture of the dopaminergic reinforcement system. However, where and how valence is first assigned to a taste remains unknown. Here, we identified a pair of subesophageal zone interneurons in *Drosophila*, termed fox, that impart positive valence to sweet taste and convey this signal to the mushroom body, the fly's associative learning center. We show that fox neuron activity is necessary and sufficient to drive appetitive behaviors and can override a tastant's intrinsic valence without impairing taste quality discrimination. Furthermore, fox neurons transmit the positive valence to specific dopaminergic neurons that mediate appetitive memory formation. Our findings reveal a circuit mechanism that transforms sweet sensation into a reinforcing signal to support learned sugar responses. The fox neurons exhibit a convergent–divergent "hourglass" circuit motif, acting as a bottleneck for valence assignment and distributing motivational signals to higher-order centers. This architecture confers both robustness and flexibility in reward processing—a principle that may generalize across species.

116 A schizophrenia/autism-associated topoisomerase complex regulates circadian rhythms and circuit development in *Drosophila*

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Topoisomerase 3b (Top3b) is a dual activity enzyme that relieves topological tension during DNA and RNA metabolism. Top3b forms a conserved complex with other RNA-binding proteins: Tudor domain containing 3 (Tdrd3) and Fmr1, the protein implicated in Fragile X mental retardation Syndrome, the most common form of autism. Top3b-Tdrd3 regulates mRNA translation and turnover, and siRNA-guided heterochromatin formation. Mutations in *Top3b* have been associated with multiple neurodevelopmental disorders (NDDs), such as schizophrenia and autism, and *Top3b*-KO and *Tdrd3*-KO mouse models similarly exhibit neurodevelopmental, cognitive, and behavioral deficits. Furthermore, *Top3b*-KO mice have shown changes in circadian activity.

Notably, sleep and circadian system defects are core pathological features of many neurodevelopmental disorders (NDDs); and disruption of circadian rhythms, whether due to genetic or environmental factors, may contribute to the development of NDDs and accelerate physical and cognitive decline. Nevertheless, the mechanisms underlying circadian dysfunctions and their potential contributions to NDD pathogenesis remain poorly understood.

Given the role of Top3b-Tdrd3 in RNA metabolisms, its links to neurodevelopment, and its interaction with FMRP, we hypothesized that the Top3b-Tdrd3 complex may resemble Fmr1 in playing a critical role in regulation of circadian rest-activity pathways in *Drosophila*. Our circadian locomotor activity assay identified that *Top3b*-KO reduces the strength of free-running rest-activity patterns; this phenotype was dependent on its RNA-binding activity. Furthermore, we found that *Tdrd3*-KO results in completely arrhythmic rest-activity patterns. We found that *Tdrd3*-KO, but not *Top3b*-KO, shows aberrant branching of small-LNvs neurons, central pacemakers which are required to drive normal circadian behavior, and tract splitting of large-LNvs, which regulate sleep and arousal. Furthermore, our RNA-seq at two timepoints, Zeitgeber Time 2 and Zeitgeber Time 14, identified 42% of transcripts in *Top3b*-KO and 36% of transcripts in *Tdrd3*-KO lost rhythmic mRNA abundance compared to WT, suggesting that Top3b-Tdrd3 regulates circadian gene expression. Together, our data suggests Top3b and Tdrd3 as key regulators of rest-activity patterns, and reinforce sleep and circadian dysfunctions as important foci for clinical intervention in NDD patients.

117 Recognition of Distinct Sleep States in *Drosophila* Uncovers Previously Obscured Homeostatic and Circadian Control of Sleep

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Understanding the mechanisms underlying homeostatic sleep regulation is a central unmet goal of sleep science. Our comprehension of such regulation in mammals has required recognizing distinct sleep stages. *Drosophila melanogaster* is an important genetic model system for studying sleep. Since the discovery of sleep-like states in the fly 25 years ago, the field has treated sleep as a unitary state consisting of any inactivity lasting 5 minutes or longer, despite convergent work suggesting the existence of multiple sleep states. Here, we establish that three distinct sleep states in flies can be classified based on simple inactivity duration criteria. We show that the daily initiation of these sleep states is temporally distinct, with long sleep occurring immediately following the largest daily period of wakefulness. We also report that rebound in response to mechanical sleep deprivation is present only in long sleep and comes at the expense of shorter sleep states. Deprivation-induced decreases in shorter sleep states obscure homeostatic sleep rebound, but only when sleep is measured using traditional methods. We observe distinctly timed ultradian oscillations of fly sleep states, reminiscent of mammalian sleep cycles. Our results indicate that the recognition of such sleep states will be necessary to fully realize the promise of the *Drosophila* model system for identifying conserved genetic mechanisms underlying such regulation.

118 Living the sweet life: How neural circuitry drives distinct features of hedonic feeding and its effects on aging

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Healthy aging may be a case of mind over matter. While longevity is often synonymous with healthy habits like eating a balanced diet, research suggests that neural states associated with feeding, the psychological or emotional component of this behavior, may be just as influential. For example, previous work has shown that modifying a fly's perception of its food environment by exposing diet-restricted animals, which normally live longer, to a nutrient-related odorant is sufficient to limit the lifespan-extending effects of restriction without affecting food intake. Additionally, aging is slowed, and lifespan significantly increased, in flies when select hunger neurons in the brain are activated by optogenetic stimulation, despite a subsequent and significant increase in nutrient intake. These examples highlight the importance of understanding how aging is modulated by feeding-related neural states. Because animals exhibit a variety of reasons and motivations for consuming food, a better understanding of their causes and consequences will likely provide opportunities for novel aging- and health-related interventions.

My research focuses on neural states related to hedonic feeding, a feeding drive originally identified in mammals and classified as "need-free" food intake, which is evoked by the incentive salience of food. My goals are to use Drosophila to better understand the neural circuitry of this drive and to determine if and how hedonic feeding experiences modulate aging. Hedonic feeding is promoted by both 'liking' (the pleasurable characteristics of food) and 'wanting' (motivation to consume pleasurable food). I pioneered a suite of behavioral assays to classify these features of hedonic feeding in the fly and used the assays to identify neural circuits that govern these features. I discovered that the α'/β' lobes of the mushroom body and protocerebral anterior medial dopaminergic neurons are involved in hedonic feeding and that distinct subsets of these neural populations influence 'liking' and 'wanting' components. Preliminary data suggest that neurons that encode 'liking' modulate lifespan, but those that only control 'wanting' do not. Flies and mammals share conserved aspects of hedonic feeding neural circuitry and signaling molecules, suggesting that interventions that target neural states related to 'liking,' the pleasurable experience we derive from eating food, may improve health and longevity in humans.

119 Cell body adhesion and electrical synapse coupling is required for the *Drosophila* backward walking circuit

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The generation of behavior relies on many neuronal types having the correct physiology and connectivity. While morphology and synapse number are well-documented to impact neuronal circuit development and function, we investigated what other aspects of neuronal identity are also required. Here, we utilize the *Drosophila* backward walking circuit. The Moonwalker Descending Neuron (MDN) is a command neuron for backward locomotion: it receives signals from the optic lobes and communicates with neurons in the ventral nerve cord. There are two bilateral pairs of MDNs, and while their cell bodies cluster together, each pair sends their axons to their respective contralateral sides. We found that anatomical location of the cell bodies is required for the circuit output, supporting a model where adhesion of the cell bodies is necessary for electrical synapses, allowing synchronous firing of the MDNs and generating backward walking. First, we identified a pathway required for MDN cell body adhesion: the transcription factor Hunchback activates the expression of the cell adhesion molecule Lar. When Hunchback or Lar is knocked down in MDN, the cell bodies are not aggregated and optogenetic activation of MDN fails to command backwards walking. Interestingly, MDN morphology and synapse number is not affected. Next, we investigated why cell body aggregation is required for circuit function. We found that the MDN cell bodies express the gap junction protein Inx8, which can function as an electrical synapse. Inx8 is required for both MDN cell body clustering and for backward walking. Electrical synapses allow ions to be shared between cells, eliciting synchronous action potentials. We used a genetically encoded voltage indicator and found that MDNs are firing synchronously in controls when all four cell bodies are touching. Knocking down Hunchback or Inx8 in MDN, thus breaking the physical contact between the cell bodies, impeded MDNs ability to fire synchronously. Considering that MDNs top downstream partners are side-specific, we believe that synchronous firing of all four MDNs is required for the backward walking circuit to function properly and generate behavior. These data provide novel insights into how electrical synapses, independent from chemical synapses, are essential for neuronal circuit development and function. This work also developed the first protocol using voltage indicators in a brain explant system, increasing the accessibility of this tool.

120 Investigating Feeding Rhythm Circuit Development in *Drosophila* Larvae

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Normal sleep and circadian rhythms during early life are important for brain development. Indeed, disruptions in sleep and rhythms during development are a common co-morbidity in neurodevelopmental disorders including ADHD and autism. Although the molecular mechanisms encoding cellular rhythms are well understood, little is known about how rhythmic behaviors first emerge. We previously determined that sleep-wake and feeding-fasting rhythms develop in early 3rd instar *Drosophila* larvae (L3). Early L3 show higher rates of feeding during the day and lower rates during the night. These diurnal differences are not observed in 2nd instar larvae or in early L3 molecular clock mutants indicating that the circadian regulation of feeding rhythms emerges at the early L3 stage. However, the neuronal circuitry responsible for feeding rhythmic behavior is not known.

Here, we demonstrate that the larval clock neurons, s-LNvs, regulate feeding rhythm development in early L3. We show that activation of SIFamide (SIFa) neurons in the larval pars intercerebralis disrupts feeding rhythm development. Finally, our preliminary data suggest that s-LNv and SIFa neurons form anatomical and functional connections at the early L3 stage but not earlier. Together, our data provide the first insights into the circuit mechanisms responsible for feeding rhythm development. Future work will examine the possible signaling mechanisms that regulate these behaviors.

121 Toll Family Receptors modulate sleep during pathogen infection

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Toll family receptors have been well established in the regulation of immune responses in both vertebrate and invertebrate model systems. Many Toll family receptors in Drosophila are expressed in specific neural circuits in the adult brain. However, functions for these receptors in the context of neuronal function are largely uncharacterized. Our lab is interested in identifying and characterizing neural circuits that regulate behavioral changes in response to pathogen exposure, infection state, and modulation of pro-immune behaviors that may allow the animal to respond or fight immune challenges. To this end, we have cultured the entomopathogenic fungus Metarhizium anisopliae and studied longevity, immune response, and behavioral changes upon exposure to its spores. We observe clear changes to sleep and grooming during an immune challenge. Furthermore, pan-neuronal knockdown of Toll 7 and specific knockdown of Toll7 in the mushroom body appear to modulate sleep during immune challenge. We seek to characterize further individual circuits that modulate illness-related behavioral states, and to neuroanatomically map and understand the modulation of neural activity during immune challenge.

122 Potential rescue of mitochondrial dysfunction by a *Wolbachia* symbiont of *Drosophila*

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Animal-microbe symbioses are ubiquitous in nature, spanning diverse interactions from facultative parasitism to obligate mutualism. Further, many of these relationships are not strictly parasitic or mutualistic, and there can be a continuum between the two states. Indeed, many facultative symbionts co-evolve with their hosts and develop obligate relationships over time. However, the transition between symbiotic states is rarely observed and little is known about the genetics and mechanisms underlying symbiotic transition points. Here, we report a case where a *Wolbachia* bacterial symbiont of *Drosophila bifasciata* is both parasitic to male hosts, but is also important for fertility in females, suggesting it may be in such a state. While the symbiont kills embryonic males, it also has an unusual trait where removal of the symbiont results in abnormal development of female reproductive organs and reduced egg laying compared to a lineage of *D. bifasciata* with no symbiont. Genomic evidence suggests that dysfunctional host mitochondria are the underlying genetic basis of the phenotype. The mitochondrial genome of the host lineage with the symbiont is highly divergent from the lineage with no symbiont. In addition, there is evidence of relaxed selection throughout the genome, and there are many SNPs in sites that are otherwise conserved in diverse *Drosophila* species. This evidence is consistent with a model where the fly lineage with the symbiont has accumulated deleterious mutations in the mitochondria through relaxed selection resulting in reduced fertility. The symbiont compensates for this to benefit females, but retains its original parasitic male-killing phenotype. This *Wolbachia-Drosophila* symbiosis may represent a new case of a symbiosis in transition to mutualism, introduces a novel mechanism of symbiosis through rescue of mitochondrial genetic defects, and allows rare insight into the molecular changes underlying a potential symbiotic transition.

123 Juvenile Hormone activity during metamorphosis and post-mating suppress antibacterial immune defenses

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Reproductive investment is associated with curtailed immune performance across insect taxa. In female Drosophila, mating reduces innate immune defenses and dramatically increases susceptibility to systemic bacterial infections. Prior work from our group demonstrated that Juvenile Hormone (JH) signaling is required for this post-mating loss of infection resistance. Because JH is a crucial regulator of both metamorphosis and oogenesis, we hypothesized that post-mating immune suppression might involve distinct JH actions at both developmental and adult life stages. Using conditional RNAi, we found that silencing the JH receptor gce in the fat body only during the pupal stages fully alleviated mating-induced infection sensitivity in adults. In contrast, adult-specific knockdown of gce in the fat body failed to rescue post-mating immune suppression. Instead, we found that in adults, gce is required both in the fat body and in the ovarian follicle cells to suppress immunity downstream of mating. Our data suggest that gce suppresses immunity via a multi-step process, first affecting the metamorphosing fat body, and then transducing mating-activated JH signaling in the fat body and ovary. To further investigate mating-induced JH functions, we used mass spectrometry and a transgenic reporter line to profile JH titers and JH signaling activity, respectively, over a post-mating time course. We found that JH titers peak within hours of mating, and then subside to relatively low but stable levels higher than those observed pre-mating. However, JH reporter expression was rapidly induced and remained elevated for days post-mating. Because sensitivity to infection is lifelong in mated females, we asked whether this sustained immune suppression would require the sustained JH signaling activity we observed. We found that transient exposure to exogenous JH failed to persistently suppress immunity in unmated females, whereas daily repeated exposures recapitulated the infection susceptibility observed in mated females. In summary, our data support a model in which gce shapes fat body development to condition a JH response that is activated upon mating in adults. This adult-specific function involves fat body-ovary interactions mediated by gce, which then establishes lasting immune suppression in female Drosophila.

124 Activation of Toll and IMD pathways in the *Drosophila* brain following local and systemic bacterial infection

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Brain infections are often life-threatening and have been linked to the development of neurodegenerative diseases. Previous studies in *Drosophila melanogaster* have shown that overactive Nuclear Factor-kappa B (NF-kB) immunity in the brain, following local bacterial infection, causes progressive neurodegeneration and impaired behavior. However, the cellular pathways through which brain-specific immune responses are mounted and the cell types in which they take place in *Drosophila* remain underexplored. We investigated how brain-specific or systemic infection with *Micrococcus luteus* and *Escherichia coli* bacteria activates the *Drosophila* NF-kB innate immune pathways, Toll, and immune deficiency (IMD) in the central nervous system of the fly. We tested the hypothesis that these pathways are acutely activated in the *Drosophila* brain and that their activation persists over time. We demonstrate that in *wild-type* (WT) controls, brain-specific bacterial infection leads to *Drosomycin* (*Drs*, Toll pathway) and *Diptericin B* (*DiptB*, IMD pathway) upregulation and that glia appears to be the primary cell type mounting this immune response at both early and later stages of infection, although some activation is observed in neurons as well. Host functional immunity cleared the pathogen load, indicating that long-term activated immunity is not associated with the presence of live bacteria in the brain. We show that the upregulation of *Drs* and *DiptB* expression also depends on canonical components of the Toll and IMD pathways, respectively. Interestingly, we found that systemic infection with *M.luteus* leads to brain-specific *Drs* activation and that signals from the fat body and hemocytes can activate the Toll pathway in the brain, pointing to an inter-organ communication. Together, these results contribute to our understanding of how non-lethal bacterial infections result in the activation of NF-kB immunity in the *Drosophila* brain that could potentially be targeted to prevent the progression of the neurodegenerative process.

125 Softening the Sting of Infection: Detoxifying and Tolerating Foreign Pathogens

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During infection, the host's responses can be organized into two categories: infection resistance and infection tolerance. Infection resistance is made up of host responses that decrease pathogen load, while infection tolerance is made up of host responses that increase ability to withstand pathogen insult. Resistance and tolerance mechanisms work concurrently to improve overall infection outcome for the host, but infection resistance effects can overshadow those of infection tolerance, which can make infection tolerance challenging to study.

I investigated infection tolerance mechanisms using *Drosophila* infection with *B. cepacia*, an opportunistic bacterial pathogen that can evade resistance mechanisms in mammalian models. I found that *Sting* mutants were upregulated for infection tolerance. The STING (Stimulator of Interferon Genes) pathway is a highly conserved innate immune pathway known to activate infection resistance mechanisms such as antimicrobial peptide induction in flies. How or whether STING regulates infection tolerance is currently unknown. Our lab previously published that *rictor* mutants are similarly upregulated for tolerance of *B. cepacia* infection. RICTOR is a component of the TORC2 or Target of Rapamycin (TOR) Complex 2, which regulates cell growth, cell proliferation, cell survival, protein synthesis, and autophagy in response to nutrient availability. Because research by other labs suggest that TOR might regulate STING, the question arises whether the increased infection tolerance seen in *Sting* and *rictor* mutants is mediated by similar mechanisms. A bulk RNA-seq analysis of both *Sting* and *rictor* mutants, compared to their respective isogenic wild-type controls in uninfected and infected conditions, reveals that while *Sting* and *rictor* mutants had relatively few identical differentially expressed genes in common, both mutants had differentially expressed genes involved in similar pathways required for detoxification and xenobiotic responses. Because many strains of *B. cepacia* deploy toxins such as hemolysin during pathogenesis, I am currently testing the hypothesis that the upregulated infection tolerance of *Sting* and *rictor* mutants is due to an increase in detoxification and xenobiotic responses using a multimodal approach that examines the infection process through the host and pathogen.

126 Crystal cells require a 'happy medium' amount of Toll pathway stimulation for normal development.

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Inflammatory pathways have been well-studied for their roles in immune response, but their role in homeostatic development is not fully understood. My project explores this current gap in our knowledge by dissecting the effects of Toll pathway signaling in the homeostatic development of immune cells in Drosophila. Drosophila immune cells are of the myeloid lineage and are in many ways conserved with human myeloid cells, making them an excellent topic of study. Similarly, the Toll pathway is highly conserved and has previously been shown to be important in both development and in immune response. In my work so far, I have provided evidence that many members of the Toll pathway, including MyD88, Tube, cactus, dorsal, and Dorsal related immune factor (Dif), are required cell-autonomously for crystal cell development during the first wave of hematopoiesis. To do this, I have used RNAi knockdowns in crystal cell specific drivers and counted fluorescently labeled crystal cells in 3rd instar larvae. However, these genes are largely not required for development of crystal cells in the lymph gland. This requirement in first-wave hematopoiesis is likely due to requirement of the Toll pathway for crystal cells differentiating in 3rd instar larvae. In contrast, overactivation of the Toll pathway results loss of crystal cells by the 3rd instar possibly due to crystal cell rupture before the third instar.

127 A host glycosylation mechanism of symbiotic bacteria recruitment

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Host species across a diversity of taxa show high specificity for certain gut bacteria that they associate with, suggesting a role for host genetics in determining microbial communities. The intestinal tract is lined by a mucosal layer rich in glycoproteins that not only protects gut epithelia but also provides glycosylated ligands for commensal or pathogenic bacteria to bind and consume. Despite our advanced understanding of the mechanisms of glycosylation, we have limited knowledge of how specific glycans are produced to selectively bind symbiotic bacteria. Recent work from our lab reveals that ligands produced by *Drosophila melanogaster* can recruit bacterial species of interest like *Lactiplantibacillus plantarum* to spatially defined niches in the foregut. *L. plantarum* is a common commensal bacterium found in animals ranging from *Drosophila* to humans. We discovered that strains of this species that co-evolved with *Drosophila* express serine rich repeat adhesion proteins that directly bind subregions of the foregut where the bacteria colonize.

We hypothesize that host glycosylation is regulated to promote selective adhesion of *L. plantarum,* which could support metabolic function and overall health of the host. To test this hypothesis, we are conducting a genetic screen using the Gal4-UAS system to knockdown expression of identified candidate genes that are (i) specifically expressed in the foregut, (ii) secreted), and (iii) highly expressed. Our screen shows that 16 RNAi knockdowns of genes responsible for N-linked and O-linked glycosylation in the foregut significantly reduce *L. plantarum* colonization. We have validated 3 of these by Bellymount intravital imaging to visualize the localization and adhesion of *L. plantarum* in the foregut. For instance, knockdown of the candidate hit *gfat1*, the enzyme that synthesizes the glycosylation substrate N-acetylglucosamine, resulted in 90% reduction of colonization. *gfat1* knockdown impaired bacterial adhesion to the crop and reduced binding of the *L. plantarum* adhesin SrpA to the foregut. We are currently performing glycomics analysis on the identified mutant lines with reduced *L. plantarum* adhesion to identify the specific enzymes which synthesize the host ligand which *L. plantarum* binds. Our results demonstrate a mechanism of symbiosis with molecular specificity.

128 Negative regulation by *Drosophila* Mute constrains histone mRNA expression to S phase

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Coupling histone gene expression to S phase of the cell cycle is essential for genome duplication and stability. Activation of Cyclin E/Cdk2 at the G1-S transition stimulates high-level expression of histone genes during S phase, but how histone genes are turned off at the end of S phase is not understood. *Drosophila mute,* homologue of human *Gon4L*, is an essential gene that encodes a large, mostly intrinsically disordered adapter protein previously shown to suppress histone mRNA accumulation, but how Mute functions during the cell cycle is unknown. We demonstrate that mutation of *mute* results in the inappropriate accumulation of both core (H3, H4, H2a, H2b) and linker (H1) histone mRNAs outside of S phase in the embryonic ventral nerve cord. Ganglion mother cells, the precursors to neurons and glia, fail to terminate histone gene expression during G2 of their final cell division cycle, a phenotype accompanied by persistence of Cyclin E/Cdk2-dependent phosphorylation of Mxc, which localizes to and activates the expression of histone genes. Measurement of histone RNAs by RT-qPCR in small collections of late-stage, whole embryos unexpectedly showed increase transcript levels only for histone H3 and H4 only, while histone H2A, H2B, and H1 transcripts remained near wildtype levels. Thus, Mute seems to play two roles in replication-dependent histone gene expression: it functions to down regulate all histone genes at the end of S phase while being required for proper level of expression of the replication dependent H3 and H4 genes. We also found that Mute localizes only to histone genes, and that *mute* mutant wing imaginal disc cells fail to proliferate. We conclude from these data that disruption of histone gene control is likely responsible for the developmental defects observed upon loss of Mute. This work enhances our understanding of the role of RD histone gene regulation in metazoans and provides insight into the mechanisms that govern the regulation of this highly conserved cellular process.

129 Syp reversibly represses translation of specific RNAs in the male germline via binding to their 5'UTRs

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Upon major waves of transcription during development, the spatial and temporal fine-tuning of the proteome is often directed by translational control. In the *Drosophila* male germline, an extended meiotic G2 prophase corresponding to the spermatocyte stage features a massive wave of transcription directed by the Aly/tMAC complex. Aly/tMAC turns on expression of ~3000 RNAs that encode proteins needed for the meiotic divisions and the subsequent 3-4 days of spermatid differentiation. Roughly 1800 genes are expressed only in spermatocytes, while another 1200 genes expressed elsewhere in the fly show expression in spermatocytes from a novel, Aly/tMAC-dependent promoter. Some of these transcripts are not translated until late spermatid development, two to three days after the meiotic divisions, suggesting that these RNAs are temporarily subject to translational repression in spermatocytes and early spermatids. A candidate translational repressor suited for acting on these RNAs is the RNA-binding protein Syp, the fly homolog of human SYNCRIP. The HA-Syp reporter protein is expressed starting in young spermatocytes, and its expression decreases after the meiotic divisions and becomes undetectable in late spermatids. Indeed, CLIP-Seq with the spermatocyte-specific version of Syp revealed that Syp was bound at high levels to the 5'UTRs of many transcripts that require Aly/tMAC for their expression, particularly those whose protein products do not appear until the late elongating spermatid stage. Syp binding was enriched at AAAUU motifs, consistent with the Syp consensus site identified *in vitro* by the Hughes lab (Ray et al., 2013). To test whether the presence of Syp at 5'UTRs could be mediating translational repression, we created eYFP reporter lines driven by the promoter and 5'UTR of transcripts bound by Syp and assayed eYFP expression live in spermatocytes and spermatids. The 5'UTRs for 8 genes (5 of the testis-specific class and 3 of the broadly expressed class) repressed translation of eYFP up until late spermatid stages in a wild-type background but not in a *syp* mutant. In fact, all three reporters were expressed robustly in spermatocytes and early elongating spermatids when Syp was absent. Intriguingly, three robustly Syp-bound AAAUU sites are unable to repress reporter translation, and all three of those sites are located no farther than 26nt upstream of the translation start site, providing a hint at a possible constraint on Syp function at target 5'UTRs.

130 Translational Regulation of Xrp1 by Upstream ORFs and Coding Sequence Features

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Chronic stress in the endoplasmic reticulum (ER) contributes to various diseases, one of which is retinitis pigmentosa (RP) caused by genetic variants in the rhodopsin gene. *Rh1*^{G69D} is an allele of the *Drosophila Rhodopsin-1* (*Rh1*) gene, encoding a misfolding-prone protein that imposes ER stress and age-related retinal degeneration similar to RP.

Upon ER stress, stress-responsive genes are induced through several transcription factors including *ATF4*. We previously reported the induction of another bZIP transcription factor, Xrp1, in response to ER stress. However, the mechanism underlying Xrp1 induction remains poorly understood. Here, we report that Xrp1 induction requires its 5' leader and protein coding sequence.

Specifically, there are seven *Xrp1* splice isoforms. Four of those (isoforms RA, RB, RF, RG) encode the Xrp1 long protein isoform (Xrp1^{long}), whereas three others (isoforms RC, RD, RE) encode a shorter Xrp1 protein (Xrp1^{short}). There are putative upstream open reading frames (uORFs) only within the exon 4, an exon that is specific for the Xrp1^{long} isoforms. To test the regulatory potential of their 5' leaders, we fused the 5' leaders of *Xrp1* isoforms (C, D, F, and G) upstream of the *DsRed* ORF. When driven through Gal4/UAS, strong *DsRed* expression was detected from the *Xrp1*-C and *Xrp1*-D reporters, but not from the *Xrp1*-F and *Xrp1*-G reporters. Disrupting the AUG of uORF2 of *Xrp1-F* reporter depressed *DsRed* expression, indicating that this uORF normally plays an inhibitory role. *Rh1*^{G69D} overexpression effectively induces the *ATF4* reporter, but the *DsRed* remained absent from the *Xrp1*-F and *Xrp1*-G reporters under equivalent conditions, suggesting a mode of translational regulation distinct from *ATF4*.

To test if *Xrp1*'s main ORF is required for its induction in response to stress, we generated HA-tagged reporters that included Xr1-F isoform's main ORF coding sequence in addition to the 5' leader. In unstressed imaginal discs, no HA signal was detected from the wild-type *Xrp1*-F transgene. In eye discs expressing *Rh1*^{G69D}, the Xrp1-HA expression was induced.

Together, our findings show that while Xrp1 translation shares similarities with ATF4, it also exhibits distinct regulatory features. Like ATF4, Xrp1 translation is controlled by two upstream open reading frames (uORFs). Unlike ATF4, however, the coding sequence of Xrp1 is essential for its translational induction, suggesting an unrecognized layer of regulation in response to stress.

131 Comparing mechanisms of histone locus body initiation and maintenance throughout development.

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Histone proteins are essential for compaction and regulation of the eukaryotic genome, and dysregulation of histone gene expression leads to aberrant development and lethality. In animals, genes encoding the replication dependent histone proteins are commonly clustered. The histone locus body (HLB) is a nuclear condensate associated with histone loci that aggregates factors that regulate histone transcription and transcript processing. The HLB initiates at the histone locus just before zygotic gene activation in early embryogenesis and is thereafter maintained at the locus throughout cell division and differentiation. However, the mechanism of HLB maintenance and latent potential for HLB initiation in non-embryonic cells is unknown. Therefore, we seek to define mechanisms of HLB initiation and developmental maintenance to gain insight into how unique genes are targeted by factors for specialized regulation. Because the Drosophila melanogaster histone genes are clustered in arrays repeated at a single locus, altering DNA sequence is challenging. We therefore employ histone transgenes that recruit HLB factors, allowing us to manipulate cis regulatory elements and determine the effects on HLB initiation and maintenance. To compare HLB initiation and maintenance mechanisms, we engineered a transgenic system in which we can remove cis elements critical for initiation during HLB maintenance. In tandem, we will perform ChIP-seq in sorted Fluorescent Ubiquitination-based Cell Cycle Indicator (Fly-FUCCI) cells to elucidate mechanisms of HLB maintenance throughout the cell cycle. Using Mosaic Analysis by gRNA-induced Crossing over (MAGIC), which generates mitotic clones in wing and haltere discs, we show that HLB initiation can also occur in differentiated cells. Overall, defining mechanisms of HLB initiation and maintenance illustrates how histone proteins are dynamically and developmentally regulated. We aim to inform the developmental contexts that support initialization of crucial gene regulation, which give insight into latent potential for gene activation in maturing tissues.

132 Regulatory Module Dissection by Reporter, EMSA, and Proteomic Analyses Reveals Combinatorial Enhancer–Promoter Interactions Controlling *Drosophila Myc* Transcription

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Transcription factor MYC is a master regulator of cell growth, biomass turnover, proliferation, and differentiation, yet its transcriptional control remains incompletely understood. In *Drosophila*, most regulatory elements driving *Myc* expression lie within noncoding upstream, intragenic, and downstream regions. To dissect *Myc cis*-regulatory modules (Myc-CRMs), we performed *Myc-lacZ* truncation assays to identify elements capable of driving reporter expression comparable to endogenous *Myc* throughout development. A 5′-proximal fragment containing a strong enhancer cluster drives *lacZ* activity in the ovary and early embryos; we termed this module the Oocyte Element. Together with the TATA and DPE promoters, the distal upstream enhancer restores tissue-specific expression resembling endogenous *Myc* in ovaries, embryos, and larval imaginal discs and brain. The DPE, combined with its adjacent enhancer cluster, shows promoter activity reflecting wild-type *Myc* expression.

To identify trans-acting factors, we developed a highly sensitive Solid Surface Magnetic Enrichment Protocol (SSMEP) to purify regulatory proteins assembling on Myc-CRMs. Gel shift assays (EMSAs) using nuclear extracts confirmed sequence-specific protein association with these modules. Proteomic analysis of the DNA-protein complexes revealed enrichment of transcriptional and other regulatory proteins, including basal transcription machinery, chromatin remodelers, transcription factors, and additional regulators. Proteomic profiling further suggests that *Myc* transcription initiates from multiple sites involving the RNA Pol II regulator complex, TBP/TFIID, Mediator, TFIIH/TFIIEα, TAF-TFIID, TAF-TRF2, chromatin remodelers, and RNA-processing proteins assembling on the TATA and DPE promoters, and the Oocyte Element. The TATA promoter is the principal initiation site producing the largest transcripts. The DPE promoter and its enhancer cluster likely recruit this machinery to form a stable pre-initiation complex, while the Oocyte Element supports promoter-independent initiation, integrating transcriptional and post-transcriptional regulation during oogenesis and early embryogenesis.

Together, these findings reveal a multilayered regulatory landscape of the *Myc* gene in which promoter- and enhancer-specific configurations of assembled transcriptional and chromatin-associated factors achieve precise developmental control of *Myc* expression in response to conserved signaling pathways and mitogenic stimuli. Finally, the SSMEP protocol provides a broadly applicable method for other model organisms and biomedical research, including isolation of RNA-protein complexes and synthetic biology studies.

133 Topoisomerase 3b promotes stabilization of maternal mRNAs under starvation stress to facilitate neurodevelopment of progeny

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Topoisomerase 3 beta (Top3b) is the only dual-activity topoisomerase in animals that can change topology for both DNA and RNA; and can facilitate transcription on DNA, as well as translation and mRNA turnover. The zygotic function of Top3b is essential for normal neurodevelopment and behaviors across multiple species. In humans, *Top3b* mutations have been linked to schizophrenia, autism, and cognitive impairment. Similarly, loss of Top3b in mice and *Drosophila* leads to behavioral and neurological phenotypes that overlap with those observed in patients with neurodevelopmental disorders. However, the functions of Top3b in maternal mRNA metabolisms are still unknown. Here we use the *Drosophila* oocyte model to demonstrate that Top3b has a crucial maternal function in stabilizing oocyte mRNAs during translation inhibition stress, thereby supporting normal neurodevelopment of the offspring.

Although *Top3b*$^{-/-}$ flies produce progeny with normal embryonic morphology under standard conditions as WT, *Top3b*$^{-/-}$ females produce embryos with abnormal neurodevelopment under translation-repression stress induced by a protein-free diet. These embryos exhibit severe disruption of ventral nerve cord (VNC) formation in maternal-, but not paternal-, dependent manner. Our RNA-seq and Ribo-seq analyses revealed that a subset of maternal mRNAs undergoes extensive degradation in stressed *Top3b*$^{-/-}$ oocytes, indicating that their stability requires Top3b. These oocytes also exhibit a robust increase in both size and number of P-bodies, cytoplasmic structures associated with mRNA degradation, and translation inhibition. Moreover, maternal RNAs in stressed *Top3b*$^{-/-}$ oocytes display significantly shorter poly(A) tails compared with WT or unstressed *Top3b*$^{-/-}$ controls, supporting a model in which Top3b promotes RNA stability by maintaining poly(A) tail length under stress. Notably, our genetic screen identified a strong interaction between *Top3b* and *pcm* (the Xrn1 ortholog), or *twin* (encoding the CCR4 deadenylase), suggesting that maternal Top3b functions in coordination with RNA degradation pathways to support proper progeny development. Together, our findings suggest that Top3b is essential for maintaining maternal RNA stability by coordinating with RNA degradation pathway during stress, that these stabilized maternal RNAs are transmitted to the embryo, and that they contribute to proper neurodevelopment of the progeny.

134 Germ granules transform into multiphasic structures by molecular condensation

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Biomolecular condensates assemble a specific repertoire of macromolecular components to promote specialized biochemical reactions. Owing to their properties as phase delimited entities, condensates rapidly form, break down, and change configuration or composition by exchanging protein content with the environment as intracellular conditions fluctuate. To investigate the relationship between condensate function and composition, we utilize germ granules in *Drosophila melanogaster* which are RNA/protein condensates that experience diverse intracellular changes during development. Drosophila germ granules concentrate mRNA regionally in the oocyte and then deploy the protein products of these mRNAs for the formation and the specification of the primordial germ cells (PGCs) in the embryo. Moreover, through mechanisms that are not fully understood, germ granules and their components ensure that the initial proteins required for PGC development are produced from germ granule mRNAs at the right place, at the right time, and to the proper levels.

We hypothesized that components of germ granules evolve as intracellular differences arise in development leading to changes in their function. To test this hypothesis, we tracked changes to proteins in proximity of germ granules across oogenesis and embryogenesis using a proximity-dependent biotinylation approach. The core germ granule protein Oskar was conjugated to TurboID, an engineered biotin ligase that catalyzes promiscuous biotinylation of proteins within a radius of several nanometers. Proteins biotinylated by Oskar-TurboID were quantified with multiplexed TMT mass spectrometry in a comprehensive time course comparing two time periods during oogenesis and six sequential time intervals after fertilization that span the first 270 minutes of embryogenesis. Our results demonstrate widespread remodeling of the germ granules and their environment upon two developmental transitions, first when the egg is fertilized and second during the formation of the PGCs.

By analyzing proteins that change significantly over time near germ granules, we discovered a novel remodeling event in the PGCs involving the recruitment of the CCR4-NOT complex to the surface of germ granules that is elicited by molecular condensation of the universal germline determinant Nanos. Assembly of a Nanos rich protein domain around germ granules promotes granule fusion and transitions germ granules into a hybrid state distinguished by a shell containing P-body components. We find that that this switch-like transformation of the germ granules is required for the robust survival of PGCs by timing a key cell state transition.

135 Regulation of spermatogenesis by Combover and the E3 ligase Mindbomb2

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Survival of higher species requires proper gamete production and differentiation. Sperm count in men has decreased significantly over the last 50 years. Understanding the signaling pathways that regulate sperm production and fertility is crucial for humanity's survival. During spermatogenesis, syncytial spermatids differentiate and individualize into mature sperm tightly enclosed by a plasma membrane. In *Drosophila*, specialized actin cones move along the sperm tails to separate spermatids during individualization. Combover (Cmb), a member of the family of intrinsically disordered proteins and an effector of planar cell polarity under the control of Rho kinase (Rok), is essential for sperm individualization and fertility in *Drosophila*. Using a germline-specific Rok CRISPR mutant, we found that loss of Rok reduced fertility, shortened testes, and disrupted individualization and elongation complexes marked by Cmb. The latter suggests that Rok regulates Cmb in the Drosophila germline. To further understand the Cmb function in spermiogenesis, we addressed the role of Mindbomb 2 (Mib2), an E3 ubiquitin ligase that physically interacts with Cmb. We find that Mib2 hypomorphic mutant males are partially. Consistently, knockdown of *mib2* with different RNAi in the germline causes a decrease in progeny, suggesting that sperm differentiation is defective upon mib2 reduction. Both Mib2 and Cmb are localized to elongation complexes and axonemes, although likely at distinct stages, as Mib2 is largely mutually exclusive with Cmb. This raises the question of which protein acts first—Cmb or Mib2, and whether Mib2 targets Cmb for degradation. To determine the relative timing of cmb and Mib2 accumulation at the distal ends of elongating cysts, we examined colocalization of Cmb, Mib2, and polyglycylated tubulin, a post-translational modification of flagellar axonemes that occurs late at the onset of individualization. Immunostaining revealed that Mib2 occasionally colocalized with polyglycylated tubulin, while Cmb never does. Strikingly, live imaging reveals that Mib2 levels increase within elongation complexes over time. Taken together, we suggest a dynamic model in which Mib2 displaces Cmb from elongating cysts to orchestrate the final steps of spermiogenesis, setting the stage for ongoing experiments to determine whether Mib2 targets Cmb for degradation in the growing axoneme, thereby promoting the shift from elongation to individualization of the mature spermatid.

136 The gut microbiome impacts both fertility and fecundity on a long-term scale in *D. melanogaster*

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Animal gut microbes impact several aspects of host physiology. In humans, these microbes, or the microbiome, contribute to a growing number of host disease states. However, the high complexity of the human gut microbiome makes it harder to isolate variables and define mechanisms. Additionally, it is nearly impossible to study how the previous generation's microbial status impacts their offspring and subsequent generations. Thus, model organisms, such as *Drosophila melanogaster* have provided a valuable tool to study host-gut-microbiome interactions. The *D. melanogaster* gut microbiome is relatively low-complex, and these microbes can be readily cultured in the lab environment. A variety of studies have reported that parental gut microbiome status can exert unexpected consequences on host development and gut physiology. There is growing appreciation that the gut microbiome can also impact more distant tissues, such as the gonads. Most studies have focused on short-term or early impacts of the microbiome on the adult fly. However, how the gut microbiome contributes to adult long-term fitness and whether these impacts span more than one generation is less well known. To address this question, we conducted long-term fecundity assays to assess whole life productivity. While the short-term fecundity of female flies is positively impacted by gut microbe status, we found that once a female fly reaches "middle age" (~12-15 days old), the gut microbiome significantly reduces their fecundity compared to age matched females without a microbiome. At the tissue level, gonads visualized at these discrete times by immunofluorescence microscopy, indicates there are changes not only in morphology, but possibly germ stem cell number, which is exacerbated depending on their gut microbe status. Together, these data suggest that over time the presence of a microbiome becomes disadvantageous, reducing both the fecundity and fertility of adult flies. We will discuss mechanisms underlying these phenotypes, including impacts on germ stem cell behavior.

137 GstS1, a prostaglandin D_2 synthase, promotes eggshell formation in Drosophila

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The multilayered *Drosophila* eggshell, a specialized extracellular matrix, acts as a barrier to prevent the embryo from dehydration and protects it from toxic macromolecules. Abnormal eggshell formation can result in impaired fertility and even embryonic lethality. The eggshell is formed during the late stages of oogenesis and is regulated by multiple signaling pathways, including prostaglandin (PG) signaling. PGs are lipids derived from arachidonic acid and exist in different bioactive forms, including PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2, and TXA_2. PGs are each synthesized by a series of enzymes, including cyclooxygenase (COX) enzymes and PG type-specific synthases. The bioactive PGs activate PG-type specific G protein-coupled receptors and downstream signaling cascades to mediate their functions. Prior work established that loss of the *Drosophila* COX-like enzyme, dCOX1/Pxt, results in defects in eggshell structure and function. One mechanism whereby PGs may regulate eggshell formation is by mediating the gene amplification of the 6 Drosophila amplicons in the follicle cells (DAFCs). Each DAFC exhibits a stage-specific pattern of amplification and encodes a cluster of eggshell proteins that are required at a specific timepoint during eggshell formation. Here we provide evidence that loss of *Drosophila* GstS1, a putative PGD_2 synthase, greatly impairs female fertility and phenocopies the eggshell defects observed in *dCOX1* mutants. Additionally, evidence supports that GstS1 regulates gene amplification pattern at the DAFCs. These findings suggest that GstS1 and PGD_2 signaling play an indispensable role in *Drosophila* eggshell formation and female fertility.

138 Dopamine production in the central nervous system is important for follicle survival and interacts with genetic background and a high sugar diet during Drosophila oogenesis

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Unhealthy diets, obesity, and reduced fertility are associated in *Drosophila* and humans. We previously showed that a high sugar diet, but not obesity, reduces female fertility in *Drosophila melanogaster* due to increased death of newly formed germline cysts and vitellogenic egg chambers. *Drosophila* strains carrying mutations in the *yellow* (*y*) and *white* (*w*) pigmentation genes are routinely used for investigating the effects of high sugar diets, but it has remained unclear how this genetic background interacts with high sugar. We therefore tested whether this genetic background influences the response to a high sugar diet. In contrast to *y w* mutants, wildtype females retain normal survival of early germline cysts and vitellogenic follicles on a high sugar diet, and rescue of *y* is sufficient to restore normal fertility to *y w* mutants. Although the precise molecular function of *y* is unknown, *y* functions with genes in the dopamine/pigmentation pathways to help produce melanin from dopamine, prompting us to investigate the roles of this metabolic pathway in the context of the ovarian response to a high sugar diet. We found that dopamine supplementation prevents follicle death in *y* mutants on a high sugar diet. Conversely, severe dopamine imbalance (i.e. increased or decreased) or lack of dopamine production in the central nervous system causes follicle death regardless of diet or genetic background, while early germline cyst survival does not depend on dopamine. We are currently investigating the potential roles of dopaminergic neurons and dopamine receptors in the control of vitellogenic follicle survival. Our findings are broadly relevant to our understanding of how the effects of unhealthy diets might differ depending on genetic factors and highlight a key connection between dopamine metabolism in the central nervous system and ovarian follicle survival.

139 Investigating kinetochore – microtubule attachments and chromosome biorientation in *Drosophila* meiosis

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During female meiotic cell division, the interactions between the kinetochores (KT) and the spindle are responsible for the correct partition of chromosomes. The KT is a proteinaceous complex that connects centromeric DNA to the spindle. The establishment of microtubule (MT) attachments at homologous KTs to opposite spindle poles is known as *biorientation* and is critical for maintaining genomic integrity. Two outer KT proteins are critical for biorientation: SPC105R and NDC80. We hypothesize SPC105R interacts laterally with spindle MTs before transitioning to stable end-on attachments at NDC80. However, the process of establishing biorientation is error prone, which can lead to chromosome aneuploidy. This can result in spontaneous abortions, birth defects, and infertility.

We are interested in determining the mechanisms regulating end-on attachments and biorientation. NDC80 is required for end-on attachments and is a proposed target of Aurora B kinase (AURKB). Additionally, our lab has previously shown that loss of NDC80 or Protein phosphatase 2A (PP2A) results in primarily lateral attachments. These results suggest that PP2A is required to dephosphorylate the KT, allowing for NDC80 to establish stable end-on attachments. However, it is not yet known which protein is phosphorylated to antagonize KT–MT attachments in *Drosophila*. The kinase that carries out this phosphorylation, as well as its cellular location, also remain unidentified.

To study how microtubules attach to kinetochores, we created transgenes that localize AURKB to different regions of the oocyte. These constructs are expressed with *Incenp* RNAi, allowing AURKB to act only at our chosen location. When AURKB was targeted to chromatin or the kinetochore, we observed mostly end-on attachments, even with *PP2A* RNAi, suggesting that KT–MT destabilization depends on central spindle localized kinase. We therefore propose that central spindle–localized kinase phosphorylates NDC80 to promote lateral attachments. Previous data has shown that loss of AURKB or Cyclin A, cofactor of CDK1, results in biorientation defects. To test this idea, we are determining whether Ndc80 is the main phosphorylation target in *Drosophila* oocytes. We designed phosphomimetic, phosphonull, and deletion mutants affecting AURKB and CDK1 sites on NDC80 to dissect how phosphorylation antagonizes KT–MT attachments.

140 A new principle of thermal adaptation: Intronic RNA thermosensors

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Temperature is a powerful environmental force influencing nearly every aspect of life, yet while ion channels are well-established thermosensors, whether RNA molecules can fulfill this role in eukaryotes—analogous to bacterial riboswitches—remains unclear. To explore this possibility, we employed an evolutionary comparative approach using *Drosophila* adapted to distinct thermal environments, examining how temperature influences splicing efficiency and intronic architecture. Using long-read RNA sequencing, single-molecule RNA imaging, and comparative genomics, we profiled temperature-dependent splicing dynamics in *D. melanogaster* (temperate, ~25 °C) and *D. mojavensis* (heat-adapted, ~35 °C). We discovered that the last intron of the core clock gene *timeless* (*tim* IntL) functions as a thermosensitive splicing switch in clock neurons of both species. IntL is efficiently spliced at each species' preferred temperature and retained below this threshold, indicating evolutionary fine-tuning of splicing to thermal niche. Notably, we found that splicing efficiency varies continuously with temperature in an analog fashion, generating either a full-length TIMELESS protein (fully spliced) or a truncated isoform (intron-retained). This discovery prompted us to investigate *tim* IntL›s physiological significance within the circadian clock, a network renowned for its temperature compensation. CRISPR-edited flies restricted to a single TIM isoform exhibited disrupted circadian rhythmicity at a broad range of temperatures, revealing that precise IntL splicing and the ratio of TIM isoforms act as a molecular timer for temperature-compensated circadian rhythms. Through a genetic screen, we identified the RNA-binding HnRNP protein, Hrb27C, as required for IntL retention at lower temperatures. Ongoing work is determining whether thermosensitivity arises from cis-encoded RNA structures or trans-acting regulatory factors to explain species-specific tuning. Together, these findings establish IntL as an evolutionarily refined eukaryotic RNA thermosensor, uncovering a previously unrecognized RNA-based mechanism for temperature adaptation and opening new avenues to explore how evolutionary forces shape RNA-based regulatory processes.

141 The thermal sensitivity and evolution of parental effects on offspring survival in *Drosophila melanogaster*

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Early development is a particularly thermally sensitive life stage in ectotherms as temperature speeds up the molecular processes of development but also compromises the cellular structures that fuel these processes. Our work in *Drosophila melanogaster* has demonstrated that exposing embryos to heat before zygotic transcription begins compromises embryo survival and that genetic manipulation of maternal provisioning of small heat-shock proteins can increase embryo thermotolerance (Lockwood, Julick, Montooth, JEB (2017) 220, 4492-4501). I will present new data showing that this provisioning effect acclimates in response to parent thermal environment, with warmer parent temperatures conferring greater embryo thermotolerance. However, the evolutionary response to temperature is somewhat distinct. Parents evolved at cooler temperatures and acclimated at their home temperature appear to better provision their embryos, which maintain high survival rates upon heat exposure. I will present additional data supporting a variety of temperature-mediated parental effects that impact offspring survivorship in outbred *Drosophila melanogaster* from Lincoln, Nebraska. For example, we have found that the relationship between parent age and offspring survivorship is thermally sensitive. Parent development temperatures of 17C best maintain offspring survivorship as parents age. I will discuss how these parent-offspring-environment interactions scale up to shape the thermal performance curves of fecundity and population growth and how thermal evolution changes these relationships. In addition to physiological effects, we are investigating how parent behavior impacts embryo survival. We have found that females significantly adjust their thermal oviposition preference in the presence of ethanol, but not in the direction that we predicted based on how temperature and ethanol are expected to impact embryo physiology. Instead, we suggest that this change in preference may be mediated by a greater than expected overlap between temperature- and ethanol-sensing neurons in the fly connectome. This research is illuminating how behavioral and physiological parental effects are integrated to impact offspring survivorship in heterogeneous environments, particularly in the early stages of embryogenesis.

142 Molecular basis of pheromone diversification in *Drosophila*

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Pheromones are chemical signals insects use to guide their behavior. Pheromones show enormous diversity even between closely related species. Yet, the molecular mechanisms driving this vast diversity remain largely unknown. Understanding this chemical diversity is vital for understanding speciation and pest control. We investigated the mechanism of pheromone diversification using CH503, an anti-aphrodisiac pheromone produced in the male ejaculatory bulb (EB) of some *Drosophila* species, as our model. Our comparative EB metabolomic analysis showed that species producing CH503 (CH503+) exhibit a more diverse EB metabolome than non-producing species (CH503-), suggesting a link between presence of CH503 and the overall metabolic diversity of EB. To test whether this diversity stems from the differential expression of genes, we compared the EB transcriptomes of closely CH503+ and CH503- species. A fatty acid elongase gene, *bond*, was found to be significantly less expressed in CH503- species. Silencing bond expression reduced CH503 levels, indicating that CH503 synthesis requires the combined activity of multiple genes. While many fatty acid synthesis (FAS) related genes also showed reduced expression in CH503- species, this reduction was not consistent across all CH503- species. This inconsistency led us to hypothesize that the metabolic diversity might be driven instead by metabolic remodeling, a possibility supported by an enrichment of lipid metabolism genes in CH503+ species. Taken together, our findings suggest that differential expression of FAS-related genes and metabolic remodeling are potential key factors underlying the pheromone diversity.

143 On the tempo and mode of transposon mobilization in *Drosophila* male germline

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Transposons are selfish genetic elements that generate copies of themselves at new genomic loci, repeatedly producing mutations that fuel evolution. While transposon mobilization may be unequivocal, it remains nontrivial to systematically characterize the tempo and mode of transposition. Here, we develop a pedigreed mutation accumulation approach in *Drosophila melanogaster*, using *rhino* mutations to disrupt piRNA production and promote transposition. Leveraging artificial selection and the lack of recombination in male flies, we followed a full complement of nonrecombining haploid genome in the male germline through 45 generations, accumulating transposon insertions on whole chromosomes. We performed single-fly Nanopore sequencing and identified a dozen families that transposed after piRNA pathway perturbation. Among them, *copia* was the most prolific and completed 63 jumps, with new insertions scattered across each chromosome arms. Using genomic DNA extracted at each of the 45 generations, we verified and dated every single *copia* insertion, revealing that *copia* transposed in bursts, departing from a Poisson process. Meanwhile, another transposon family, *roo*, transposed with high frequency irrespective of piRNA pathway perturbations, likely by exploiting a unique developmental niche. Collectively, by experimentally minimizing selection, drift, recombination, and piRNA-mediated control, this work defines the tempo and mode of transposon mobilization with unprecedented resolution. Given the ubiquity of transposons across the tree of life, these insights into their mobilization have broad implications for understanding the mutational processes that shape evolution.

144 Thrifty Gene Signatures Underlying Diet-Dependent Variation in *Drosophila* Development

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Nutritional and developmental plasticity are essential for organisms adapting to fluctuating environments, and diet-induced phenotypic variation has long been a major target of natural selection. However, the genetic basis of diet-induced developmental plasticity remains unclear. The thrifty gene hypothesis proposes that alleles once advantageous for energy storage now increase disease risk in nutrient-rich environments. In this study, we quantified key metabolic, physiological, and life-history traits in a subset of lines from the Drosophila Genetic Reference Panel, measured in larvae and adults raised on diets with differing nutritional content. We estimated trait heritabilities and correlations across diets and life stages, identifying development time as the trait with the highest heritability and the strongest diet effect. We therefore prioritized development time for genetic mapping, complementing the DGRP with a Drosophila recombinant outbred population to increase power and sensitivity. Genome-wide association analyses within and across diets revealed candidate loci enriched for conserved metabolic regulators, including several with human orthologs associated with metabolic diseases. Consistent with predictions of the thrifty gene hypothesis, we also detected diet-specific shifts in development time, suggesting the presence of “thrifty” variants that respond sensitively to nutritional availability. These findings advance our understanding of the genetic architecture of diet-induced developmental plasticity and highlight the value of combining natural panels with outbred mapping populations to dissect gene-by-environment interactions.

145 Experimental evidence of Fisher’s second-site modifiers of dominance in a Mendelian trait.

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Allelic dominance is a fundamental factor that shapes the genotype-phenotype map. By changing the phenotype of heterozygotes, evolutionary reversal of dominance can alter the trajectory of adaptation. Since about 100 years ago, there have been two conflicting hypotheses about why some alleles are dominant and others are recessive. Wright (1929) and Haldane (1930) hypothesized that dominance relationships between alleles are determined by inherent properties of alleles, without requiring selection. Alternatively, Fisher (1928) hypothesized that dominance relationships evolve by selection on second-site modifier loci that reduce the effects of deleterious mutations by increasing the dominance of the wild-type allele. Although theoretical models have predicted that dominance can be reversed through the evolution of modifier loci, especially when balanced polymorphisms are maintained over a long time, experimental evidence has been difficult to obtain. We tested for the existence of Fisher’s modifier loci using the Mendelian color dimorphism found in many species of the *Drosophila montium* subgroup. In most species with discrete dark and light morphs, the Dark allele is dominant, but the Light allele is dominant in some species. Using GWAS and selective genotyping, we found that *POU domain motif 3* (*pdm3*) locus, which encodes a transcription factor that represses pigmentation, is responsible for or linked to this dimorphism in both Dark-dominant and Light-dominant species. We show that dominance reversal is due to changes in phenotypic plasticity. In Dark-dominant species, the Dark *pdm3* allele is dominant across all developmental temperatures. In Light-dominant species, the Light *pdm3* allele is increasingly dominant at higher temperatures. To test whether evolutionary changes in dominance are due to evolution of modifier loci, we introgressed alternative *pdm3* alleles into the genomic background of different species. We found that a Light *pdm3* allele that showed temperature-dependent dominance in the genomic background of its own species became almost recessive at all temperatures in the genomic background of different species. Therefore, evolutionary transition between fixed dominance and temperature-dependent dominance is mediated by second-site modifier loci. Our results provide a novel insight that evolution of Fisher’s modifier loci can reverse dominance through evolution of phenotypic plasticity.

146 Genetic Basis of Sensitivity and Resistance to *Stellate* Meiotic Drive

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Meiotic drivers are selfish elements that bias gametogenesis to increase their own transmission. The selective costs imposed by drivers on their hosts may trigger intragenomic conflict, leading to the emergence of suppressors and subsequent arms race dynamics between the driver and suppressors. We recently demonstrated that the *Stellate* (*Ste*) meiotic drive system of *Drosophila melanogaster*, previously proposed to be an inactive meiotic drive relic, is in fact still active in contemporary populations. In natural backgrounds, low copy-numbers of X-linked *Stellate* genes are wholly repressed by piRNAs produced by the Y-linked *Suppressor of Stellate* (*Su(Ste)*) locus, while high copy-number driving alleles (>150 copies) overcome this suppression in a dose-dependent manner, leading to the accumulation of Stellate protein in spermatocytes and subsequent preferential killing of Y-bearing sperm. Consequently, the X chromosome is overtransmitted in driver males, resulting in female-biased broods. We also found that driving *Stellate* alleles are common in most populations (10-40%), but there is considerable variability in its penetrance among populations and genetic backgrounds. Surprisingly, for driving alleles, *Ste* transcript abundance is uncorrelated with drive penetrance and strength, indicating that most variation functions downstream of *Su(Ste)*-mediated repression. To elucidate the basis of this variation, we characterize drive and associated phenotypes in a panel of lines which are congenic aside from having different wild-derived Y chromosomes, allowing for precise discrimination of Y-specific modification of drive. With these lines, we establish that: 1) most but not all contemporary functional variation in the system appears Y-linked; 2) strong autosomal dominant suppression occurs in some populations; 3) population-of-origin predicts drive substantially better than SNP-based Y chromosome ancestry, potentially indicating that population-specific pressures shape the rapid evolution of the Y-linked factors mediating drive and resistance; 4) the abundances of several Y-linked simple satellites are strongly predictive of drive magnitude, indicating that these satellites may mediate and modulate Stellate protein's targeting of and/or toxicity to Y-bearing sperm.

147 Transposable elements shape genome evolution via modulating gene expression level and noise

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Transposable elements (TEs) are genomic parasites that can proliferate at the expense of host fitness. Host genomes typically deposit repressive marks at TEs; this suppression reduces TE replication and is considered beneficial. However, repressive marks often spread from silenced TEs to nearby genes, leading to the enrichment of repressive marks at adjacent genic alleles. Population genomic inference has demonstrated that these "epigenetic effects of TEs" are deleterious and subject to purifying selection. Yet previous studies in *Drosophila* bulk embryos and other systems have found no evidence that TE-mediated enrichment of repressive marks reduces expression of adjacent genes. This raises a critical, but unresolved, question: If TE insertions that lead to the enrichment of repressive marks at adjacent genes are deleterious, where do these fitness costs originate? One possibility is that TEs› epigenetic effects on the total output of gene expression are tissue- or cell-type specific. Additionally, because the spreading of repressive marks is stochastic, TEs› epigenetic effects may generate cell-to-cell heterogeneity in the expression of adjacent genes, thereby increasing expression variability, or expression noise, which was shown to be selected against. To investigate how TE-mediated spreading of repressive marks influences nearby gene expression and whether the effects are context-dependent, we analyzed single-cell RNA-seq data from the *Drosophila* Fly Cell Atlas. Supporting our hypothesis, we found that TEs reduce mean expression in a subset of tissues, particularly in the head. Moreover, the presence of TEs indeed increases the variability of expression. Among TE families, long terminal repeats (LTRs), which elicit the strongest enrichment of repressive marks, exert the strongest expected effects. Notably, the magnitude of these effects varies across tissues in association with the chromatin environment: tissues exhibiting stronger effects also have higher expression of heterochromatin regulators. We are investigating TEs' epigenetic effects on gene expression by inter-strain comparison, as well as using population genomics to infer the associated fitness consequences. Overall, our findings highlight the context-dependent influence of TEs' epigenetic effects on gene expression, by both reducing the mean expression and increasing expression noise, both of which are selected against, shaping the evolutionary trajectory of both TEs and host genomes.

148 Sleep-dependent clearance of brain lipids by peripheral blood cells

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Sleep is essential for maintaining physiological homeostasis and influences processes such as memory, longevity in animals. While sleep is primarily regulated by the brain, recent evidence suggests that peripheral tissues also contribute to sleep regulation. Among these, immune cells are known to influence sleep, but most studies have focused on conditions of sleep deprivation or immune activation. In this study, we investigated the role of immune cells in regulating sleep under normal physiological conditions using the *Drosophila* model. We discovered that macrophage-like blood cells (hemocytes) take up lipid droplets from glial cells via the scavenger receptor Eater, reducing metabolic stress in glia and promoting sleep. Disruption of lipid droplet clearance through hemocytes led to their accumulation in glial cells, resulting in increased Acetyl-CoA levels, reduced NAD+ levels, and inducing acetylation of proteins such as PGC1α and Drp1. These findings highlight the importance of sleep in maintaining metabolic homeostasis in the brain and reveal a novel role for circulating immune cells in this process.

149 Disrupted Mitochondrial Metabolism Links LIPT1 and GLUD2 Variants to Neuronal Dysfunction

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Mitochondrial metabolism is essential for neuronal function, and disruption of the tricarboxylic acid (TCA) cycle components often results in severe neurodevelopmental and neurodegenerative disorders. *LIPT1* and *GLUD2* encode mitochondrial enzymes required for TCA cycle function. LIPT1 catalyzes the lipoylation of the pyruvate (PDH) and α-ketoglutarate dehydrogenase (OGDH) complexes, and its deficiency causes a rare metabolic disorder characterized by reduced PDH/OGDH activity, elevated lactate and elevated α-ketoglutarate (α-KG), leading to a progressive neurodegeneration. We are studying patients with compound heterozygous *LIPT1* variants who exhibit sporadic acute lactic acidosis, α-KG accumulation, and neuronal abnormalities. We are also studying seven LIPT1 disease associated variants described in the literature.

GLUD2, encodes glutamate dehydrogenase 2, which catalyzes the conversion of glutamate to α-KG. We have identified six individuals carrying potentially pathogenic *GLUD2* variants who present with cardiac and neurological symptoms, including syncope and, in some cases, sudden death.

The *Drosophila* orthologs *Lipt1* and *Gdh* are essential genes whose loss cause embryonic lethality. Using CRiMiC T2A-Gal4 alleles, we show that *Lipt1* and *Gdh* are expressed in neurons and glia. Neuronal but not glial knockdown of *Lipt1* results in severe neurodegeneration, locomotor defects, and shortened lifespan, recapitulating human phenotypes. Photoreceptor-specific knockdown of *Lipt1* causes retinal degeneration, rhabdomere engulfment by glia, and accumulation of large lipid droplets in photoreceptors. Metabolomic analysis of neuronal *Lipt1*-deficient flies revealed a significant downregulation of dopamine metabolites, suggesting a novel connection between LIPT1 deficiency and dopaminergic signaling.

Ongoing experiments utilize transgenic flies expressing human *LIPT1* or *GLUD2* reference and patient-derived variants to assess functional rescue, variant pathogenicity, and metabolic alterations. In parallel, we are characterizing mitochondrial function and metabolic profiles in patient-derived fibroblasts.

Finally, we are testing pharmacological and dietary interventions including rapamycin, N-acetylcysteine amide (NACA), and Triheptanoin to determine their potential to restore metabolic balance and alleviate disease phenotypes in variant-expressing flies. Together, our work establishes conserved metabolic links between mitochondrial dysfunction, α-KG imbalance, and neuronal degeneration.

150 Increased Glycine-*N*-methyltransferase expression disrupts light-dependent rhythms in the *Drosophila* eye

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Glycine-*N*-methyltransferase (Gnmt) is highly upregulated in the aging *Drosophila melanogaster* eye. Gnmt regulates *S*-adenosylmethionine (SAM) and *S*-adenosylhomocysteine (SAH) availability by catalyzing the transfer of a methyl group from SAM to glycine, producing sarcosine and SAH. Gnmt is well studied in the liver, however little is known about the consequences of increased Gnmt in neurons. We overexpressed Gnmt in the eye and profiled rhythmic gene expression and histone methylation in photoreceptor neurons. Here, we show that eye-specific Gnmt overexpression altered rhythmic gene expression without impacting core clock gene expression or photoreceptor viability. Gnmt overexpression decreased global repressive histone methylation but had no major effect on H3K4 methylation, suggesting that methylation reactions are selectively inhibited by Gnmt and that Gnmt disrupts rhythmic gene expression independent of H3K4 methylation. Moreover, overexpressing wild-type or mutant Gnmt in clock cells suppressed day/night activity differences under diurnal light conditions. These results suggest that Gnmt has roles in the regulation of light-dependent rhythmic gene expression in the eye and behavior that are partially dependent on its catalytic activity, but do not impact age-dependent retinal degeneration.

151 Nutrition-sensitive peptide hormone orchestrates lipid-steroid metabolism for sexual maturation

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Steroid hormones are ancient and conserved signaling molecules that play central roles across development, reproduction, and aging. Because steroidogenic tissues have limited hormone reserves, the rapid mobilization of stored sterol precursors by peripheral metabolic and stress cues is crucial for timely steroid release. Here, we identify a conserved peptide hormone-JAK/STAT-lipolysis axis that rapidly couples metabolic state to steroid hormone biosynthesis, revealing a fast and reversible metabolic mechanism. During early third-instar larval growth, the rapidly growing imaginal discs act as key metabolic sensors of nutritional state and secrete optimal levels of the peptide hormone Unpaired (Upd). Upd activates the JAK/STAT signaling pathway in the steroidogenic prothoracic gland (PG), functioning as a molecular brake that suppresses lipid droplet (LD) accumulation through the activation of hormone-sensitive lipase (Hsl). The transcription factor Fruitless (Fru) serves as a key effector of this pathway, acting as a lipid-storage gatekeeper that links extracellular signaling input to metabolic output. The Upd–JAK/STAT–Fru axis prevents premature accumulation of cholesterol precursors and suppresses ecdysteroid synthesis. At the late third-instar stage, when larvae stop feeding, Upd expression in the imaginal discs and fat body sharply declines. The release of this inhibitory brake allows rapid LD accumulation in the PG, providing substrates for massive ecdysteroid production that triggers metamorphosis and sexual maturation. Nutritional deficiency during mid-third instar larvae induce precocious inactivation of JAK/STAT signaling in the PG, leading to early metamorphic transition. Once sufficient steroid levels are reached in the prepupal stage, the ecdysone signaling pathway induces programmed cell death in specific tissues such as the salivary glands and fat body. Dying cells subsequently secrete Upd, which feedback to the PG to reactivate the JAK/STAT-Fru-Hsl brake, thereby promoting lipid droplet hydrolysis and downshifting steroid hormone levels to a stable physiological range. Cross-species analyses in German cockroaches and mice reveal a conserved coupling among peptide hormone, lipolysis, and steroidogenesis, recapitulating the vertebrate hormonal trajectory from prepubertal quiescence to pubertal onset. Together, these findings define an evolutionarily conserved metabolic-endocrine feedback architecture, in which the peptide-based regulatory circuit operates faster than neural pathways, allowing immediate integration of nutritional deficiency, tissue injury or tumor stress to dynamically adjust endocrine output, steroid homeostasis, and reproductive fitness.

152 Glycerol-3-phosphate dehydrogenase regulates Target of rapamycin (Tor) activity to prevent ectopic growth of *Drosophila* larval brain.

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Animal development requires integration of nutritional cues with signaling pathways and gene expression networks. One of the key nutrient-sensing proteins that coordinates growth and metabolism is the highly conserved protein Target of rapamycin (Tor), which famously regulates developmental progression in response to amino acid availability. Recent studies, however, also reveal that the glycolytic intermediate dihydroxyacetone phosphate (DHAP) promotes Tor activity, thus revealing that this ancient nutrient sensor also coordinates glycolytic flux with growth and development. To better understand how glycolysis influences Tor activity in vivo, we are examining this relationship in the fruit fly *Drosophila melanogaster* using a combination of genetic and metabolomics approach. Our studies reveal a novel mechanism by which glycerol-3-phosphate dehydrogenase 1 (GPDH1), a key enzyme that regulates abundance of the glycolytic intermediate DHAP, functions in a feedforward loop with Tor signaling. Specifically, we discovered that Tor inhibition induced significant elevation in GPDH1 expression, raising the possibility that GPDH1 serves a role in Tor-dependent growth regulation. Consistent with this hypothesis, we found that GPDH1 is required in neuroblasts to restrict Tor activity under growth limiting conditions. *Gpdh1* mutant larval neuroblasts not only exhibit inappropriate Tor activation following either starvation or treatment with a sublethal dose of rapamycin, but also undergo ectopic divisions – resulting in brain overgrowth and the eventual formation of tumorous brains. Overall, our findings hint a Tor - GPDH1 feedforward mechanism during *Drosophila* larval brain development, where Tor inhibition promotes GPDH1 expression. In turn, GPDH1 limits the DHAP pool size and further suppresses Tor-dependent growth signaling, allowing animals to optimize survival in stress conditions. Based on this model, we hypothesize that sublethal doses of rapamycin induces brain tumors in *Gpdh1* mutants due to elevated DHAP levels, which results in appropriate Tor signaling and ectopic neuroblast division. Overall, our studies demonstrate how changes in glycolytic flux affect growth signaling and hold the potential to uncover a fundamental mechanism by which GPDH1 and Tor regulate tissue growth in response to changes in carbohydrate metabolism.

153 Expected and surprising developmental requirements of juvenile hormone degradation enzymes in *Drosophila melanogaster*

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Precise control of hormones is essential to development and reproduction. Hormone bioavailability is regulated by their synthesis and transport, as well as sequestration, natural turnover, and programmed degradation, the latter of which are poorly understood. Here, we use *Drosophila melanogaster* to investigate the enzymes that degrade the retinoic acid-like

Juvenile hormones (JH) that functionally oppose the steroid hormone, ecdysone. While JH titers must decrease to ensure metamorphosis, the developmental requirements of both classes of JH degradation enzymes, JH esterases (JHEs), and JH epoxide hydrolases (JHEHs), have not been determined due to genetic redundancies. To overcome this knowledge gap, we generated double knockout animals lacking both JHEs as well as triple knockout animals lacking all three JHEHs. While both classes of JH degradation enzymes have higher levels of the JH target gene Kr-h1, we found that neither class is required for viability. They have unexpectedly distinct requirements in developmental timing and growth. Animals lacking JHEs pupariate at the expected time but are larger, indicating that JHEs restrain developmental growth but not timing. In contrast, animals lacking JHEHs are smaller and severely delayed in their development, indicating that JHEHs regulate both developmental timing and growth. To investigate this unexpected separation of requirements between JH degradation enzymes, we analysed the endocycling larval tissues including the hormone-producing glands and salivary glands, performed genetic and hormone rescue experiments, and characterized dysregulated gene networks using white prepupae RNA-sequencing. 45 ribosomal genes were dysregulated in *JHEH* WPP, including 5 associated with the Minute phenotype. Consistently, in addition to slower development and smaller body side, *JHEH* adults have shorter and thinner thoracic bristles, a signature of the *Minute* syndrome. Together, our data reveal that despite unique developmental phenotypes and transcriptional signatures, loss of JHE and JHEHs share requirements in Cytochrome P450 regulation and the size of hormone-producing glands. With these new genetic tools and phenotypic characterizations, we have shed light on the complexities of programmed hormone degradation in development.

154 mTORC1 and the Integrated Stress Response pathway regulate the IGF1 homolog Dilp6 in response to variable protein in a sex-dependent manner in *Drosophila*

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Hormones such as insulin, insulin-like growth factors (IGFs), and insulin-like peptides (ILPs) convey information about nutrient availability to tissues to regulate energy demanding processes such as growth. *Drosophila melanogaster* ILPs, also known as Dilps, are produced in specific tissues, like insulin-producing cells in the brain (Dilps 2, 3, and 5) or fat body (Dilp6). However, it is not well understood how dietary nutrients drive individual Dilp secretion in a tissue-specific manner. Our previous work investigated how Dilps are regulated by diet using a nutritional geometry approach. We found that starvation, as well as diets high in sucrose, but low in protein, increased Dilp6 production from the fat body. In contrast, high protein, low sucrose diets suppressed Dilp6 production. Our current project focuses on determining how starvation and dietary protein regulate fat body Dilp6 expression and production. Two major signaling pathways, GATOR2/1-mTORC1 and the Integrated Stress Response, respond to dietary nutrients to control protein synthesis, cellular metabolism, and hormone production. We examined the regulation of *Dilp6* by dietary protein in male and female mid-third instar larvae with fat body-specific loss of GATOR components or the Integrated Stress Response kinases Gcn2 and PERK. We found nutrients act via these pathways to regulate *Dilp6* in a sex-dependent manner. In particular, the GATOR2 component Wdr59 regulates *Dilp6* with both high protein and starvation diets in females. However, males regulate *Dilp6* with high protein diets through Gcn2, a kinase that canonically senses un-methylated tRNAs. Here, Western blotting showed that phosphorylation of the Gcn2/PERK target eIF2α was largely unresponsive to diet in female fat body. Surprisingly, in male fat body, eIF2α phosphorylation was suppressed in response to starvation but increased by dietary protein. Future efforts will determine how mTORC1 and Gcn2 regulate transcription factors, including cryptocephal, the fly ortholog of ATF4, to regulate *Dilp6* in response to dietary protein or starvation. Ultimately, our preliminary data suggests growth hormone regulation relies on sex-biased nutrient sensing strategies to compensate for variable amino acids to maintain juvenile development.

155 Sexual identity of adipokinetic hormone (Akh)-producing neuroendocrine cells determines nucleobindin 1 (Nucb1)-dependent metabolic and reproductive plasticity

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Sex differences in metabolic physiology are widespread across species, yet the cellular mechanisms that generate sex-specific energy allocation remain poorly understood. We identified nucleobindin 1 (Nucb1), a conserved calcium-binding protein, as a female-specific regulator of adipokinetic hormone (Akh)–producing cells (APCs) in *Drosophila melanogaster*. APC-specific knockdown of *nucb1* significantly altered energy storage in females but not in males. These metabolic effects were also accompanied by reduced *akh* transcript levels. Cellular sex reversal experiments demonstrated that the requirement for nucb1 depends on the intrinsic sexual identity of the APCs. Strikingly, *nucb1*-deficient females failed to suppress ovarian follicle proliferation under nutrient restriction, continuing to lay mature eggs even when maintained on a poor diet. Together, these results establish Nucb1 as a female-specific regulator that links Akh-dependent energy mobilization to nutrient-sensitive control of reproduction.

156 FGF is attenuated to promote stem cell recovery in the *Drosophila* germline

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Maintenance of adult stem cells is critical for tissue homeostasis across an organism's lifetime. Challenge to the tissue – including nutrient deprivation and aging – cause a significant decline in stem cell numbers and necessitates mechanisms to replenish the dwindling stem cell pool. One mechanism is dedifferentiation, in which differentiating progenitors migrate back to the stem cell niche and revert to bonafide stem cell identity. Given the complex architecture of most niches, the dedifferentiation process is still poorly understood. Our lab utilizes longitudinal live imaging of the testis niche to directly interrogate cellular responses to stress. In this system, germline stem cells (GSCs) and differentiating daughters are spatially and morphologically distinct. While GSCs are single cells adherent to a pool of quiescent niche cells, differentiating daughters are displaced from the niche and divide in a syncytium. These groups of differentiating germ cells, or cysts, are formed by mitosis followed by incomplete cytokinesis, generating stable intercellular bridges known as ring canals (RCs). Incomplete cytokinesis is a highly conserved hallmark of gametogenesis across kingdoms, and yet, poses an issue for plastic processes like dedifferentiation. How do germ cell cysts break their connections, migrate back to the stem cell niche, and assume GSC identity? Here, we find that nutrient deprivation alters soma-germline signaling, driving dedifferentiation by promoting completion of cytokinesis in germline cysts. Under homeostasis, we have previously found that the FGF ligand Pyramus is produced by surrounding somatic support cells and transmitted to germline cysts, activating FGFR Heartless and promoting stability of RC F-actin. Loss of either FGF, or FGFR, induces instability in the germline cyst RC and allows cysts to complete cytokinesis. We find that FGF activity decreased in germ cells during dedifferentiation due to a loss of tight soma-germline contact. Critically, we find that constitutive activation of FGFR within germ cells prevents restoration of the GSC pool following injury, suggesting that diminished FGF signaling and subsequent RC instability are essential steps required for dedifferentiation. Once dedifferentiation completes, FGFR activity is restored and cysts no longer complete abscission outside of the niche. Thus, somatic cells play a fundamental role in regulating germline cysts to maintain homeostasis and promote stem cell recovery.

157 Investigating the cell-specific function of *LSD1-interacting non-coding RNA-3* (*LINR-3*) in regulating fly oogenesis

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Cellular differentiation is tightly regulated to ensure proper tissue development and homeostasis. Lysine-specific demethylase 1 (LSD1), an epigenetic modifier, modulates chromatic dynamics to promote cell proliferation, and its interactions with specific long non-coding RNAs (lncRNAs) have been implicated in tumorigenesis. However, the physiological functions of LSD1-associated lncRNAs remain poorly understood. Our laboratory recently identified specific *LSD1-interacting noncoding RNAs* (*LINRs*) as novel regulators controlling *Drosophila* egg production. Among these *LINRs*, we found that knocking out *LINR-3* impaired oogenesis. While clonally knockdown (KD) of *LINR-3* in early somatic cells and follicle stem cell lineages also leads to ovarian structural defects, such as enlarged germarium. Moreover, abnormal differentiation was found to be widespread among different types of ovarian cells and seemed not to be restricted to only the *LINR-3* KD cells, suggesting a non-cell-autonomous function of *LINR-3*. Consistently, knocking down *LINR-3* in early follicle cells increased the number of germline stem cells (GSCs), indicating a long-range impact of *LINR-3* in controlling GSCs differentiation. Interestingly, in germarium, we observed the presence of non-nuclear *LINR-3* transcripts and extracellular vesicles (EVs) signals released from follicle cells enriched around the germline stem cell niche. These results support our model that specific EVs released from early follicle cells may carry *LINR-3* to affect niche cells, thereby impacting GSC differentiation remotely *in vivo*.

158 Transcriptional co-conspirators: Tai and Yki cooperate in intestinal homeostasis

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As organisms age, the proliferative capacity of stem cells declines, which in turn impairs tissue renewal and can lead to organ dysfunction and disease. How local and systemic cues precisely regulate stem cell-based tissue renewal remains unclear, particularly in the rapidly cycling intestinal epithelium. A pair of transcriptional coactivator proteins – the Hippo pathway protein Yorkie (Yki) and the Ecdysone nuclear receptor (EcR) interactor Taiman (Tai) – are each implicated in the promotion of intestinal stem cell (ISC) renewal. We previously determined that a physical interaction between Tai and Yki, mediated via respective PPxY (proline-proline-x-tyrosine) motifs and WW (tryptophan-tryptophan) domains, allows these factors to cooperatively induce target genes associated with regenerative growth of disc epithelia (e.g., *Ilp8*). As Tai and Yki each drive ISC proliferation in the adult *Drosophila* midgut, we sought to test the hypothesis that Tai:Yki binding is required for this gut renewal function, and that this interaction allows the Hippo and EcR pathways to cooperatively regulate gut homeostasis via a set of as yet undefined target genes in midgut cells, e.g., absorptive enterocytes (ECs) and ISCs. To address this question, we have generated a CRISPR mutant fly stock in which the PPxY motifs of endogenous Tai are converted to PPxA, blocking Tai's binding to Yki and allowing precise and selective decoupling of the Tai/EcR and Yki/Hippo pathways *in vivo*. Here we show these *tai*^{PPxA} homozygous animals are viable, sensitive to gut damage, and have reduced ISC pools in the adult midgut, which is predicted to dramatically lower their regenerative potential. We also observe a dramatic upregulation of the Tai-Yki coregulated gene *piwi*, which is an ISC-specific gene required for gut renewal. Surprisingly, this *piwi* upregulation is accompanied by an increase in transposable element mobility in *tai*^{PPxA} heterozygous guts. At present, we are mapping the source of this *tai*^{PPxA} effect to ISCs or to ECs that transmit damage signals to induce ISC asymmetric divisions. Ongoing lineage tracing experiments will also assess the renewal capacity of *tai*^{PPxA} ISCs. Collectively, we hypothesize that the Tai-Yki axis may have a dual role in ISCs: to repress renewal genes such as *piwi* in the absence of gut damage but then switch to activation of these genes in the presence of damage signals (e.g., upd3).

159 Adhesive cell interactions mediated by Rho1 direct assembly of a functional niche

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Stem cells are required to maintain and regenerate tissue in adult organisms. Stem cells reside in a cellular microenvironment, also known as the niche, which sends signals responsible for regulating surrounding stem cell fate. Damage to the niche is often associated with heritable disease, and constructing a functional niche during tissue development is essential for adult homeostasis. Studying mechanisms that form a niche has proven challenging, as most niches form internally during embryogenesis and are inaccessible to imaging. Our lab developed a method to enable *in vivo* live imaging of testis niche formation at its inception. This imaging, coupled with the genetic tractability of *Drosophila* and the well-defined architecture of this tissue, makes the testis an ideal model to study niche formation. We previously showed that niche assembly requires niche cell accumulation of the conserved transcription factor Islet, which is required for niche cells to polarize their F-actin cytoskeleton and assemble at the anterior of the embryonic gonad (Anllo & DiNardo, 2022). In many contexts F-actin polarization drives cell motility, but cells sorting by differential adhesion often polarize their cytoskeleton in response to establishment of cell contacts. Thus, it remains elusive whether F-actin directly mediates niche cell assembly, and what specific regulators of F-actin are required for the assembly process. We have established a method that employs molecular optogenetics to directly manipulate F-actin regulatory mechanisms during specific stages of niche development. Here, we demonstrate precise temporal control of the Cry2 CIBN system in the embryonic testis, using blue light to direct localization of cytoskeletal modulators to the cell cortex. Using *in vivo* live imaging with these optogenetic techniques, we show a direct role for polarization of cortical F-actin in niche assembly. Our data support a requirement for the cytoskeletal modulator Rho1 in niche assembly. In the absence of Rho1 during late gonadogenesis, niche cells fail to accumulate stereotypical niche adhesion proteins, and do not assemble an organized anterior structure. We find that these disrupted niches also lack the ability to signal to neighboring stem cells. Together, our work defines dynamic contributions of the F-actin cytoskeleton and its regulators in the establishment and functionality of a stem cell niche.

160 Lactate dehydrogenase as a metabolc regulator and potential anti-aging target in the *Drosophila* intestinal stem cell niche

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Tissue-resident stem cells are required for the constant generation of the specialized cell types present in tissues and organs. In the *Drosophila* intestine, intestinal stem cells (ISCs) maintain epithelial integrity throughout life through tightly regulated proliferation and differentiation. With age, ISCs hyper proliferate, and daughter cells fail to properly differentiate, leading to epithelial dysplasia and loss of barrier integrity. However, the mechanisms driving this age-associated increase in stem and progenitor cells remain poorly defined.

Age-related changes in metabolism are known to influence multiple aspects of stem cell physiology and regenerative capacity, and such metabolic remodeling represents a conserved hallmark of aging across tissues. Most stem cells rely on glycolysis for energy generation, while their differentiated progeny favor oxidative phosphorylation. We identified the glycolytic enzyme Lactate dehydrogenase (Ldh) as a potential metabolic regulator of ISC behavior and potential anti-aging target. Our data show that *Ldh* expression increases within the intestines of aged flies and demonstrate the requirement and sufficiency of *Ldh* expression to regulate ISC maintenance under both homeostatic and aged contexts. Moreover, lineage-tracing experiments suggest that *Ldh* expression controls stem cell fate choice, as *Ldh* overexpression impaired the ability of progenitor cells to differentiate appropriately. Together, these findings contribute to define a mechanistic role for *Ldh* in coupling metabolic state to stem cell maintenance and lineage commitment in aging. As alterations in metabolism and stem cell behavior are conserved features of aging across tissues, this work positions *Ldh* as a target for metabolic regulation of epithelial homeostasis and regenerative decline.

161 4D imaging of the germarium suggests that Follicle Stems Cells and Follicle Cells self-organize around germline cysts

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Follicle Stem Cells (FSCs), located in the germarium of the Drosophila ovary, give rise directly to adult Escort Cells and Follicle Cells (FCs), both of which are essential for germline cyst maturation. Lineage tracing experiments have shown that FSCs maintain their population through population asymmetry, a mechanism in which the collective population sustains appropriate numbers of differentiated cells, while the fate and behavior of individual stem cells remain stochastic. To investigate FSC behavior in more detail, live imaging of ex vivo ovarioles was conducted. We were interested to know when and how an FSC becomes an FC, and if there is any coordination with germline cyst movement or shape. Individual FSCs, FCs, and germline cysts were segmented in confocal image stacks and custom software was used to create interactive 4D animations. Germline cysts exhibited a consistent sequence of morphological transitions: moving from the 2a to 2b region while flattening into a disc shape, followed by rounding on the posterior side of the cyst while moving into region 3, followed by rounding into a ball and moving posterior before budding off into an egg chamber. Trajectories of individual FSCs and FCs, including daughters of cell divisions of FSCs and FCs, were mapped at all positions in the germarium, revealing how cysts become coated with FCs. We found that FCs do not bind tightly to germline cysts but are mobile along the cyst surface. When cells divided, their nuclei moved away from the basement membrane, deforming germline cysts, before the daughter nuclei returned close to the basement membrane. Daughters of FSC divisions generally returned to the basement membrane in non-adjacent positions. FSCs and early FCs often jostled back and forth in position, frequently moved one cell diameter anterior or posterior in relation to other cells, and could move many cell diameters around the circumference of the germarium. FSCs and early FCs exhibited highly variable patterns of movement and division, suggesting great flexibility among individual cells. We propose that self-organization of FSCs and FCs complements long-range positional cues, with local interactions and feedback enabling flexible organization around germline cysts that are continually changing shape and position.

162 mTORC2-Chinmo Axis Regulates Germline Stem Cell Aging via Niche Remodeling in *Drosophila*

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Stem cells reside within niches that regulate their self-renewal and differentiation, thereby maintaining tissue homeostasis. However, aging progressively impairs niche function, disrupts stem-cell behavior, and contributes to tissue decline. The molecular mechanisms underlying stem-cell aging—and how stem cells adapt to deteriorating nichesremain incompletely understood. Our previous studies utilized Drosophila male germline stem cells (GSCs) as a model and found that the transcriptional repressor Chinmo declines with age, resulting in the aberrant secretion of the extracellular matrix protein Perlecan (Pcan). Pcan accumulates in the testicular lumen, perturbs GSC–niche interaction, and ultimately drives GSC loss during aging. Remaining aged GSCs adapt by upregulating Dystroglycan (Dg), which binds Pcan to maintain adhesion within the remodeled niche. Building on this, we identify mTORC2 as an upstream regulator of Chinmo. We show that Tor protein levels are relatively reduced in aged GSCs, and germline depletion of the mTORC2 core component Rictor decreases GSC number, lowers Chinmo expression, increases Pcan deposition, elevates Dg levels, and attenuates JAK/STAT signaling. Moreover, the simultaneous depletion of Rictor and Pcan in germline cells rescues GSC loss in aged testes, demonstrating that mTORC2 maintains GSC homeostasis at least in part by regulating extracellular matrix remodeling. Given the evolutionary conservation of mTORC2 and its relatively understudied role compared to mTORC1 in stem cell aging, these findings highlight a previously unrecognized stem cell–intrinsic signaling pathway that controls niche function during aging.

163 Function of the PinX1 homolog, Chigno, in the *Drosophila* testis stem cell niche

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Stem cells play a critical in tissue homeostasis by maintaining the balance between stem cell self-renewal and differentiation. Disruption of this balance can cause cancer or loss of stem cells leading to organ dysfunction. The *Drosophila* testis provides a model for studying stem cell regulation with two stem cell populations: germline stem cells (GSCs), which generate sperm, and somatic cyst stem cells (CySCs), which provide support and signaling cues for germline differentiation. Previous work from the Wawersik lab identified the novel gene, Chigno (CG11180), as a regulator of somatic CySC behavior. Somatic knockdown of Chigno leads to over-proliferation of stem cells, undifferentiated germ cells, and reduced fertility. Building on these findings, the current study further characterizes the function of Chigno in the CySC and GSC lineages. Soma-specific RNAi knockdown of Chigno results in CySC expansion and reduction of differentiated cyst cells. Germline-specific RNAi knockdown of Chigno results in reduced GSC and spermatogonia numbers. This data suggests that Chigno inhibits CySC self-renewal while promoting GSC maintenance and possibly differentiation. Given that Chigno shares homology with the human PinX1, a telomerase inhibitor and RNA-binding protein expressed in reproductive tissues, these results may have broader implications for understanding human reproductive disorders and cancer. Insights into Chigno function could reveal conserved mechanisms by which stem cells regulate self-renewal and genome stability. Understanding these pathways in *Drosophila* provides a foundation for exploring how similar processes may contribute to infertility and cancer in humans, advancing our knowledge of stem cell regulation and its links to disease.

164 A High-Throughput, Artificial Intelligence-Powered Pipeline for *Drosophila* Behavioral Phenotyping

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Genetics and environment influence animal behavior in subtle, complex, and hard-to-predict ways. While traditional behavior assays are highly targeted, modern artificial intelligence (AI) allows deeper, holistic phenotypic profiling and quantification. Here, we present our AI-powered, general-purpose *Drosophila* behavior phenotyping pipeline. We built a custom platform that simultaneously records 150 isolated flies in full color with 59 pixel/mm resolution and 40 frames/sec. We use DeepLabCut to track 30 body part positions, from which we engineer 512 postural features per video frame. A custom 3.2-million-parameter Transformer-based neural network then predicts behavior labels for each frame with 90% accuracy. Per-frame data and labels are aggregated to define behavior profiles with ~6000 behavior-specific features per fly. Using these aggregate profiles, we train explainable AI (XGBoost) models to distinguish genotypes (eg: control vs mutant). Feature importance analysis then identifies the specific behavior features which drive these distinctions. We can also apply these models to additional genotypes or treatments (eg: mutant with transgene, suppressor, etc.) to reveal whether their behavior profile is similar to control or mutant, and how each behavior feature contributed to that determination. We validated our approach in several ways. First, we analyzed flies with heat-activated TRPA1 expressed in various neurons, and were able to detect previously reported behavioral changes (eg: increased wing grooming). Second, we analyzed humanized *UBA5* flies with either wild type or patient mutations and found that mutants exhibit unstable inverted locomotion with jerky leg movements and a prolonged recovery response upon falling, consistent with previously reported climbing and seizure defects. Third, we analyzed *spd2* mutants, revealing differing patterns of limb movement during walking and grooming. We applied the model to *spd2* mutants with rescue constructs from different species – consistent with our previous cell-biological work, our behavior analysis revealed strong rescue with *Dmel spd2*, moderate rescue with *Dwil spd2A* which is expressed in the brain, and no rescue with *Dwil spd2B* which is testis-specific. Altogether, our behavior analysis pipeline includes high-throughput recording, behavior prediction, reveals genotype-relevant aspects of behavior, and will facilitate behavior research by decoding complex phenotypes that human observation misses.

165 An Interactive, 3-D Developmental Atlas for *Drosophila melanogaster*

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A developmental atlas is one of the most fundamental tools of any biologist, and current *Drosophila* developmental references are used by hundreds of researchers and students each year. For example, Hartenstein's *Atlas of Drosophila Development* has been the foundational resource in our community for the last three decades. However, recent advances in tomography-based imaging technologies provide an opportunity to update these resources with accurate 3D representations of animals in their native state at unparalleled resolution. Using phase-contrast X-ray imaging, we have created a developmental atlas that spans over 30 stages of wildtype *Drosophila* development, from cellularization of the embryo to 10 days after eclosion. This atlas enables visualization of the native conformation of every tissue during each stage of development at sub-cellular resolution, allowing precise morphological analysis of the fly. Our images of the pupal stages, the most understudied stage of *Drosophila* development, are especially illuminating, providing an unparalleled glimpse into metamorphosis as larval tissues transform into their final adult form. We were also successful in preserving and visualizing small and delicate structures such as the heart and nephrocytes in their native configuration, along with understudied organs such as the ring gland. We anticipate this atlas to be a useful tool for any *Drosophila* scientist and are excited to share it in its current form, with future plans to incorporate images of mutant animals into this resource to expand its utility within the fly community.

166 FlyPredictome: A Structurally Resolved Atlas of the *Drosophila* Protein Interactome

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Large-scale genetic and proteomic screens are instrumental in mapping cellular networks but often fail to resolve the domain- or residue-level details of the direct, physical mechanisms underpinning functional relationships. Here, we present FlyPredictome, a publicly accessible resource of structurally resolved *Drosophila* interaction predictions, currently totaling ~1,200,000 predictions. This resource was built using our novel computational pipeline, which couples AlphaFold-Multimer with an integrated Local Interaction Score (iLIS) calculation to accurately predict a broad range of high-confidence interactions, from stable intracellular complexes to transient interactions such as the secreted ligand and their receiving receptor pairs. We validated the ability of our iLIS scoring to accurately identify direct interactions by benchmarking against existing protein-protein interactions (PPIs) including large-scale datasets (FlyBi, DPiM, and DPiM2). We also applied this pipeline to evaluate all the genetic interactions annotated in FlyBase, demonstrating its power to explore the potential mechanism for some genetic interactions as physical interactions—exemplified by providing a structural basis for classic genetic alleles. We further showcased the utility of this pipeline for hypothesis generation by showing how it builds extended networks from annotated pathways, like autophagy, or functional genomics data from CRISPR screens. The FlyPredictome integrates the structure-based protein-protein predictions, offering the research community a valuable tool to systematically reveal the residue-level details underlining the functional relationships.

167 Endocytome profiling uncovers cell-surface protein dynamics underlying neuronal connectivity

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Endocytosis actively remodels the neuronal surface proteome to drive diverse cellular processes, yet its global extent and effects on neural circuit development have defied comprehensive interrogation. Here, we introduce endocytome profiling: a systematic, cell-type-specific approach for mapping cell-surface protein (CSP) dynamics *in situ*. Quantitative proteomic analysis of developing olfactory receptor neuron (ORN) axons generated an endocytic atlas comprising over 1,000 proteins and revealed the extent to which the surface proteome is remodeled to meet developmental demands. Targeted interrogation of a junctional CSP showed that its endosome-to-surface ratio is precisely balanced to enable developmental axon pruning while preserving mature axon integrity. Multi-omic integration uncovered widespread transcellular signaling and identified a growth factor secreted by neighboring neurons to direct ORN axon targeting via endocytic regulation of its receptor. Endocytome profiling provides unprecedented access to cell-surface proteome dynamics and offers a powerful platform to dissect proteome-scale remodeling across diverse cell types and contexts.

168 Development of a novel high-throughput system to study *Drosophila melanogaster* metabolism

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Metabolomics has rapidly emerged as a powerful and indispensable tool for investigating the complex metabolic mechanisms that underpin a wide array of biological processes. Among model organisms, the fruit fly *Drosophila melanogaster* has proven exceptionally well-suited for metabolomic studies due to its genetic tractability, short life cycle, and well-characterized biology. Increasingly, researchers are leveraging this system to explore how metabolic pathways are influenced by developmental stages, disease onset and progression, genetic mutations, and environmental stressors. Despite its promise, the full potential of metabolomics in Drosophila research remains underutilized. Many previous studies have relied on small-scale approaches, often focusing on the metabolic impact of single genes or isolated environmental conditions. These limited methodologies restrict the scope of discovery and hinder the ability to draw broader, systems-level conclusions. To address this gap, we present a streamlined and cost-effective metabolite extraction method coupled with a novel high-throughput analytical platform. This system is capable of processing up to 94 samples simultaneously using a 96-well plate format, with each sample requiring only a one-minute run time. Due to the low cost (approximately $10 per sample), this system is highly accessible for large-scale studies. Our platform utilizes ultra high-pressure liquid chromatography-mass spectrometry (UHPLC-MS), adapted from protocols originally developed for human blood sample analysis. This adaptation ensures high sensitivity and precision while maintaining scalability and reproducibility. This system was validated using previously characterized metabolic mutants. Further, the integration of this method into Drosophila research enables comprehensive metabolite profiling across diverse experimental conditions, paving the way for more robust and expansive metabolic investigations.

169 ChIP-SMF: Scalable single-molecule profiling to reveal protein co-binding dynamics on DNA

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Protein-DNA interactions are a key aspect of nuclear processes such as gene transcription and chromatin organization. Current methods for mapping these interactions are limited by their bulk nature. These approaches average across cells and thus cannot capture the co-occurrence and dynamics of proteins on single DNA molecules. To address these limitations, we introduce ChIP-SMF, a method that combines single-molecule footprinting with chromatin immunoprecipitation. ChIP-SMF retains the scalability of a ChIP-seq bulk assay while embedding single-molecule information and providing high-coverage single-molecule footprints of genomic regions bound by a protein of interest. By locally increasing sequencing coverage, this method enables the detailed characterization of protein interactions and their co-binding dynamics on DNA. ChIP-SMF is particularly well suited for studying transcription factors and larger complexes such as the transcription machinery at promoters. To showcase the method, we performed ChIP-SMF on multiple components of the Pre-Initiation Complex (PIC) in *Drosophila melanogaster* Kc167 cells. We uncovered co-dependencies across promoter types and revealed the relationship between RNA Polymerase II pausing and the positioning of the +1 nucleosome, within a single dataset. We also developed flexible computational tools tailored for single-molecule resolution data to quantify and visualize the results. In summary, we developed an experimental and analytical framework to decode the combinatorial rules by which transcription factors and other proteins bind simultaneously on DNA.

170 Nanopore Sequencing in *Drosophila*: Length and Bias Limits Progress in Completing the Genome

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Long-read DNA sequencing technologies have enabled chromosome-level and telomere-to-telomere genome assembly. Substantial progress has been achieved with human, yeast, and plant genomes following the introduction of Oxford Nanopore Technologies ultra-long sequencing methods. However, complete assembly of Drosophila genomes remains challenging despite their smaller genome sizes. We sequenced two *D. melanogaster* strains, one *D. simulans* strain, and one *D. pseudoobscura* strain using the Oxford Nanopore Technologies PromethION platform. Read N50 values were 22 kb, 23 kb, and 28 kb for each species using the Ligation Sequencing Kit (LSK114) without Blue Pippin size selection, whereas the same method yielded 33 kb and 44 kb with mammalian DNA. The highest read N50 generated by transposase-based methods (Ultra-long Kit [ULK114] and Rapid Sequencing Kit [RAD114]) was 19 kb in *D. melanogaster*.

While copy number and motif sequence content varied between strains when reads were divided into length partitions of N10, N20, etc., no clear trends in composition were observed for 2-mers, 3-mers, and satellite motifs (e.g., AATAT). Most short satellite repeats were underrepresented in Nanopore reads regardless of read length, and ribosomal DNA (rDNA) regions remain unresolved. Consistent with previous findings, AAGAG was rarely sequenced, while its reverse complement CTCTT was detected more frequently. LSK114 yielded more uniform coverage across the reference genome, whereas ULK114 was biased toward euchromatic regions. Although de novo assemblies exceeded current reference genome sizes, no complete chromosomes were recovered. The combination of shorter read lengths in *Drosophila* compared to other systems and the inability to capture satellite sequences means that Nanopore sequencing cannot fill gaps in current *Drosophila* reference assemblies. *Drosophila* species, particularly those with abundant repetitive heterochromatic sequences, present obstacles to complete genome assembly that exceed those encountered in larger genomes such as humans.

171 *'Flying' through the local and long-range functions of the intestine in health and disease.*

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The adult intestine is a major barrier epithelium and coordinator of multi-organ functions. Stem cells constantly repair the intestinal epithelium by adjusting their proliferation and differentiation to tissue intrinsic as well as micro- and macro-environmental signals. How these signals integrate to control intestinal and whole-body homeostasis is largely unknown. Addressing this gap in knowledge is central to an improved understanding of intestinal pathophysiology and its systemic consequences.

Combining *Drosophila* and mammalian model systems my laboratory has discovered fundamental mechanisms driving intestinal regeneration and tumourigenesis and outlined complex inter-organ signaling regulating health and disease. We have three interrelated areas of research in the lab, which I will discuss during my seminar.

1- *Identify and characterise stem cell intrinsic adaptations underpinning intestinal regeneration and tumourigenesis.*

2-Elucidate interactions between the intestine and its microenvironment influencing intestinal regeneration and tumourigenesis.

3-Characterise how long-range signals from the intestine impact whole-body in health and disease.

172 Ageing and metabolic dysfunction - insights from *Drosophila*

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Our research leverages *Drosophila* as a model to investigate mechanisms underlying ageing. We are particularly interested in redox signalling, which operates through the post-translational modification of cysteine residues on proteins. This cysteine oxidation acts as a reversible redox switch, selectively modulating the biological function of the target (1). Dysregulation of redox homeostasis has been implicated in the pathophysiology of many diseases, as well as in the ageing process itself. We combine biochemical, molecular and genetic knock-in approaches, together with state-of-the-art redox proteomics to profile redox signalling *in vivo* (2). Ultimately, we aim to uncover novel redox-sensitive targets and therapeutic strategies to achieve health and longevity benefits.

We also explore the impact of dietary interventions and metabolic dysfunction on survival (3). Recently, we revealed the importance of pH imbalance, which is appreciated in diseases such as cancer, but fundamentally overlooked in ageing. We show that *Drosophila* hemolymph acidifies with age, and that this systemic extracellular acidification correlates to mortality rate, independently of sex. Environmental, genetic and pharmacological paradigms of lifespan extension all delay the age-related decline in hemolymph pH. Older flies are more susceptible to acidotic stress, while alkalotic treatment can extend fly lifespan in unstressed conditions. The age-dependent hemolymph acidification is attributed to insufficient acid elimination, associated with downregulation of genes in the fly excretory tract that control pH. Overall, we propose systemic extracellular acidification as a novel hallmark of ageing (4).

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(3) van Dam *et al.* (2020) *Cell Metabolism*, 31:710-725

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173 Themes and variations in *Drosophila* social circuits

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Animals exhibit astonishing variation in their behavior, both within and across species. We have been using the elaborate courtship rituals of *Drosophila* to gain insight into the circuit mechanisms of adaptive behavior over different timescales, from how social interactions unfold on a moment-to-moment timescale to how species-specific mating displays arise over evolution. By applying an interdisciplinary toolkit to probe the concise circuits of the fly, we have begun to shed light on how the interplay between internal arousal states and external sensory signals shapes behavior, allowing animals to flexibly navigate complex social landscapes.

174 Regulatory logic of neuronal identity specification in *Drosophila*

M. Neset Özel Stowers Institute

Regulatory logic of neuronal identity specification in Drosophila

175T Exploring the role of PDZ-GEF in embryonic wound healing

Olivia Fortman, Katheryn E Rothenberg Biology, University of Iowa

Collective cell migration is critical for embryonic development and tissue repair. However, cells can also use the same migration machinery for spreading cancer, resulting in metastasis. To understand the molecular mechanisms that drive collective cell migration, we investigate wound healing in epithelial tissue using the *Drosophila* embryonic epidermis. Wounds are generated by ablating 4-5 epidermal cells in stage 14 embryos using a high-powered laser. The lesions heal rapidly without scarring or inflammation, forming a supracellular actomyosin cable connected by reinforced cell-cell adherens junctions at the leading edge of the migrating cells. Our previous work has demonstrated a role for the small GTPase Rap1 in coordinating the cell adhesion and cytoskeletal rearrangements required for rapid wound healing. Rap1 GTPase is also misregulated in many metastatic cancers, and the Rap1 signaling pathway plays a role in epithelial integrity. Thus, understanding the factors that promote Rap1 activity is critical for important questions in health and disease. Rap1 is known to be regulated by specific GEFs - PDZ-GEF, Epac, and C3G - and a GAP - Rapgap1. In unwounded epidermis, endogenously tagged PDZ-GEF:GFP localizes to cell-cell boundaries. RNAi-mediated knockdown of PDZ-GEF, verified via Western blotting and loss of PDZ-GEF:GFP fluorescence, had no effects on the rate of embryonic wound closure or cytoskeletal polarization. Together, this suggests that other Rap1 GEFs and GAPs are playing a more prominent role in controlling Rap1 activity to drive wound closure. Future work will examine localization and RNAi-mediated knockdown of Epac, C3G, and Rapgap1 to better understand the regulation of Rap1 during collective cell migration.

176T Examining the in vivo dynamics of Ds-Ft signaling

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A fundamental question in developmental biology is how organ size and cell number are regulated. The Hippo pathway regulates organ size by restricting the activity of the transcriptional co-activator Yorkie, which promotes growth. Several independent upstream inputs regulate Hippo signaling, including the protocadherins, Fat (Ft) and Dachsous (Ds), which bind heterophilically and together promote Hippo pathway activity to restrict growth. Loss of Ft leads to overgrowth and stabilization of the key downstream effector Dachs, an unconventional myosin. Two additional regulators, the palmitoyltransferase Approximated (App) and the SH3 domain containing protein Dachs Ligand with SH3s (Dlish) are required for Dachs stabilization and its localization at the apical junctional cortex. Together, these proteins form a stable 'core complex' that functions to promote growth. Current models suggest that Ds organizes the core complex at junctional puncta to protect them from degradation in competition with Ft, which promotes degradation or dissociation of the core complex. However, it remains unclear how Ds and Ft regulate localization and abundance of core complex at apical junction for growth control. To understand the function of Ds and Ft in regulating the core complex, here we explore the localization and dynamics of App as well as Ds and Ft in living imaginal discs. To examine its dynamics *in vivo*, we tagged App extracellularly with the V5 epitope using CRISPR-Cas9. Using antibodies as well as transgenically-encoded nanobodies, pulse-chase experiments revealed that App is initially uniformly distributed across the apical surface but later becomes stably maintained in junctional puncta together with other core complex proteins. In addition, App is internalized via endocytosis and co-localizes with late endosome marker Rab7. These data suggest that junctional App is stabilized in active signaling puncta and that excess App is removed from apical surface via endocytosis. Since Ft activity prevents core complex formation at the junctional cortex, we examined the effects of Ft on App. App accumulates more stably in junctional puncta in ft^{sum} mutant tissues than in wild type, indicating that Ft activity prevents core complex formation and stabilization. Our data indicate that multiple biological process including protein-protein interaction, recruitment to the junctional cortex, degradation and endocytosis are involved to control the Ds-Ft signaling pathway.

177T Searching for Binding Partners of the ck/Myosin VIIA Tail Domains

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Unconventional myosins play essential roles in linking the actin cytoskeleton to membrane-associated complexes, thereby regulating cell shape, adhesion, and signaling. *ck*/Myosin VIIA is critical in building actin-based protrusions across species, yet the molecular mechanisms governing its interactions with binding partners in *Drosophila* remain incompletely understood. Here we investigate potential binding partners of the C-terminal tail Myth4FERM and SH3 domains of *ck*/MyosinVIIA using transgenic fly lines containing a triple affinity tag – ck tail construct and GFP-fusion proteins of potential cargo proteins including *singed* and *shavenoid*. Preliminary studies have optimized the purification of the tagged *ck* tail constructs but have not found either cytoskeletal-associating protein to be a binding partner. Future studies will examine additional actin-associated proteins.

178T A secreted bacterial effector drives cell death by interfering with Rab35 activity

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Intracellular bacteria secrete proteins called effectors to promote host cell infection. We use *Drosophila* as an in vivo cell biology platform to discover novel effector functions and to study effector-host interactions. The bacterial STI *Chlamydia trachomatis* (*Ct*) grows within a specialized vacuole called an inclusion and secretes numerous inclusion membrane-bound effector proteins. One such protein, called CpoS, is important for *Ct* intracellular development and has been shown to bind and recruit numerous Rab GTPases, such as Rab35, to redirect vesicular cargo to the growing inclusion. However, the consequence of this direct binding to the activity state of Rab35 has not been explored. To address this, we transgenically express CpoS in flies, which allows us to study CpoS-Rab35 interactions without the confounding effects of active infection. CpoS expression in the whole fly is lethal; expression in various tissues result in strong tissue degeneration. Restricted CpoS expression in a wing compartment, using *ptc*-GAL4, leads to a reduction in wing compartment area. This reduction is driven by increased cell death in the corresponding region in the larval wing disc. Interestingly, the dominant-negative Rab35 S22N mutant, when expressed with *ptc*-GAL4, phenocopies the CpoS-induced cell death and reduction in wing area. Therefore, we hypothesize that the *Ct* effector CpoS promotes the inactive state of Rab35. In support of this, genetic interaction experiments between CpoS and different Rab35 mutants result in the following: 1) lethality upon co-expression of CpoS and Rab35 S22N "off" mutant, indicating enhancement; and 2) amelioration of the Cpos-induced wing reduction phenotype upon co-expression with Rab35 Q67L "on" mutant. Also, expression of Rab35 Q67L reduces CpoS-induced cell death in the larval wing disc. Other intracellular pathogens such as *Legionella* and uropathogenic *E. coli* produce effectors that alter Rab35 activity to promote its infection. Further experiments are planned to determine whether CpoS is a novel bacterially-encoded RabGAP of Rab35.

179T Functional interactions between Kibra, a Hippo pathway component, and the apical polarity kinase aPKC

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The Hippo signaling pathway coordinates tissue growth with morphogenesis in animal development. Hippo signaling also has a complex relationship with apical-basal polarity in epithelial cells - disruptions in Hippo signaling components result in a larger apical domain and disruptions of polarity components affect Hippo signaling. Kibra, an upstream regulator of Hippo signaling, is thought to interact with aPKC, a key apical polarity protein via a substrate motif, suggesting that Kibra could be a focal point for interactions between Hippo signaling and apical polarity. Previous work in mammalian cells suggests that Kibra inhibits aPKC's kinase activity, though the physiological relevance of this is poorly understood. Conversely, recent work in our lab indicates that aPKC represses Kibra's ability to promote Hippo signaling.

We are examining the relationship between Kibra and aPKC, and how they function together to control cell polarity and the cytoskeleton, apart from Hippo signaling. Using the follicular epithelium, which has been used extensively to study epithelial polarity and Hippo pathway function, we are examining the effects of gain and loss of Kibra function on follicle cell polarity and morphogenesis. Notably, ectopic Kibra expression leads to abnormal cell shape and loss of epithelial integrity. This effect requires Kibra's aPKC binding domain and is enhanced by decreased aPKC dosage, suggesting that Kibra can antagonize aPKC function in vivo independent of its functions in Hippo signaling. In contrast, reducing Kibra dosage in aPKC hypomorphic flies improves egg chamber development. Further studies will seek to better understand how Kibra controls aPKC function. In particular, we plan to examine the possibility that Kibra represses aPKC activity either by acting as a competitive substrate or by promoting aPKC degradation. Together these experiments should elucidate the importance of Kibra-aPKC interaction for apical polarity and tissue morphogenesis during development.

180T Building Epithelial Barriers with Synaptic Tools

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Septate junctions (SJs) form a barrier essential for epithelial integrity, yet the mechanisms that initiate their assembly remain unclear. SJs share molecular components with synapses, the best studied of which in *Drosophila* is the neuromuscular junction (NMJ). Remarkably, placozoans, which possess epithelia but lack neurons, express a suite of neural adhesion genes. These observations suggest that SJs may represent an evolutionary precursor to synapses, and therefore that there may be overlap in the machinery that builds each junction. We are exploring this possibility in the *Drosophila* follicle epithelium, which forms SJs during later stages of development. Transcriptomic data reveal a coordinated increase in the expression of multiple genes normally associated with synaptic assembly—including but not limited to Liprins (α, β, γ), CASK, Ankyrin 2, and the signaling regulators Tao and Pdk1 — at stages 9–10A, preceding visible SJ formation. These same data implicate BMP signaling, through Wit and Tkv, in junctional morphogenesis. Consistent with this model, nuclear phosphorylated Mad (pMad) accumulates at the onset of SJ formation. Together, these findings support an ancestral link between epithelial and synaptic assembly programs integrating BMP signaling with scaffold organization.

181T Defining the functions of p53 nuclear bodies

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p53 is best known as a tumor suppressor in humans. Although its tumor suppressor activity was initially attributed to its regulation of cell death and cell cycle, it is now known that p53 contributes to a growing list of other cellular pathways. Yet, much remains unknown about the different functions of p53. To address this knowledge gap, we have been studying *Drosophila* p53. We have previously shown that different *Drosophila* p53 protein isoforms have overlapping and distinct functions in apoptosis, autophagy, meiotic DNA repair, and oocyte quality control. As part of these previous analyses, we showed that fluorescent fusions of different p53 isoforms localize to one prominent and several minor nuclear bodies. These observations are similar to those for p53 nuclear condensates in humans, the function of which are still being defined. Although p53 nuclear bodies have been previously reported in *Drosophila*, their structure and function remain largely unknown. Here, we report on our investigation into the dynamics and function of the major p53 body in *Drosophila*.

We have observed p53 nuclear bodies in all cells that we have examined, including male and female germline and in the nuclei of early embryonic cleavage cycles. The largest p53 body appears close to but not coincident with the DAPI-bright heterochromatic chromocenter, which suggests that the body has a distinct nuclear location. In ovarian nurse cells, p53 forms one body early in oogenesis and then evolves into closely-spaced clusters of multiple bodies later in oogenesis when nurse cell polytene fibers disperse. In proliferating cells, the major p53 body persists into mitosis during which it associates and segregates with mitotic chromosomes. Altogether, these observations suggest that the p53 nuclear body forms at a specific chromosome locus. Consistent with its nuclear location near the DAPI-bright chromocenter, double labeling indicates that the p53 body is contained within the heterochromatic HP1 nuclear body. Moreover, FISH mapping indicates that the p53 body associates with a centromere proximal chromosome locus. Other evidence suggests that p53 DNA binding is important for nucleation of the p53 body at this locus. We will report on our progress to define the relationship of these nuclear bodies to specific functions of p53 in *Drosophila* and its relevance to nuclear p53 bodies in humans.

182T Endosomal protein CG14767 limits tissue growth by inhibiting the Hippo pathway regulator Yorkie

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The Hippo/Yorkie (Yki) tumor suppressor pathway is an evolutionarily conserved system in metazoans that controls cell proliferation, differentiation, and cell death. The Hippo pathway is regulated by endosomal trafficking; however, the underlying mechanism is not well understood. In a screen for *Drosophila* Yki-interacting proteins, we identified a novel interactor, CG14767, that may be involved in endocytic regulation of Yki function. The mammalian orthologs of CG14767 belong to the lysosomal-associated protein transmembrane (LAPTM) family. They are localized in late endosomes and play multiple roles in mammalian cells, including a capacity to promote tumor growth. We have found that the CG14767 and Yki proteins bind each other via the L/PPxY motif/WW domain interaction. Genetic tests have revealed that unlike mouse LAPTMs, CG14767 limits tissue growth in *Drosophila*. CG14767 is involved in endolysosomal trafficking, and it colocalizes with Yki in cultured *Drosophila* S2 cells. Further colocalization and protein-protein binding assays have revealed that CG14767 also interacts with the previously characterized endosomal protein in the Yki network, Leash. Interestingly, CG14767 may limit Leash's association with Yki and can promote the turnover of both Yki and Leash in S2 cells by an unknown mechanism. We are investigating the relationships between CG14767, Yki, and Leash using a combination of genetics and cell biological approaches. This research will expand our knowledge of the mechanisms of Yki regulation and will provide further insights into the regulation of Hippo signaling by the endolysosomal system.

Keywords: *Drosophila*, Hippo pathway, LAPTM, endolysosomal trafficking, lysosome

183T The Rho GTPases pay an essential role in proper caudal visceral mesoderm (CVM) migration during embryogenesis

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Proper organ formation is dependent upon the migration of cells from one location to another during embryonic development. The intracellular mechanism of actin polymerization which allows cells to move is similar in different cell types and organisms. When a migratory cell receives a signal to move, actin fibers push on the cell membrane which extends out the leading edge (front) of the membrane while the trailing edge (back) retracts. This process of actin polymerization repeats until the cell reaches its destination. One class of proteins that regulates actin polymerization is the Rho family of GTPases. Rho GTPases are known for regulating essential components of cell migration, including cell morphology and actin polymerization. Rho GTPases are regulated by GEFs (Guanine Exchange Factors) and GAPs (GTPases-Activating Proteins), two classes of proteins that respectively activate and inactivate Rho GTPases. The gene *crossveinless-c (cv-c)* encodes a RhoGAP protein that regulates the GTPase Rho. Caudal visceral mesoderm (CVM) cells are one type of migratory cell that forms the longitudinal visceral muscles which surround the midgut of Drosophila. Currently, we have shown that loss of *cv-c* results in mis-migration of CVM cells and stalled CVM migration. We also show that expressing a constitutively active (CA) version of Rho1 displays an extremely stalled CVM migration. Conversely, expressing a dominant negative (DN) version of Rho1 shows a slightly stalled CVM migration. In addition to Rho, we are currently investigating the roles of Rac1 and Cdc42 on CVM migration. Preliminary results show that altering Rac1 activation results in a severe stalling of CVM migration whereas altering Cdc42 activity displays a mild stalling defect. Continuation of this research will serve to characterize the effects of Rho GTPase activity on actin polymerization and CVM migration.

184T A single amino acid substitution in a novel secreted protein specifies variation in a rapidly evolving male reproductive structure

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Understanding the molecular mechanisms that specify phenotypic diversity across species is one of the principal goals of evolutionary developmental biology. The male terminalia of the *Drosophila melanogaster* species complex— *D. melanogaster (mel), D. simulans (sim), D. sechellia (sec), and D. mauritiana (mau)*— provides a powerful opportunity to study these mechanisms. In particular, males of these species have evolved novel reproductive structures called the epandrial posterior lobes (ePLs), which are essential for successful copulation. Although a handful of genes that specify species divergence in reproductive structure morphology have been identified, we know little about how their divergence directs the molecular mechanisms by which these genes function to give rise to variation among species. We identified a previously uncharacterized gene, *Goldilocks (Glds)*, that negatively regulates ePL size. The protein encoded by *Glds* possesses a 17 amino acid (AA) long Signal Peptide (SP) at the N-terminus. We show via a functional cell secretion assay that the SP is both necessary and sufficient for secretion for all four species. Interestingly, we found that one unique AA substitution in the *mau* SP directs a significantly greater rate of Glds secretion compared to the other three species. Based on this result, we performed a CRISPR-Cas9 genome editing experiment to introduce the unique AA substitution for the *mau* SP into the *mel* allele of *Glds*. We found a significant decrease in the ePL size in the edited genotype that supports the previous result of Glds is a negative regulator of growth. To identify potential interactors of Glds, we performed an AlphaFold *in-silico* protein-protein interaction screen and identified strong candidates that are known to be expressed in the developing genitalia. One of these genes encodes a candidate receptor for Glds called FMRFaR. Deletion of the FMRFaR gene gives rise to a significant decrease in ePL size, which suggests a negative interaction between the ligand and the receptor. AlphaFold predictions also suggest that Glds binds the extracellular ligand binding site of the receptor, blocking FMRF-amides from binding as a potential mechanism for growth inhibition.

185T Investigating mechanisms of v-ATPase-mediated autolysosomal acidification

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Macroautophagy (henceforth autophagy) is a process by which cellular components are sequestered into a double-membraned autophagosome and degraded upon fusion of the autophagosome with a lysosome. Efficient degradation requires a low pH to activate the multiple types of acid hydrolases present in the lysosomal lumen. Autophagy is regulated via mechanistic Target of Rapamycin (mTOR), a nutrient-sensing kinase. In the presence of ample nutrients, mTOR is active and inhibits autophagy, whereas under nutrient poor conditions, mTOR is inactive, allowing for increased autophagy. mTOR inhibits autophagy in part by phosphorylation of Autophagy-related (Atg) proteins, which are necessary for autophagosome formation, with some having roles outside this canonical function. The early steps of autophagy have been well studied, but the mechanisms of subsequent events such as autophagosome-lysosome fusion and acidification are still obscure.

The lysosome is predominantly acidified via the Vacuolar H^+-ATPase (v-ATPase). In the literature, v-ATPase inhibitors have been shown to block fusion as well as acidification, suggesting that fusion is dependent on acidification. However, the Neufeld lab has shown that genetic inhibition of v-ATPase in the *Drosophila* larval fat body does not block autophagosome-lysosome fusion. In addition, we find that knock down of Atg proteins or activation of mTOR disrupts lysosomal acidification, suggesting that acidification of the lysosome may be dependent on its fusion with a functional autophagosome. Consistent with this hypothesis, I have found that genetic depletion of key fusion proteins, such as Syntaxin 17 and Snap29, results in a lack of acidification as well as a lack of fusion. Atg proteins have been shown to interact with v-ATPase subunits, and v-ATPase assembly at the lysosomal membrane has been shown to be influenced by mTOR in some cell types. To address the mechanisms by which autophagosomes and their fusion influence lysosomal acidification, I am investigating the dependence of v-ATPase trafficking and assembly on Atg proteins and other autophagy factors such as mTOR, using microscopy techniques on *Drosophila* larval fat bodies.

186T Dominant-Negative DRP1 variants reduce Tau Toxicity through rebalanced organelle dynamics and redox function

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Mitochondrial dynamics and organelle communication are essential for neuronal stability, and disruptions in these processes contribute to tauopathies, including Alzheimer's disease. DRP1 (DNM1L), a key regulator of mitochondrial and peroxisomal fission, plays a central role in coordinating ER, mitochondria, peroxisome crosstalk; however, its influence on tau pathology remains poorly understood. Previous human genetic studies have shown that heterozygous missense variants in DNM1L impair mitochondrial and peroxisomal fission, and functional assays in *Drosophila* have recapitulated these organelle-morphology defects. Further, studies have shown that Tau accumulation alters DRP1 activity leading to defective mitochondrial dynamics.

Here, we investigate how human Tau overexpression perturbs organelle homeostasis, revealing pronounced defects in the trafficking of peroxisomes and mitochondria, as well as disruptions in ER organization within neuronal cell bodies and axons. To determine whether these cellular disturbances are relevant in a disease context, we extended our analysis to a Drosophila tauopathy model. By expressing dominant-negative human DNM1L (DRP1) variants (G350R, R403C, L700del) in flies overexpressing human Tau, we observed a remarkable reduction in both total Tau and phospho-Tau levels. This suppression was accompanied by improved redox balance, partial restoration of organelle function, and enhanced neuronal integrity. Together, these findings reveal that dominant negative human DRP1 variants can mitigate Tau-induced cellular stress and neurotoxicity in vivo, highlighting a previously underappreciated role for mitochondrial fission machinery in modulating tauopathy progression. To assess whether these protective effects translate into human systems, we examined fibroblasts derived from patients carrying DNM1L mutations (R403C, L700del). Consistent with our fly findings, patient-derived cells exhibited reduced tau and phospho-tau expression, indicating a conserved protective role for these variants across species.

Together, our data demonstrates that dominant-negative human DNM1L variants attenuate tau pathology by restoring redox balance and partially improving mitochondrial and peroxisomal function. These results identify DRP1 as a conserved suppressor of Tau-induced neurotoxicity and underscore its potential as a therapeutic target in tau-mediated neurodegeneration.

187T Apocrine secretion in the prepupal salivary glands of *Drosophila* utilizes a novel non-canonical and non-vesicular transport and secretory machinery

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We recently described a novel noncanonical and nonvesicular cellular signaling pathway, apocrine secretion (AS), and re-defined it with respect to how it accomplishesthe *en mass* transport and externalization of proteins and cytoplasmic material. It uses a strikingly different mechanism to traffick and secrete material than exocytosis. Our recent studies in the salivary glands (SGs) of *Drosophila melanogaster* have started to reveal its molecular and genetic foundations. Within the SGs, a massive AS occurs during the late prepupal stage, well after the exocytotic secretion of the Sgs-glue. This AS is essential for providing a fully immunocompetent exuvial fluid that surrounds the metamorphosing pupae throughout the pupation process and pupal stage. An examination of more than 30 *Drosophila* species along with two more distantly related fly species for the occurrence of AS in the prepupal SGs indicated that, unlike the production and release of Sgs-glue, AS is a function that is conserved evolutionarily across all fly SGs. Comprehensive proteomic analyses uncovered over 1500 proteins present in the AS. Notably, these proteins originate from microsomes, membranes, the cytoskeleton, mitochondria, the ER- and Golgi-, as well as nuclei and nucleoli. A comparison between the *Drosophila* SG proteome and human AS proteomes demonstrated that their ontological categories are evolutionarily closely related, despite these proteins having diverse biological and molecular roles and different sub-cellular origins. During this research, we discovered two previously unidentified organelles involved in AS: collector sacculae and corridors. Both are formed from apical microvilli that significantly invaginate into SG cells during the AS process. A genomic screen in *Drosophila* to systematically search for AS effectors revealed more than 20 loci that when manipulated either prevent, up-regulate or precociously initiate AS, or alter the transport of a cargo. Many of these loci are previously uncharacterized *CG*-genes with novel functions. We found that the main transport and secretory structures used during the process of AS are novel previously overlooked organelles, and which require the cooperation of several apical factors.

188T Phenotypic severity and subcellular localization depend on Bruno1 isoform expression levels in indirect flight muscle

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RNA binding proteins play critical roles during muscle development in regulating isoform-specific expression profiles of structural proteins that determine fiber-type specific morphology and function. Notably, the level of expression of regulatory proteins is tightly regulated between muscle fiber-types and across myogenesis. The CELF family of RNA binding proteins are key regulators of alternative splicing and muscle development in vertebrates that are misregulated in patients with Myotonic Dystrophy Type 1 (DM1), where CELF activity and nuclear localization are increased. The relationships between CELF expression level, activity, and nuclear localization, as well as how this effects the balance of CELF nuclear and cytoplasmic function is only partially understood. We have shown that the conserved CELF protein Bruno1 (Bru1) regulates alternative splicing and promotes a transition to mature splice isoforms in *Drosophila* indirect flight muscles (IFMs), establishing the fly as a model to understand CELF function in myogenesis. Here we show that the IFMs are sensitive to the expression level, or dosage, of specific Bru1 isoforms. We show that endogenously-tagged Bru1^{eGFP} localizes in the cytoplasm as well as the nuclei at early stages of IFM development from 24 h to 48 h after puparium formation (APF), and is progressively enriched in the nucleus during later pupal and adult stages. Using a set of isoform-specific GFP-tagged transgenes, we investigate isoform-specific localization and find that Bru1 isoforms are actively exported from nuclei in early development. This effect is independent of the N-terminal disordered region of Bru1, as Bru1-isoD as well as a construct with an N-terminal deletion show similar behavior as longer Bru1 isoforms. By using UASp and UAS-T promoters which drive lower and higher levels of expression in somatic muscle, we show that high Bru1 expression promotes irregular trafficking and localization and disrupts the balance in nuclear versus cytoplasmic pools of Bru1. We further investigate the effect of isoform-specific overexpression of untagged Bru1 isoforms on endogenously tagged Bru1^{eGFP} across IFM development. Our data reveal that Bru1 expression and localization are dynamically regulated across IFM development, enabling the muscle to finely tune the level of Bru1-mediated splicing activity throughout myogenesis. Forced overexpression of select Bru1 isoforms at high levels disrupts this finely tuned balance, altering levels of nuclear Bru1 and leading to changes in gene expression that alter muscle structure and impair flight ability.

189T Generation and maintenance of apical rib-like actin fibers in epithelial cells of the *Drosophila* eye

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Heterogeneity and complexity of cytoskeletal structures, and how these are regulated, is poorly understood. We use cells of the *Drosophila* pupal eye as models to explore diversity in the actin cytoskeleton. We found that different F-actin structures emerge in primary (1°), secondary (2°), and tertiary (3°) pigment cells as they mature. 1°s become characterized by dense accumulations of F-actin that we term Apical Ribs of Actin Fibers (ARAFs). The formins Diaphanous and Dishevelled Associated Activator of Morphogenesis are essential for generation of ARAFs, which are connected into a network by a-Actinin, the villin Quail, and Spectrins, and linked to the apical membrane by Quail and Spectrins. ARAFs are stress-fiber-like and connect to adherens junctions. Impairing ARAFs indicated that this network maintains cortical tension and is crucial for 1°s to achieve their characteristic shapes. Our evaluation of the three-dimensional shape of 1°s revealed that ARAFs are essential for the shape of the curved apical membrane. Hence, a toolkit of conserved actin regulatory proteins builds and maintains a network of apical stress fibers that govern the 3D morphology of 1° cells.

190T Genetic dissection of motor proteins mediating dense core vesicle axonal trafficking

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Neuropeptides modulate diverse developmental and behavioral processes, but the mechanisms controlling their trafficking and release are not well understood. A key step in this process is the bidirectional transport of neuropeptide-containing dense core vesicles (DCVs) along axons. Although many studies of DCV transport in *Drosophila* have focused on motor neurons, here we use the pigment dispersing factor (PDF)-releasing peptidergic ventral lateral neurons (LNvs) as a model system to study axonal DCV transport.

Through transcriptomic analysis, live imaging, and behavioral screens, we confirmed that Unc-104 and Dhc64c, two canonical motor proteins, are required for anterograde and retrograde transport of PDF, respectively, consistent with findings in other peptidergic neurons, Notably, we also identified additional motors that may contribute to DCV transport in LNvs, including two kinesins Klp68D and Khc-73, and most surprisingly, the axonemal dynein subunits CG9313 and Dhc93AB. While axonemal dyneins are generally not implicated in cytoplasmic transport, we hypothesize that these subunits could nevertheless regulate DCV transport and dynamics in LNvs through their interactions with cytoskeleton components. Additionally, live imaging in third instar larval LNvs suggests that axonal DCV transport is under circadian regulation, potentially contributing to the daily oscillation of PDF levels at release sites. Together, these findings provide new insights into the mechanisms that governs DCV transport in central peptidergic neurons.

191T Age-dependent requirement of EMC4 function in the fat body for female fertility and fecundity in *Drosophila melanogaster*

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The endoplasmic reticulum (ER) membrane protein complex (EMC) plays a vital role in the proper folding, insertion, and trafficking of membrane proteins, yet little is known about its cell type-specific function. An important system that relies on signaling between different cell types is the regulation of fertility and reproduction. In *Drosophila melanogaster*, the fat body is a central metabolic and endocrine tissue that communicates nutritional and physiological status to the ovaries, making it a key regulator of female reproductive output. We hypothesized that EMC function in the fat body is required to maintain fertility and fecundity, particularly with age. To test this, we used the GAL4–UAS system to selectively knock down the EMC subunit EMC4 in the fat body. Fat body–specific EMC4 knockdown led to a reduction in fertility and fecundity in aged females and relatively minor effects in younger females. These results suggest an age-dependent requirement for EMC4 in the fat body to support normal reproductive capacity. To investigate whether reduced reproductive output is associated with changes in ovarian development, we assessed ovary size and the expression of functional markers of oogenesis. Ultimately, we aim to determine how disruptions in ER protein biogenesis within the fat body alter ovarian structure and function. A key unresolved question is which EMC-dependent membrane proteins fail to be properly processed in the fat body following EMC4 knockdown and how this misregulation contributes to impaired fertility and fecundity. Together, these findings highlight a critical role for EMC4 in the fat body in sustaining female fertility during aging and emphasize the importance of inter-organ communication between metabolic tissues and the reproductive system.

192F Characterizing the Role of Perlecan on Drosophila Border Cell Migration

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Collective cell migration is an integral process in wound healing, development, and some types of cancer. This highly regulated process - in which a group of cells receive and interpret stimuli from their environment to guide migration, control cell adhesion, and invade tissue - is observed in various contexts, but is not fully understood. The consensus regarding the role of the extracellular matrix (ECM) on collective cell migration has focused on cell adhesion and structural/ mechanical features of migrating cells' substrate. However, the ECM has also been shown to modulate growth factor (GF) distribution and impact GF receptor signaling, and little is known about how this impacts collective cell migration . The secreted heparan sulfate proteoglycan, Perlecan (encoded by trol in flies), is one component of the ECM that is capable of all these functions, but its role in collective cell migration is unknown. Border cell (BC) migration in Drosophila oogenesis offers a means to investigate the conserved signaling pathways governing collective cell migration and determine if Perlecan has a role in this process. To begin, we confirmed expression of Perlecan in the follicle cells (FC), BC cluster and the migratory domain of the BCs – between germline cells. Knockdown of Perlecan in all follicle cells produced delayed migration of the BC cluster, reduced cluster cohesion , and changed cluster polarity. These migratory defects were not present with reduced Perlecan expression in medial FC populations, suggesting that Perlecan impacts pertinent signaling cascades for BC migration and/or specification . Utilizing live-imaging, we elucidated cluster dynamics and differentiation when Perlecan levels were reduced in the BC cluster. We also assessed if Perlecan genetically interacts with a key BC migration regulator and guiding chemoattractant, Platelet Derived Growth Factor- and Vascular Endothelial Growth Factor- Related Factor 1 (PVF1). Changes in PVF1 expression alone result in migratory defects that are exacerbated by changes in Perlecan , suggesting the ECM may modulate signaling. We are currently investigating possible physical interactors of Perlecan to characterize the signaling components it impacts. This project elucidates the role Perlecan plays in collective cell migration, providing a better understanding of the mechanisms guiding grouped cell migration. This research is funded by NSF grant IOS-2303587.

193F Rapgap1 negatively regulates Drosophila embryonic epithelial wound healing

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Collective cell migration is essential for embryonic development, wound healing, and cancer invasion. In epithelial wound healing, cells migrate as a cohesive sheet, maintaining cell-cell adhesion while dynamically remodeling their cytoskeleton to close the wound. Ras-associated protein-1 (Rap1), a small GTPase of the Ras family, serves as a signaling hub that integrates upstream mechanical and biochemical cues to regulate cytoskeletal dynamics and intercellular adhesions. In Drosophila embryos, Rap1 is necessary and sufficient for rapid epithelial wound closure, promoting actomyosin polarization via Ephexin-Rho1 and E-cadherin accumulation at tricellular junctions via Canoe. However, the spatiotemporal regulation of Rap1 activity during epithelial sheet migration is unknown. Rap1 is negatively regulated by its GTPase-activating protein (GAP), Rapgap1, which stimulates GTP hydrolysis. We hypothesize that Rapgap1 spatially and temporally inhibits Rap1 activity to maintain balanced migration. Using spinning disk confocal microscopy to ablate and record wound closure in stage 14 Drosophila embryos, we found that Rapgap1 knockdown accelerates wound closure, elongates wound morphology, maintains F-actin at the wound edge, and increases internal protrusions. These phenotypes suggest that Rapgap1 restrains Rap1-driven cytoskeletal activity to ensure balanced migration. Understanding the spatiotemporal regulation of Rap1 signaling is critical for identifying drivers of pathological migration and for developing targeted therapies to improve wound healing outcomes.

194F Generalizable non-canonical secretion of a novel amphipathic α-helical peptide

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An understudied class of peptides, those translated from short open reading frames (sORFs), are becoming increasingly appreciated for their roles in development and disease. Dafcin is an evolutionarily conserved amphipathic alpha-helical peptide that is translated from one such 63 nucleotide sORF. This sORF is found in a *Drosophila Amplicon in Follicle Cells* (*DAFC*) locus, a genomic region of high gene copy amplification during oogenesis. Dafcin-GFP fusion protein, expressed from the endogenous *dafcin* gene, localizes to the Golgi in ovarian follicle cells and is trafficked into the egg cell via endocytosis, localizing inside yolk granules. The yolk granules in *dafcin* mutants are larger than wildtype, suggesting Dafcin's potential role in limiting vesicle size. Pure Dafcin peptide inhibits the size of liposomes formed *in vitro* in the peptide's presence. These data suggest Dafcin regulates yolk granules via lipid interactions that induce curvature. However, Dafcin's mechanism of *in vivo* secretion and uptake is unclear, since the peptide lacks a canonical signal sequence for ER-Golgi trafficking and secretion. To investigate the mechanism, I designed a transgenic line with the Dafcin-GFP fusion protein under control of a UAS promoter. When this transgene was expressed in ovarian follicle cells, Dafcin-GFP was taken up into the oocyte, but not at levels scaling with its expression in the follicle cells. Preliminary experiments suggest this is due to limitations in the rate of secretion of Dafcin-GFP, rather than in the rate of uptake. I am currently using the transgene to express Dafcin-GFP in specific regions of larval imaginal wing discs to see if the protein is secreted and taken up by neighboring wing disc cells. The detection of soluble secreted Dafcin-GFP is aided by an anti-GFP nanobody localized to the surface of nearby cells. These results will help inform how the secretion and uptake of this non-canonical small peptide is controlled.

195F Optogenetic Control and Characterization of Rab11 Trafficking during Tissue Morphogenesis

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During Drosophila gastrulation, actomyosin-dependent apical constriction drives invagination of mesoderm primordium by forming a furrow at the ventral side of the embryo. Recent work showed that apical actomyosin activity promotes dynein mediated transport of vesicle-like Rab11-positive compartments ("Rab11 vesicles") to the apical side. The resulting accumulation of Rab11 vesicles reinforces adherens junctions (AJs) and stabilizes the supracellular actomyosin network during invagination.

To further elucidate how Rab11 vesicle trafficking is regulated and functions during morphogenesis, we examined two Rab11 effector proteins: Nuf and Rip11. We found that both proteins co-localize with Rab11 vesicles at the apical side during ventral furrow formation, although their general localization patterns are not entirely identical. Consistent with previous findings, depletion of Nuf caused early developmental arrest during cellularization, precluding the analysis of its function in gastrulation. In contrast, Rip11 knockdown embryos completed cellularization normally but exhibited slower and less coordinated ventral furrow invagination. These findings suggest that, unlike Nuf and Rab11, Rip11's function is more critical during gastrulation than at earlier stages.

To directly perturb Rab11 trafficking, we employed an optogenetic approach to acutely recruit dynein to Rab11 vesicles. Upon stimulation, dynein recruitment resulted in rapid clustering of Rab11 structures at both perinuclear and basal locations within minutes. In addition, Rab8, which functions downstream of Rab11, co-clustered with Rab11, consistent with a general impact on Rab11-mediated trafficking. Interestingly, optogenetic clustering of Rab11 during mid-to-late cellularization resulted in slower and shallower ventral furrow invagination. In contrast, stimulation at or shortly after the onset of gastrulation had little effect. These results suggest that proper distribution and trafficking of Rab11 compartments at mid-late cellularization plays a critical role in subsequent ventral furrow invagination.

In summary, our results demonstrate that regulated Rab11 trafficking plays a stage-specific role during epithelial morphogenesis and reveal a critical function for Rip11 in this process. These findings motivate further investigation into how dynamic vesicle trafficking supports the complex cell behaviours that drive gastrulation.

196F Nuclear pore complex abundance regulates the specificity and kinetics of nuclear-cytoplasmic transport in early *Drosophila* embryos

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Communication between the cytoplasm and the nucleus is mediated by the nuclear pore complex (NPC), a large macromolecular assembly that governs selective molecular transport across the nuclear envelope. Once considered to be relatively static and invariant, recent studies suggest that NPCs are dynamic structures that vary in both number and protein composition during development. However, the mechanisms by which NPC abundance regulates nuclear transport remains elusive. We have recently shown that NPC abundance progressively declines during early *Drosophila* embryogenesis. This provides a powerful model to examine how developmental modulation of NPC number influences nucleocytoplasmic transport and downstream processes, including cell cycle control. Using STED super-resolution microscopy, we quantify the number of NPCs across early embryonic cell cycles with single-pore precision. To quantitatively characterize the relationship between NPC number and nuclear transport, we generate synthetic fluorescent cargos of defined molecular sizes carrying nuclear localization (NLS) or export (NES) signals. Quantitative live imaging of these synthetic cargo proteins reveal that changes in NPC abundance have selective effects on specific cargo rather than affecting all cargos uniformly. Mechanistically, we show that NPC number differentially regulates active transport and passive diffusion across the nuclear envelope, underscoring this cargo specificity. Finally, we show that partial depletion of NPC in the early embryos leads to an overall delay of cell cycle progression, suggesting that NPC number sets the pace of early embryonic development. Together, these findings establish NPC as an active regulator of the kinetics and specificity of nuclear transport, demonstrating that modulation of NPC number selectively shapes transport dynamics and governs embryonic progression.

197F The LINC complex and microtubule network are altered in larval muscles prior to tumor induced wasting

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Cachexia is a wasting disease that affects approximately 80% of cancer patients by causing muscle and fat loss, and unfortunately there is no effective treatment for it. The *Drosophila melanogaster* larval and adult tumor models have become an emerging platform for studying cellular changes that occur in cachexia. Previously, we observed altered nuclear positioning and disorganization of sarcomeric proteins prior to the onset of muscle wasting in a larval model of tumor induced cachexia. It is known that nuclear positioning in muscles, organization, and trafficking of proteins to the sarcomere is heavily dependent on the microtubule (MT) network. Furthermore, it is known that members of the LINC complex help tether nuclei to the MT network. Therefore, we hypothesized that the MT network and LINC complex may be altered in pre-cachexic muscle.

When comparing the muscles of wild-type to tumor-bearing larvae, I observed decreased MT coverage of the fibers during pre-cachexic stages. However, in fully cachexic larvae, I observed robust MTs wrapping around the nuclei instead of interlocking with its surrounding networks. Surprisingly, these results reveal that the MT network responds differently in pre-cachexic and cachexic muscles, meriting further exploration into the regulation of the MT network in both pre-cachexic and cachexic muscles. The reduction of MTs in early stages may be due to either altered nucleation, polymerization or catastrophe. These results suggest a mechanism for the observed nuclear positioning and protein trafficking defects, namely that they originate from the MT network collapse.

To assess if the nuclear positioning defects may also be due to altered tethering to the MT network, we examined expression of the LINC complex members. We selected Klarsicht (Klar) due to its involvement in nuclear positioning in striated muscle, where reduction of Klar results in defects. Surprisingly, we observed that Klar is strongly upregulated in pre-cachexic and cachexic muscles. These results suggest that components of the LINC complex have altered expression in pre-cachexic and cachexic muscles, and that excess Klar may also affect nuclear positioning, which we are currently testing. Taken with the alteration to the MT network, these results suggest that alterations to the MT network and its connections to organelles may be early tumor-induced changes in muscles that significantly precede wasting.

198F Signaling and mechanics influence cell identities during *Drosophila* pupal wing vein patterning

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Morphogenesis relies on cell signaling and mechanical forces for precise tissue patterning. How these two inputs integrate is a key question in developmental biology. *Drosophila* wing vein refinement is primarily mediated by the Notch pathway, which relies on cell-cell interactions and tension in the signal-sending cell to activate signaling. During this process, broad primordial vein (provein) regions, defined by EGFR activity, are progressively narrowed between 16 and 32 hours after puparium formation (h APF). Provein cells, expressing Delta ligand, activate Notch receptors in the adjacent intervein cells, which represses EGFR signaling and vein fate.[1] Though signaling defines vein and non-vein cell identities, how the structural and mechanical changes each wing cell type experiences contribute to the morphogenetic program remains poorly characterized.[2,3]

Using quantitative confocal imaging, I have found that wing cells experience cell identity-specific shifts in apicobasal shape, actomyosin localization, and adherens junction localization, which emerge as Notch signaling drives vein pattern refinement. Notably, these structural and mechanical changes display intriguing spatiotemporal correlations to Notch signaling activation levels. To probe the functional relationships between these structural and mechanical changes and signaling activation states, I am investigating how Notch signaling pathway perturbations impact wing cell structural and mechanical features. I am also utilizing genetic manipulation to examine how perturbed actomyosin dynamics and junctional connectivity impact Notch signaling, structural morphology, and vein/non-vein cell fates. My preliminary results support a model where cellular mechanics spatiotemporally integrate with signaling to govern vein versus non-vein cell identities during wing vein patterning.

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199F Border cell secreted basement membrane is required for on-time migration

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Cell migration is a key physiological process in embryonic development, wound healing, and immune cell invasion, and is co-opted during cancer metastasis. One factor that influences cell migration is the basement membrane (BM), a type of extracellular matrix. The BM is network of proteins and carbohydrates that serves as the substrate for migration, and its organization and mechanical properties impact the speed and direction of migration. One model used to study collective cell migration *in vivo* is Drosophila border cell migration. Drosophila border cell migration occurs during Stage 9 of oogenesis, when a group of follicle cells, termed border cells, delaminate and migrate between the nurse cells to reach the posteriorly located oocyte. Border cell migration shares numerous conserved properties with mammalian cell migration. However, the presence of a BM facilitating border cell migration has not been previously identified. To investigate the presence of this network, we used fluorescently-tagged BM components Collagen IV, Laminin A, Laminin B, Nidogen, and Perlecan. We find Collagen IV, Laminin A, Laminin B, and Perlecan surround the border cell cluster during migration. Having established the presence of the BM, we next sought to determine whether it facilitates migration and if the border cells produce it. Border cell knockdown of Collagen IV or Laminin A results in delayed border cell migration, while knockdown of Laminin B or Perlecan does not impact migration. As a control, we also knocked down Nidogen, which is not in the border cell BM, and find, as expected, that border cell migration is on-time. These results reveal that the border cells produce their own BM and suggest that reducing Collagen IV or Laminin A results in a defective BM that cannot serve as an adequate substrate for border cell migration, while knockdown of Laminin B or Perlecan creates an altered but functional BM able to support normal migration. Together, these findings reveal another conserved element between Drosophila border cell migration and mammalian collective cell migration – a BM – and further supports using this model for studying the mechanisms regulating cell migration.

200F Relating gene expression dynamics to actin remodeling during *Drosophila* cellularization

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Quantitative changes in the kinetics of gene expression, rather than complete loss or gain of function, can result in morphogenesis failures and congenital defects. These subtle yet consequential changes are frequently associated with transcriptions factors (TFs) that fine-tune the outputs of developmental networks. But it remains unclear how quantitative changes in the expression of cytoplasmic machinery might contribute to morphological phenotypes. The goal of this project is to determine how subtle changes in the transcriptional kinetics of genes encoding actin regulators will impact the kinetics of actin remodeling and, ultimately, of morphogenesis. In *Drosophila* embryos, actin remodeling is critical during cellularization, the embryo's first tissue-building event. Cellularization happens uniformly and synchronously in the entire embryo and its success requires expression of five zygotic genes, *serendipity-alpha*, *slam*, *nullo*, *bottleneck* and *dunk*, which all act as actin regulators. Previous whole embryo fractionation showed that these genes are expressed in a pulse, showing highest levels at early cellularization, and being sharply turned off by late cellularization. Here we used RNA FISH to establish a spatio-temporal transcriptional profile of the cellularization genes from nuclear cycle 10 through the end of nuclear cycle 14. Our preliminary data suggests that each of these genes reaches a maximal transcriptional behavior for several nuclear cycles prior to cellularization. In these peak cell cycles, the genes are expressed uniformly throughout the anterior, trunk and posterior of the embryo, with 50-100% of nuclei showing transcriptional foci. Similar transcriptional behaviors have previously been described for cell fate determinants, and rely on pioneer TF activity, RNAPolII pausing and clustered TF binding: signatures also detected in the regulatory elements of all the cellularization genes. Experiments are ongoing to determine the consequences of perturbing the uniformity, amplitude and timing of transcription of the cellularization genes; and also to identify mechanisms shutting these genes off by late cellularization.

201F Assessing changes in non-centrosomal MTOC number and position as a mechanism for microtubule alterations in cachexic muscle

Cody Griffin[1], Mardelle Atkins[2] [1]Sam Houston State University, [2]Biological Sciences, Sam Houston State University

Cancer cachexia is a severe muscle-wasting syndrome that affects up to 80% of patients with advanced cancer. It is characterized by severe weight loss, skeletal muscle atrophy, fatigue, and a significant decline in quality of life. This syndrome is driven by tumor-released factors that disrupt normal metabolism and cell function; however, the exact mechanisms remain poorly understood. *Drosophila melanogaster* has, over recent years, emerged as a popular model for studying cancer, cachexia, and tumor-host interaction due to its short lifecycle, high reproductive rate, and extensive genetic tools. In our *Drosophila* tumor-induced muscle-wasting model, we have identified early disruptions and loss in the microtubule network. These non-centrosomal microtubules (MTs) are generally anchored to organelle membranes at structures called ncMTOCS and, in turn, are thought to be important for organelle positioning. Concomitantly, we observe mislocalization of nuclei. We hypothesize that mispositioning and MT loss may result from disruption of ncMTOC formation at the nuclear envelope or at other known sites in the endoplasmic reticulum (ER) or Golgi bodies (GB). To address this, our project aims to visualize the ncMTOC proteins, ER, and GB in *Drosophila* larval muscle under both control and tumor-bearing conditions and quantify changes in ncMTOC position with MT formation, or lack thereof. By examining ncMTOC sites and MT polymerization at different stages of tumor development, and correlating this with changes in the nuclear envelope, ER, and GB positioning or morphology, we will increase our understanding of how organelle reorganization may influence ncMTOCs and overall muscle structure during cancer cachexia.

202F Investigating light-dependent redox regulation of protein kinase C as a phototransduction tuning dial in insects.

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Drosophila phototransduction is one of the fastest G-protein-coupled-receptor phosphoinositide cascades. This speed and adaptability are achieved by a precise balance between initiation and termination at each step of the cascade. While the events that terminate the cascade at the GPCR are well characterized, the downstream events that terminate the later steps are not fully understood. The protein kinase C (PKC) INAC is required for the rapid termination, and is essential for light adaptation. INAC is activated by calcium and diacylglycerol, which are generated by this GPCR cascade, suggesting how INAC activity could terminate the activated cascade. However, this canonical activation of INAC does not allow to adaption to increasing light intensities as DAG and calcium are quickly removed from the rhabdomere to prevent excitotoxicity. We propose that there is an additional mechanism to enhance termination of phototransduction involving INAC under high light intensities that utilizes redox signaling triggered by the blue-light sensing flavoprotein Cryptochrome (CRY). While CRY is best recognized for its role in circadian entrainment, CRY also localizes to the rhabdomere which contains the phototransduction machinery. We have identified oxidation of INAC at three cysteines in the zinc finger motifs of the DAG binding domain after prolonged blue light exposure and observed a decrease in light sensitivity. Additionally, we have purified recombinant INAC and performed kinase assays in both oxidizing and nonoxidizing conditions. Finally, we have generated a mutant fly line that contains C>S mutation in INAC at the three oxidized cysteines and have performed *in vivo* activity assays.

203F Exploration of lipid profiles in mitochondria of *Drosophila melanogaster* spermatogenesis

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Mitochondrial membranes have distinct lipid profiles due to curvature of the inner membrane which is in part caused by cardiolipin, a specialized four-tailed phospholipid. Fission and fusion processes require modulation of the lipids present in the membranes to accommodate metabolic and physical changes which can include alterations of existing lipids or synthesis of new ones through complex regulatory pathways. Drosophila spermatogenesis offers a particularly useful model to examine such pathways due to the dramatic morphological changes the nebenkern, a specialized cellular structure composed of a major and minor mitochondrial derivative, undergoes during the process. This development occurs in discrete stages, which we have visualized through phase contrast and fluorescence microscopy and used to explore how lipids are synthesized or altered. To understand how lipid profiles are developed between pre-meiotic spermatocytes and post-meiotic spermatids, we have used genetic constructs to halt meiosis and assess them via LC-MS and cardiolipin specific staining with 10-nonyl-acridine-orange. This fluorescent staining has shown promise in visualization of the distribution of cardiolipin in cellular structures. To further explore the presence of cardiolipin in spermatogenesis, we have knocked down enzymes that are a part of the biosynthesis and remodeling pathway and found significant morphological defects in spermatids. Additionally, in assessing post-meiotic spermatids, we have manipulated proteins involved in oxidative phosphorylation to understand how membrane lipids respond to mitochondrial dysfunction. Others have shown that lipids are active players in facilitating mitochondrial membrane structure and that embedded proteins, such as those part of the OXPHOS complex, rely on specific membrane lipid makeup to maintain functionality. We are exploring whether this is consistent in post-meiotic spermatids through LC-MS. We have observed morphological differences in spermatids with altered OXPHOS complexes as well as implemented 10-NAO fluorescent staining to visualize cardiolipin. These data give us insight into mitochondrial lipid dynamics across Drosophila spermatogenetic processes and will lead to a greater understanding of mitochondrial dynamics.

204F Uncovering functional divergence of human co-chaperone UNC45 paralogues by expression in *Drosophila* muscle

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Muscle myofibril assembly and contraction require chaperone proteins that fold clients into their functional conformations. Uncoordinated-45 (UNC45) is a conserved chaperone involved in the folding of myosin, a key muscle component. Within vertebrates there are two UNC45 paralogues: UNC45A, which is found in a variety of tissue types, and UNC45B, which is restricted to striated muscle. In invertebrates, however, only one isoform exists. Using *Drosophila*, we investigated the functional differences among UNC45 paralogues by determining their effects on muscle structure and function. While UAS-GAL4 induced over-expression of *Drosophila* UNC45 in indirect flight muscles had no negative effects, expression of either human paralogue during early muscle development resulted in muscle structural defects and dramatically decreased flight ability; however, expression later in development or during aging had negligible consequences. RNAi knockdown of *Drosophila* UNC-45 in indirect flight muscles disabled flight. When combined with *Drosophila* UNC-45 over-expression, flight ability was rescued; conversely, neither human paralogue was capable of rescue. Interestingly, the human paralogues differed in their localization within flight muscle sarcomeres, alluding to functional differences. To gain mechanistic insight, chimeric fusion proteins were generated to explore the function of each sub-domain of these proteins. We found that the myosin-binding UCS domain of the UNC45A paralogue impaired muscle function as severely as full length UNC45A. This was not the case for the UNC45B paralogue. Our work indicates that the human UNC45 paralogues are functionally unique and have evolutionarily diverged from the invertebrate paralogue.

205F Investigating the role of collagen IV in collective cell migration during embryonic epidermal wound healing

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Collective cell migration is a fundamental biological process that underlies tissue morphogenesis, repair, and metastasis. Understanding how cells coordinate movement within a tissue is essential to explaining the mechanisms of these processes. We use wound healing in Drosophila embryonic epidermis as a model system to study collective cell migration. The extracellular matrix (ECM) plays a central role in wound healing by providing both structural support and biochemical cues that guide migrating cells. However, the remodeling of key ECM components during wound repair remains incompletely defined. To address this knowledge gap, we are investigating basement membrane (BM) remodeling during embryonic wound repair. Embryonic wounds heal rapidly and without scarring. To close wounds, cells rely on integrin-based adhesion for forward movement and to properly reinforce the F-actin cytoskeleton and cell-cell adhesions. Wounds also show an accumulation of the key BM protein collagen IV (viking) at the wound site. We aim to understand the role of collagen IV remodeling in the wound closure process. Using the Gal4-UAS system, we disrupted collagen IV expression through RNAi. Our initial experiments expressing ubiquitous collagen IV RNAi resulted in early embryonic lethality, highlighting the protein's developmental importance. When collagen IV disruption was restricted to the epidermis using the 69B-Gal4 driver, embryos survived long enough for wound healing assays. We made wounds using a high-power laser and visualized wound repair dynamics using spinning disk confocal microscopy. Under collagen IV knockdown conditions, wound closure was impaired compared to controls. This suggests that collagen IV expression specifically in the epidermis is essential for rapid embryonic wound healing. Future work will examine the effects of collagen IV epidermal knockdown on BM accumulation and remodeling at the wound site.

206F Characterizing the roles of Cdk8 in epithelial tissue closure

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My research focuses on investigating the developmental roles of cyclin-dependent kinase 8 (Cdk8). Cdk8 is a protein kinase which acts as part of the Mediator complex regulating RNA-polymerase II mediated transcription. Although its role in the transcription has been well established in different model organisms, the Verheyen lab has found unrelated novel roles in regulating Drosophila mitochondrial dynamics and thorax formation.

Tissue closure is a phenomenon that is widespread across the animal kingdom. It generally involves the fusion of two separate epithelial tissues, which is analogous to wound healing. Some developmental processes that involve epithelial tissue joining include: dorsal closure, palate closure, and neural tube closure. Complications in any of these processes result in either lethality or developmental complications. Spina bifida, the protrusion of the spinal cord and nerves, result from defects in neural tube closure. Phenotypes from palate closure defects range from a cleft lip to an opening between the nasal cavity and the mouth. While the physical process of these events have been well characterized, their regulation is less understood.

I use Drosophila's thorax closure process to investigate the effects of Cdk8 on tissue fusion. Thorax closure is the process where the notum domains of the two larval wing imaginal discs migrate and fuse to form the adult thorax structure. Changing the levels of Cdk8 results in a malformed cleft thorax. Many signalling pathways that regulate tissue closure are conserved. Investigating how Cdk8 affects tissue closure can reveal a novel function in the protein and better understand tissue closure as a whole.

207F Lysine acetylation regulates non-muscle myosin II filament assembly

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Non-muscle myosin II (NMII) is a pivotal cytoskeletal motor protein that assembles into bipolar filaments, facilitating the conversion of the chemical energy derived from ATP hydrolysis into mechanical work. This process is necessary for exerting tension on actin filaments, thereby generating cellular contractility. The activity of NMII is primarily regulated by phosphorylation. Specifically, the phosphorylation of the regulatory light chain induces a conformational shift from an “inactive” state—characterized by a folded tail that obstructs the head domain from filament assembly and actin binding—to an “active” state wherein the myosin can aggregate into bipolar filaments. While the phosphorylation-dependent regulation of NMII is well characterized, the effects of acetylation as a post-translational modification aren’t as well understood. Recent studies suggest that the acetylation of lysine residues within the NMII heavy chain may significantly influence its regulatory mechanisms and subsequently impact cellular contractility. In this current study, we addressed the functional implications of lysine acetylation on NMII by creating mutations in a cluster of three lysines in the heavy chain. These lysines were substituted with either glutamines (Q), which mimic the acetylated state, or arginines (R), which are resistant to acetylation and maintain a positive charge. Observations revealed that both the Q variants and the wild type (WT) NMII exhibited distinct punctate distributions characterized by well-defined and aggregated myosin filaments. Conversely, the R mutant displayed a markedly diffuse distribution, suggesting a failure to form bipolar filaments. To further elucidate the impact of charge on this behavior, we created two additional mutants: glutamic acid (E), which introduces a negative charge, and isoleucine (I), a neutral amino acid of similar size to lysine but nonpolar. To deepen our understanding of the role of lysine acetylation in modulating NMII function and its influence on cell contractility, we employed RNA interference (RNAi) to deplete lysine-acetyltransferases in an effort to identify which are likely involved in NMII regulation. This approach aims to help understand the networks and pathways controlling NMII activity and its potential cascading effects on cellular function.

208F A DNA quality control program eliminates defective haploid sperm nuclei

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Genome integrity is fundamental to cellular and organismal health with the germline genome bearing the most profound impact for survival across generations. Yet, sperm genomes face unique and long-lasting challenges to their DNA integrity as they mature. During spermatogenesis, post-meiotic germ cells undergo a series of morphological changes over an extended period of time to produce mature sperm. Post-meiotic cells lack critical DNA templates for accurate DNA repair, such as homologous chromosomes or sister chromatids, but little is known about how genome integrity is maintained in these cells that pass the genome to the next generation. Here, we use irradiation as a model to examine how post-meiotic spermatids respond to DNA damage to ensure genome quality for the next generation. Strikingly, we find that DNA-damaged sperm nuclei are effectively eliminated via a mechanism involving the histone variant H2Av and the cellular individualization process at the end of spermatogenesis. We find that haploid spermatid nuclei can respond to DNA damage through phosphorylation of H2Av, a well-known DNA damage response signal. Upon depletion of H2Av, DNA-damaged spermatids are not eliminated, leading to decreased fertility. H2Av is necessary for sperm elimination after DNA damage, and we find the S137 residue specific to DNA damage signaling is responsible for triggering sperm elimination. Ongoing experiments aim to elucidate the cellular mechanism facilitating elimination as well as functional consequences in early embryos from H2Av-depleted fathers. Overall, we reveal a haploid-specific DNA quality control program that prevents DNA-damaged sperm nuclei from being passed to the next generation. These insights shed light on the intersections between DNA damage responses, genome architecture, and sperm developmental dynamics to untangle crucial quality control mechanisms that guard genomes across generations.

209F From Surface to Signal: Substrate-Dependent Formation of the Axon Initial Segment in Drosophila Neurons

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Dysregulation of neuronal polarity and excitability contributes to numerous neurodegenerative diseases. The axon initial segment (AIS) is a critical structure that maintains neuronal polarity and initiates action potentials. Proper AIS formation is essential for normal neuronal function, and disruptions to its organization have been implicated in various neuropathologies. Our study investigates how extracellular cues influence AIS formation in Drosophila neurons. We dissected third instar larval brains, dissociated the neurons, and plated them on coverslips coated with either neuronal extracellular matrix (nECM), which mimics the natural neuronal environment, or concanavalin A (ConA), a plant-based substrate that promotes surface adhesion. Neurons grown on nECM adopted a bipolar morphology and formed an AIS at the expected location. In contrast, neurons plated on ConA displayed a unipolar structure and showed little to no AIS formation. Together, these findings suggest that substrate composition directly influences both neuronal morphology and AIS organization, highlighting the importance of extracellular signaling in shaping neuronal architecture and function.

210S Dual wing and follicle cell screen for bioactive protein traps

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Hostile takeover (*Hto*) FLAG-RFP protein traps are expressed via the GAL4 system and may include either the full-length target protein or a C-terminal fragment. In cases where a fragment is expressed, the protein trap inherently provides protein structure-function information. We have found that two types of biological activity can be identified in forward *Hto* genetic screens, developmental disruption and subcellular localization. We performed screens for disruption of wing development using a very strong wing GAL4 driver derived from *scalloped* (*sd*), recovering 12 lines with unique combinations of wing phenotype and RFP localization. The most severe wing defect occurs with a line targeting *diablo*, encoding an E3 ubiquitin ligase; its fusion is full-length and shows a punctate cytosolic pattern. A wing reduction phenotype and a membrane RFP pattern were obtained from an insert targeting Protein Tyrosine Phosphatase Meg2, a known trafficking regulator. To identify fusions with useful localization patterns, we performed a live confocal microscopy screen of new *Hto* hops in the embryo epidermis. Of 79 recovered embryos, we obtained a final collection of 41 lines. 17 (41%) show a «diffuse nuclear-enriched» pattern; these tend to be out-of-frame fusions to short peptides. 6 (15%) are predicted in-frame fusions with targets including *RhoGAP100F*, *PXo*, *heph*, and *Zdhhc8*. Based on these results, we implemented a two stage screening process with the strong *sd* driver to recover both the wing disruptors and the well-expressed ‹localization› inserts from a single batch of new *Hto* hops. The *sd>Hto* flies with wing defects were retained, yielding 9 unique lines. The *sd* driver also expresses in late (St.13) ovary follicle cells (FC), with RFP stably accumulating in shed follicle cell epithelia in the posterior ovary. Thus the normal-winged females were re-screened for ovary fluorescence. 14% (93/687) had high RFP expression in the body; 60 of these were used to initiate lines, then dissected for confocal imaging of late FC. 24 (40%) displayed patterns of interest and their lines were further tested for localization in mid-stage FC, with 11 retained. Another six lines were retained for a total of 17 ‹localization› lines (2.5% of the screened females). Of these, 6 lines have GAL4-independent RFP expression in various regions of the adult body, apparently due to ‹enhancer & protein traps›. The insertion sites will next be determined and the lines made available to the community.

211S Decoding Organogenesis: Unraveling the Role of E-Cadherin in Malpighian Tubule Elongation

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Organs generate and maintain consistent shape, form, and physiological function through complex cellular processes. E-Cadherin (E-Cad), a key cell-cell adhesion protein, is critical for cell shape, arrangement, and tissue structure. In this study, I investigate the role of E-Cad in the morphogenesis of the Drosophila Malpighian tubules. In this system, tubules extend four times in length purely by cell rearrangements and cell shape change without any cell proliferation. Previous work using full embryo E-Cad mutants showed that the Malpighian tubules separate from the hindgut epithelium and subsequent convergent extension movement is blocked (Tepass, 1996). Thus, while E-Cad is a known critical player, we do not know how E-Cad facilitates cell-cell adhesion during tubule extension, nor do we know the tissue specific requirement of E-Cad. To analyze in vivo spatiotemporal localization dynamics of E-Cad, I constructed a stock with fluorescently-labeled E-Cad and tissue-specific labeling of the tubule cell membranes and imaged tubule elongation. At the beginning of elongation when the tubules are short and wide, E-Cad is enriched at apical contacts of the tubule cells. As the tubules elongate, E-Cad is more strongly enriched at the apical contacts of all tubule cells. To analyze the spatiotemporal dynamics of E-Cad, I am developing an image analysis pipeline that will enable me to correlate E-Cad intensity and localization with specific cell behaviors such as cell intercalations and cell shape change. To assess the requirement of E-Cad in organ formation, I will reduce its expression using tissue-specific protein degradation of E-Cad. By degrading a vital adhesion protein for cells, we hope to observe potential changes in cell intercalation behavior, such as change in intercalation speed and initiation. I will quantify these defects by comparing changes in cell and organ shape in control and E-Cad-reduced tubules. Developing these tools not only enables our work in these tubular organs, but also allows for comprehensive image analysis of other tubular 3D organ forms. Elucidating the precise mechanisms behind cell behavior, shape, and cell-cell interaction has important human health implications and will enable work in many other fields.

212S Molecular remodeling of the oosome during pole cell formation in *Nasonia vitripennis*

Md Eleus Hussain Bhuiya, Jeremy A Lynch Biological Sciences, University of Illinois Chicago

Germ cell specification in the parasitoid wasp *Nasonia vitripennis* relies on a uniquely large, RNA protein-rich germ plasm known as the oosome. Contrasting the polar granules of *Drosophila melanogaster*, the oosome is composed of a mesh-like RNA network, a distinctive *Nv*-Tudor (*Nv*-Tud) protein shell, and almost complete exclusion of mitochondria. Despite its unusual architecture, the molecular changes accompanying oosome fragmentation and pole cell formation remain poorly defined. We used multiplexed HCR RNA-FISH combined with super-resolution SRRF imaging to analyze oosome organization in both control and RNAi-treated embryos. Knockdown of *Nv-oskar* (*Nv-osk)*, *Nv-vasa* (*Nv-vas*), or *Nv-aubergine* (*Nv-aub*) transformed the normal mesh-like oosome into small punctate granules, indicating that each of these factors is essential for maintaining oosome integrity. *Nv-tud* knockdown reduced oosome size and significantly impaired pole cell formation, suggesting that *Nv*-Tud contributes both to oosome stability and germ cell development. Comparative analyses of before (0–3 h) and after (3–6 h) pole cell formation revealed distinct spatiotemporal localization patterns of different transcripts and proteins. In pole cells, *Nv*-Osk decreased remarkably, *Nv*-Tud dispersed from its shell-like arrangement, *Nv*-Vas shifted to a shell-like distribution, and *Nv*-Aub formed larger granules with higher intensity. Moreover, *Nv-osk* and *Nv-coronin* transcripts decreased remarkably, and *Nv-rrm* moved outward after the formation of pole cells. In contrast, *Nv-bark* RNA maintained a stable mesh structure across both stages. These results provide the first high-resolution evidence of coordinated transcript–protein remodeling during oosome-to-pole-cell transition in *N. vitripennis*.

213S Elucidating the Role of Different Integrin Heterodimers in Epidermal Homeostasis and Repair

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Cell migration underlies many physiological and pathological processes, including embryonic development, metastatic disease, inflammation, and wound healing. Although cell migration is such fundamental behavior, it is not entirely understood on a molecular scale. To migrate, cells must rearrange their connections to their environment and to neighboring cells, structures known as cell adhesions. The extracellular environment largely consists of the extracellular matrix (ECM). Cells are anchored to the ECM by heterodimeric transmembrane proteins called integrins. Integrins have been strongly implicated in many processes that involve cell migration, such as wound healing and embryogenesis, while *in vitro* studies have characterized roles for integrins in mediating actomyosin contractile forces, organizing tissues, and driving cell movement. We aim to bridge this gap by understanding how different integrin heterodimers contribute to tissue homeostasis and cell migration in an intact living tissue – the *Drosophila* embryonic epidermis. Integrins heterodimers consist of an a and a b subunit. *Drosophila* have five a and two b subunits that are representative of the ECM-binding capabilities of mammalian integrins. Previous work from our lab has demonstrated a role for the β subunit myospheroid (mys), which is known to form heterodimers with three of the a subunits, in determining epidermal mechanics and driving efficient wound healing. We have performed immunofluorescent staining for mys alongside the three a subunits: mew, if, and scab. Surprisingly, we did not measure strong co-localization between mew and mys. Additionally, we detected localization of inflated on the anticipated basal surface of the cells, but also the apical and lateral sides of cells. These results challenge our understanding of integrin heterodimer function in living epidermis. Future work will continue exploring these subunits, as well as the additional two a and one b subunits, in homeostatic and wounded epidermis.

214S Role of α-Catenin actin binding domain (ABD)-based mechanosensing in tissue morphogenesis

Samantha B Delios[1], Milena Pellikka[2], Ulrich Tepass[2] [1]Cell & Systems Biology, University of Toronto, [2]University of Toronto

Epithelial morphogenesis relies on the coordination of cell adhesion and cytoskeletal remodeling to preserve tissue integrity while allowing cell rearrangements. The cadherin–catenin complex (CCC) mediates these processes, with α-Catenin (α-Cat) linking adherens junctions (AJs) to F-actin through its actin-binding domain (ABD). The α1 and α2 helices within the ABD act as force-sensitive elements that regulate actin binding through a catch-bond mechanism. Prior in vitro studies have shown that removal of either the α1 helix or the α1/α2 helices increases F-actin binding. This suggests that one component of the mechanosensing and mechanotransduction mechanism of the CCC is a force-induced conformational change of the α-Cat-ABD that causes an enhanced interaction with F-actin.

To investigate how ABD force-sensing contributes to epithelial morphogenesis in vivo, I replaced endogenous α-Cat with siRNA-resistant constructs lacking α1, α2, or both helices. Embryos expressing α-CatR-Δα1/Δα2 displayed elevated E-cadherin levels and delayed neuroblast ingression and germband extension, indicating that hyperstable F-actin binding of α-Cat disrupts epithelial dynamics and coordinated morphogenetic movements. These findings suggest that force-responsive modulation of the α-Cat–F-actin interactions is essential for epithelial plasticity during gastrulation. I am currently assessing the dynamic properties of E-cad::GFP and junctional actomyosin in α-CatR-Δα1/Δα2 embryos using FRAP analysis and laser cutting experiments to further assess how mechanosensing of α-Cat-ABD impacts key molecular components of the morphogenetic machinery.

215S Testing the Cell-Penetrating Potential of a Novel Drosophila Peptide in Mammalian Cells

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Peptides translated directly from short open reading frames are an emerging class of microproteins with diverse cellular functions. Dafcin is one such 21-amino acid peptide discovered recently in Drosophila melanogaster that is predicted to form an amphipathic alpha-helix. It most resembles the cell-penetrating HA fusion peptide that coats influenza virion particles. Synthesized in ovarian follicle cells, Dafcin traffics out of the follicle cells and enters the oocyte via endocytosis. If GFP is fused to Dafcin, it has this capability as well. Whether Dafcin can traffic other cargo proteins into any cell type remains to be determined. We are attempting to determine if Dafcin can traffic fused cargoes into mammalian cells. To test this, Dafcin peptide fused to CRE recombinase was purified from E. coli cells. As a control, untagged CRE was also purified from bacteria. HEK293T cells were transduced with a constitutive reporter gene in which RFP flanked by loxP sites is upstream of GFP. If CRE protein is present, the RFP segment is floxed out and cells express GFP. We are incubating purified Dafcin-CRE protein or CRE alone in the HEK293T cell media. Successful cell penetration by Dafcin-CRE will result in GFP fluorescence. Determining if Dafcin enables cytosolic cargo delivery would identify a new mechanism, expanding understanding of peptide-mediated membrane trafficking, and have potential applications in protein delivery and therapeutics.

216S The functions of the FERM protein Yurt in epithelial morphogenesis

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Yurt, a FERM-domain protein, plays a critical role in regulating epithelial cell polarity, morphogenesis, and tissue organization during development. Yurt acts as a basolateral polarity regulator together with Coracle, Neurexin IV and the NaK-ATPase. Yurt antagonizes apical determinants such as Crumbs, and is a phosphorylation target of aPKC, preventing its apical recruitment in most epithelial cells. We show that Yurt genetically interacts with myosin II and E-cadherin to support epithelial integrity in the Drosophila embryo. Additionally, Yurt localizes to AJs during early embryogenesis where we suspect it physically interacts with myosin II and members of the cadherin-catenin complex. Loss of Yurt leads to decrease in junctional myosin and E-cadherin levels. Using new transgenes where Yurt is tagged with fluorescent probes, we are investigating Yurt dynamics in gastrulation embryos using live imaging and FRAP analysis. We also examine the role of Yurt in morphogenetic movements that rely on adherens junctions. We hypothesize that Yurt plays a role in maintaining epithelial integrity and epithelial morphogenesis by linking the adherens junction to the actomyosin network.

217S Uncovering Genetic Drivers of Midgut Lipid Droplet Accumulation in the Mex II *Drosophila* Model

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Lipids are essential to cellular function, acting as energy reservoirs and key regulators of metabolic and signaling pathways. Triglycerides (TGs), stored in lipid droplets (LDs), play a central role inmaintaining energy balance and cellular homeostasis. Our research focuses on a unique *Drosophila melanogaster* strain, **Mex II**, which displays elevated LD accumulation in the midgut due to an insertional mutation driven by the **Mex II-Gal4** enhancer trap. This system provides a powerful model for dissecting the molecular mechanisms that govern lipid metabolism and LD dynamics.

Phenotypic analyses across the Mex lines (Mex I, Mex II, and Mex III) revealed distinct LD accumulation patterns compared with wild-type flies. Oil Red O staining confirmed substantial LD enrichment in the midgut, while buoyancy assays demonstrated that Mex larvae remain floating in sucrose solution due to increased lipid content. In contrast, starved larvae sink as lipid stores are depleted, reinforcing the relationship between buoyancy and triglyceride abundance.

Genetic mapping localized the Mex II mutation to **Chromosome 2**, and similar LD phenotypes in Mex II homozygotes and Mex II/OreR heterozygotes indicate disruption of pathways regulating lipid homeostasis. We have isolated genomic DNA from mutant flies, performed PCR amplification and gel electrophoresis, and are now preparing samples for sequencing to identify the affected gene(s). Future work will employ **inverse PCR (iPCR)** to precisely map the insertion site and define the gene loci associated with the Mex mutations.

Overall, this research deepens our understanding of lipid metabolism and LD regulation. By leveraging the genetic tractability of *Drosophila*, our study offers valuable insights into molecular mechanisms relevant to metabolic disease, with potential implications for obesity, diabetes, and disorders linked to defects in lipid storage and trafficking.

218S Structure-function analysis of a phosphate transporter using Hostile Takeover protein trapping

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Phosphate homeostasis is essential to cellular metabolism and signal transduction, yet the mechanisms underlying inorganic phosphate storage and regulation remain poorly understood. The PXo protein in *Drosophila* functions as a phosphate exporter that has recently been found to aid in the formation of multilamellar phosphate storage structures termed “PXo bodies” (Xu et al, 2023). Here we investigate the cellular localization and trafficking behavior of a PXo protein trap (Ve02) made using the *Hostile takeover* (*Hto*) protein trapping system. Ve02 has replaced residues 1-23 with the FLAG-mCherry tag and the N-terminal deletion has resulted in the loss of key conserved residues in the cytosolic phosphate sensing domain of the protein. Typically, *Hto* cannot make functional fusions with transmembrane proteins since an N-terminal signal peptide would be bypassed or capped by mCherry, but PXo is an 8-TM protein with an internal membrane insertion signal. Our predictive modeling has shown significant impacts to the proteins tertiary structure, however the structural changes have not affected Ve02’s ability to insert into the plasma membrane. Confocal imaging in the follicle cell epithelium has revealed polarized localization of Ve02 to apically biased vesicles and the basolateral plasma membrane. Currently, the understanding of PXo body biosynthesis suggests formation through de novo membrane synthesis that originates from the Golgi (Xu et al, 2023). However, a co-expression assay revealed strong colocalization of Ve02 with the early endosomal marker GFP-FYVE. The presence of Ve02 in early endosomes denotes a trafficking route that differs from the endogenous route defined in gut epithelial cells. This alternative routing reveals trafficking variations resulting from either the RFP tag and N-terminal truncation, or tissue specific differences in trafficking routes.

219S Regulation and Function of the Basolateral Polarity Determinant Discs Large 1 in *Drosophila* Germband Extension

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Epithelial morphogenesis requires precise coordination of cell polarity, junctional integrity, and actomyosin contractility across scales. In early *Drosophila* embryogenesis, the body axis elongates through germband extension (GBE), where cell intercalation is driven by myosin II-dependent remodeling of adherens junctions (AJ). We recently identified an essential role for the polarity determinant Discs large 1 (Dlg1) in this process. Loss of Dlg1 disrupts myosin localization during GBE, reducing junctional myosin while increasing medioapical and lateral myosin, producing a characteristic “lateral spread.” Interestingly, although classically viewed as a basolateral scaffolding protein, Dlg1 becomes enriched in the subapical domain near AJs during gastrulation, suggesting a developmental shift in its localization and function.

To understand how Dlg1 prevents lateral myosin spreading, we analyzed AJ components in *dlg1* RNAi embryos. Live imaging revealed that E-cadherin, normally enriched subapically, becomes laterally mislocalized in *dlg1* knockdown embryos and partially colocalizes with ectopic myosin. We also examined the Rho1 GEF Cyst, which activates junctional myosin via the Rho1-Rok pathway. Cyst displayed a similar lateral spread as myosin in *dlg1* RNAi embryos, consistent with our prior finding that Cyst depletion suppresses lateral myosin accumulation. Together, these data support a model in which Dlg1 restricts Cyst, and thus myosin activation, to the junctional region.

Because Dlg1 recruitment in follicular epithelial cells involves interactions with PI4P and $PI(4,5)P_2$, we investigated whether similar mechanisms operate during early embryogenesis. We found that PI4P and PIP_2 exhibit dynamic localization: both are largely complementary to Dlg1 during cellularization, but become strongly enriched and colocalized with Dlg1 at the subapical region during GBE. These results suggest that phosphoinositides may contribute to Dlg1’s subapical targeting in a stage-specific manner.

In summary, our findings support a model in which Dlg1 maintains proper junctional contractility during GBE by preventing lateral spread of Cyst-myosin activity and by coordinating AJ positioning. The stage-specific colocalization of Dlg1 with PI4P/PIP_2 further suggests a lipid-polarity mechanism that spatially organizes contractility. Future studies will further define how lipid biosynthesis, polarity, and contractility are mechanistically coordinated during epithelial morphogenesis.

220S Investigating the Molecular Interconnectivity of Z-Disc Protein Accumulation in Drosophila Indirect Flight Muscles

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The Z-disc is a structurally and functionally complex region of the sarcomere that anchors actin filaments and transmits force during muscle contraction. In *Drosophila* indirect flight muscles (IFMs), the Z-disc serves as a mechanical anchor for many major Z-disc components. Although several major components of the Z-disc, such as Zasp52, α-Actinin, and Sals, have been identified, the interdependencies among these proteins remain to be fully defined and understood.

This study investigates the molecular interdependence of major Z-disc proteins in *Drosophila* IFMs. By utilizing antibodies that are specific to six distinct Z-disc proteins, we carried out quantitative immunofluorescent mapping of their localization and distribution in the Z-disc in response to targeted RNAi-mediated knockdown of Z-disc components. When knocking down major Z-disc components, such as Zasp52, that are required for protein-protein interactions, we saw a decrease in the localization of other Z-disc proteins, especially in the localization of α-Actinin. When we knocked down other components of the Z-disc, such as Mlp84B or Sals, we did not see a significant change in localization of Zasp52 or α-Actinin.

Using this systematic knockdown of major Z-disc components approach, we found that there is a direct interaction between Zasp52 and α-Actinin that characterized the network-level effects of how knockdown of individual proteins influenced the expression and distribution of other Z-disc components within the myofibrils of the IFMs. By mapping these molecular interactions, our findings provide a deeper understanding of sarcomere organization and may explain how alterations of this organization contributes to defects and degenerative muscle disorders.

221S Systemic propagation of STING signaling via generation of large extracellular vesicles

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Large extracellular vesicles (EVs) derived from tumor cells play important roles in tumor formation and progression. However, it remains unclear why malignant cells produce these EVs and how they act *in vivo*. We employ a well-characterized *Drosophila* tumor model and demonstrate that large EV biogenesis from malignant cells is an evolutionary conserved process. Our study uncovers an essential role for the cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway, which mediates the innate immune response to cytosolic DNA, in driving large EV biogenesis and inducing a systemic immune response to tumors. STING engages a signaling axis comprising JNK and FAK—independent of TANK-binding kinase 1 (TBK1) and inhibitor of nuclear factor κB kinase (IKKβ)—to drive large EV biogenesis in both *Drosophila* and human malignant cells. Transplantation of large EVs from *Drosophila* tumors to wild type larvae is sufficient to recapitulate the systemic immune response to tumors by activating STING signaling in macrophage-like cells. Thus, our study establishes a novel animal model for studying large EVs derived from malignant cells and provides insights into how STING signaling propagates from tumor cells to the immune system via large EV biogenesis, inducing a systemic immune response to tumors.

222S How Does Membrane Tension Alter Epithelial Cell Division and Macrophage Infiltration Dynamics?

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To evenly disseminate throughout an organism and mount a robust immune defense, macrophages must navigate through diverse environments lined with obstacles and barriers. This migration often involves infiltration into normally sealed, tightly packed, cell-dense tissues such as epithelia. The mechanical interactions that underlie such infiltration are not yet well characterized, leaving a significant gap in our knowledge. Particularly, understanding these processes could help us elucidate mechanisms allowing macrophages to invade epithelial tumor microenvironments

Drosophila hemocytes exhibit properties and behaviors of vertebrate macrophages, making them a great model to study these cellular dynamics in real time. During embryogenesis, hemocytes follow stereotyped migratory paths through epithelial barriers, notably infiltration of the germband (GB) between mesodermal and ectodermal tissues at Stage 12.

Previous studies have identified two factors required for the success of hemocyte GB invasion: 1) an adjacent ectoderm cell to start division which downregulates cell-cell adhesions; 2) Eiger protein (ortholog of TNF-alpha) signaling which downregulates phosphorylated myosin levels at the apical membrane of ectoderm, consequently decreasing apical tension. Thus, if ectodermal divisions are inhibited or apical membrane tension increased (as in *egr* mutants), hemocyte invasion is impaired. However, how exactly mechanical changes on the apical membrane promote hemocyte invasion at the basal side of the ectoderm is not understood.

In the current project we aim to uncover the interplay between these two factors controlling hemocyte invasion. We recorded timelapses of the invasion process in WT and *egr* mutant embryos via two-photon imaging of fluorescently labeled hemocytes (srp-H2A-3xmCherry), as well as membranes and adhesion molecules of GB tissue (DECad-GFP; Vinc-mCherry). Analyzed *egr* mutant embryos display more columnar ectodermal cells compared to WT, with a longer and narrower cell shape.

We hypothesize that tissue morphology changes in *egr* null mutants due to differential tension alter the position and frequency of ectodermal cell divisions at the edge of the GB tissue, which hemocytes use as entering points, in turn affecting hemocyte invasion efficiency. Future work will be focused on validating these findings via alternative induction of tension with a higher degree of spacio-temporal control such as tissue-specific, inducible expression of constitutively active Rho1.

223S Mitochondrial inner membrane protein MITOP functions as mitochondrial proton-calcium exchanger

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Mitochondrial Ca^{2+} plays a critical role in regulating diverse cellular processes, from cell growth to cell death, by modulating mitochondrial metabolism and cellular bioenergetics. In recent decades, the core mitochondrial Ca^{2+} uptake machinery has been identified as the MCU complex, while the sodiumcalcium exchangers NCLX and TMEM65 have been characterized as mediators of mitochondrial Ca^{2+} efflux. In parallel, TMBIM5 has been proposed to function as a putative mitochondrial protoncalcium exchanger, though its precise role remains controversial. To further elucidate the regulatory network governing mitochondrial Ca^{2+} homeostasis, we performed a wholegenome ORF screen in *Drosophila* and identified a previously uncharacterized mitochondrial protoncalcium exchanger, which we named MITOP. Expression of this inner mitochondrial membrane protein was sufficient to accelerate mitochondrial Ca^{2+} efflux and rescue the Ca^{2+} overload induced by MCU complex overexpression. Using a liposomebased reconstitution assay, purified MITOP protein demonstrated pHdependent Ca^{2+} transport activity. Genetic ablation of MITOP disrupted mitochondrial ultrastructure and function, leading to behavioral deficits and reduced lifespan in flies. Together, these results expand the known framework of mitochondrial Ca^{2+} transport and nominate MITOP as a potential therapeutic target for disorders linked to mitochondrial Ca^{2+} dysregulation.

224S *Drosophila* e and g subunits of the ATP synthase contribute to fly development and Ca^{2+} homeostasis

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Besides ATP production during oxidative phosphorylation, ATP synthase in its multimeric forms induce inner mitochondrial membrane (IMM) curvature, contributing to the formation of mitochondrial *cristae*. Furthermore, ATP synthase was recently demonstrated to be one of the key candidates of the so-called permeability transition pore (PTP). PTP is a Ca^{2+}-activated, unselective channel that permeabilizes the IMM to ions and solutes. In mammals and yeast, this channel is involved in both physiological Ca^{2+} homeostasis and cell death, while in *Drosophila melanogaster* PTP homolog appears to operate as a selective Ca^{2+}-release channel (CrC). Remarkably, in this model organism it has been shown that dimers of ATP synthase can generate Ca^{2+}-activated channels of a peculiar 53 pS conductance, much smaller compared to the mammalian counterpart (1.2 nS). This channel can be considered a relative of the *bona fine* PTP, since it reacts to the same set of inducers and inhibitors of the mammalian and yeast pores. Recently, it emerged that subunits e and g which are essential mediators of dimerization of the enzyme are also critical for PTP formation. These findings come from studies in mammalian cells and yeast, while whether these roles apply also *in vivo* remains unknown. Thus, we down-regulated either subunit in *Drosophila* and found that ubiquitous knockdown caused larval developmental arrest, whereas tissue-specific suppression in muscle or neurons led to severe locomotor impairment. ATP synthase dimerization was markedly reduced, altering mitochondrial ultrastructure with, however, slight effects on respiration. This indicates that impairment in other functions of ATP synthase may be involved in the generation of the observed phenotypes. Notably, mitochondria from both ubiquitous knockdown lines accumulated substantially more Ca^{2+} than controls, suggesting impaired CrC function. We also found that the levels of ecdysone in the hemolymph, which release relies on Ca^{2+} signaling and masters metamorphosis, were nearly abolished in knockdown *larvae*. Furthermore, in neuron-specific knockdown flies, a decrease in mitochondrial Ca^{2+} efflux and alterations in the synaptic organization at the neuromuscular junction were observed. These findings firstly reveal an essential role for ATP synthase subunits e and g in CrC activity and organismal Ca^{2+} homeostasis, which in turn regulates fly development and proper neuronal functionality.

225S Preventing cracks in the foundation: Investigating how basement membranes control Collagen IV levels

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Basement membranes are sheet-like extracellular matrices that underlie epithelia and surround muscles. Basement membranes conduct many specialized, crucial roles to tissues including structural support, filtration, and signaling. These roles are affected by the unique composition and structure of each specialized basement membrane. For example, the levels of the most abundant component of basement membranes, Collagen IV (Col4), has been correlated with supporting tissue development, proper filtration in the kidney, as well as mechanosensitive and ligand-associated signaling pathways. Col4 levels are also independently altered with aging and many disease states. The distinct changes in Col4 levels suggest a degree of biological regulation, but the regulation of Col4 under homeostatic conditions in adults is not well understood. We seek to characterize how Col4 is regulated in basement membranes of *Drosophila*, primarily using the gut basement membrane due to the mechanically active environment and available tools to genetically or pharmaceutically alter this tissue. The levels of Col4 in the gut basement membrane can be visualized with a Col4-GFP (Vkg-GFP) protein trap. Col4-GFP levels are quantified through a novel analytical pipeline utilizing the interactive machine learning tool ilastik and python coding. This analysis shows that total Col4 increases in the gut basement membrane over weeks, indicating that incorporation of Col4 into the basement membrane is faster than the loss of Col4. To specifically analyze this loss, we conditionally turn off Col4-GFP in adulthood while a wild-type allele of Col4 is unaffected. Col4-GFP loss is imperceptible, indicating that Col4 is very stable in the gut basement membrane. During these experiments, we also quantified Col4-GFP in the Malpighian tubule basement membrane and found that total Col4-GFP and Col4-GFP loss is identical in this tissue, indicating that incorporation of Col4 is imperceptible while loss of Col4 is steady. This finding is intriguing since the gut and Malpighian tubule basement membranes were from the same animals and thus exposed to the same pool of available Col4 which is secreted into the hemolymph. Future directions will be discussed including a screen to address possible effects on Col4 levels from circadian cycling, mechanical activity, and endocytic recycling.

226S Investigating ER stress caused by glial-specific knockdown of the ER membrane complex, subunit four (EMC4)

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The endoplasmic reticulum (ER) plays a crucial role in ensuring the quality control, folding, and insertion of membrane proteins, while also maintaining cellular homeostasis in eukaryotic cells. Within the ER membrane, the endoplasmic reticulum membrane complex (EMC), composed of 8-10 subunits, supports key ER functions. This complex is highly conserved between yeast and humans, but its functional range across specific cell types remains poorly understood. Using RNA interference, we knocked down EMC4 in glial cells, resulting in severe phenotypes, including delayed development, locomotor impairment, and a shortened lifespan of 5-6 days. To further understand the impact of EMC disruption in glial cells, we measured protein aggregation using western blot analysis and found increased ubiquitinated insoluble protein in heads, suggesting the presence of ER stress. Using additional analytical measures, we quantified protein and mRNA levels of genes related to ER stress pathways, such as the unfolded protein response (UPR), autophagy, and heat shock response. We observed changes in the inositol-requiring enzyme 1 (IRE1) pathway of the unfolded protein response, heat shock protein expression, and increased levels of both insoluble and soluble Ref(2)P, indicating upregulation of autophagy. Further investigation will examine additional ER stress markers, as well as how EMC interacts with specific cellular stress pathways. These findings provide insight into the critical role of the EMC4 subunit in regulating glial cell stress to maintain cellular homeostasis.

227T A conditional Flp/FRT screen on chromosome 2R identifies growth and developmental mutants as part of the Fly-CURE

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An F2 mutagenesis screen was conducted utilizing the Flp/FRT system to screen mosaic eyes for regulators of cell growth and development on chromosome 2R. This was a “two-hit” screen in which the starting chromosome harbored an allele of *Dark* (*Dark*^{82}). Homozygous *Dark*^{82} cells block the canonical apoptotic pathway, allowing for the identification of conditional regulators of cell growth and development. 5007 F2 mutants were screened, and 136 growth mutants were identified, and stable stocks were generated. To understand the breadth of genes and pathways impacted by this screen, we utilized deficiency mapping, whole-genome sequencing, and other techniques to characterize and map these mutants. Different categories of mutants were identified including previously characterized growth mutants (*Egfr, Hpo*), growth mutants of known pathways, but identified as conditional regulators of growth (*Ptc, Cos2, Shn*), and identification of novel genes involved in regulation of cell growth and development (*Cliff*). The mapping and characterization of these mutants was accomplished by over 3,000 undergraduate students conducting research within their undergraduate research laboratory course as part of the Fly-CURE consortium. This screen provides new insight into genes that regulate cell growth and development in the *Drosophila* eye and provides support for the collaboration of undergraduate researchers working collaboratively.

228T Live imaging and bioinformatic analysis reveal the molecular mechanism of direct nuclear-to-cytoplasmic ratio sensing in early *Drosophila* development

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The first steps of embryonic development are conserved across different eukaryotes, with maternally deposited mRNAs and proteins initiating embryonic development upon fertilization. Later, the zygotic genome activates and takes over the control of subsequent development. While the timing of zygotic genome activation (ZGA) is crucial for proper embryonic development, the mechanisms by how this timing is well-regulated have not been fully understood. Over the past few decades, studies have suggested that the increase in nuclear-to-cytoplasmic (N/C) ratio following each cell division serves as a timer for the ZGA in *Drosophila*. However, since the increased N/C ratio is tightly linked to lengthened cell cycle duration, identifying direct N/C ratio-responsive genes and investigating the underlying mechanism is challenging without detangling the two factors. In this study, we curated a list of 280 N/C ratio-sensitive candidate genes based on RNA-seq data from early *Drosophila* embryos with arrested cell cycles. Utilizing in vivo live imaging on *wild-type* (diploid and normal cell cycle), *sesame mutant* (haploid and short cell cycle), and *checkpoint mutant* (diploid and short cell cycle) embryos, we quantitatively verified N/C ratio-sensitive genes by separating the effect of N/C ratio manipulation from cell cycle length. We found that the activation of *frühstart* (*frs*) and *rhomboid* directly responds to the N/C ratio. We further confirmed that the *frs* 1kb and 300bp regions upstream from the transcription start site retain complete response to N/C ratio, identifying the minimal regulatory region that confers N/C ratio sensitivity. Through motif enrichment analysis, we identified potential transcription factors (TFs), such as Cropped and BEAF-32, that may convey N/C ratio measurement to gene activation decisions. Our study provides the first quantitative characterization of the role of N/C ratio in transcriptional regulation in *Drosophila* embryos. In addition, our identification of potential N/C ratio-sensing TFs proposed a novel mechanism of N/C ratio-mediated ZGA regulation during early embryonic development.

229T Coordinating New DNA Synthesis during Double-Strand Break Repair in *Drosophila*

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DNA integrity is crucial for faithful replication, cell division and viability. Various damage events that go unrepaired can lead to a multitude of disorders and diseases. The DNA double-strand break (DSB) has no adjacent DNA strand to use as a repair template and therefore is a dangerous lesion that can lead to mutations. DSBs repaired by end-joining tend to incorporate errors, whereas repair via homologous recombination (HR) is more accurate. The HR process involves invading a template (sister chromatid or homologous chromosome) and undergoing nascent DNA synthesis to recover any lost information. This project studies HR using the *P{w^{e}}* genetic assay where excision of the transposon generates a single 14-kilobase gap, thereby requiring a large amount of nascent synthesis, which allows us to assess which DNA polymerases and clamps are utilized in this process. Previously, we shown that both replicative and translesion synthesis DNA polymerases compete in the repair process, with replicative polymerases being utilized in longer synthesis tract lengths and translesion polymerases in shorter repair synthesis. In our current efforts to determine if clamps are utilized, we have studied *PCNA-Ub* and *PCNA2* mutants as well as *hus1* mutants (part of the 9-1-1 complex). Similar to the polymerase counterparts, there also appears to be a division of labor among clamp usage, with data suggesting PCNA/PCNA2 usage in short tract synthesis and 9-1-1 in long tract synthesis.

230T Wingless and Yorkie Drive Tumor Growth Through JNK: Insights from *Drosophila* and Human Cancers

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Recently, we uncovered a tumor specific regulatory network using the ***Drosophila Ras^{V12},scrib$^{-/-}$*** **tumor model**. This network is comprised of **Wingless (Wg)**, the effector caspase **Dronc**, and a feed-forward loop between J**un N-terminal Kinase** and **Yorkie** that promotes tumor growth. Wg is ectopically induced in *Ras^{V12},scrib$^{-/-}$* clones. First, we modulated Wg signaling by reducing activity of the pathway transcription factor dTCF in tumor clones (*dTCF^{DN};Ras^{V12},scrib$^{-/-}$*), and found it significantly reduces tumor clone size, suppresses survival and decreases apoptotic activity. We hypothesized that Yorkie (Yki), is the key downstream regulator of this network. Consistent with this hypothesis, heterozygosity of yki (*yki^{B5}/+;Ras^{V12},scrib$^{-/-}$*) reduced clone size, Wg expression, apoptotic response, and survival signaling. To further dissect the roles of Yki and Wg**,** we simultaneously suppressed Wg and *yki* activities in tumor clones (*dTCF^{DN},yki^{B5};Ras^{V12},scrib$^{-/-}$*). We evaluated the following: (a) Cancer Hallmarks: Invasion, cell adhesion, and survival signaling assessed by IHC, (b) Transcriptional effects: mRNA expression profiling via qRT-PCR, c) Pathway Activities: Western blot analysis of Hippo (Mst, p-Mst), JNK (JNK, p-JNK), and Wg signaling. We observed that dual suppression of Wg and Yki leads to significant changes in tumor growth dynamics, and suppression of aggressive tumor growth. Reduction of Wg and Yki activities affects JNK activity in the tumors, suggesting intricate signaling interactions in this network. Furthermore, to assess whether this tumor-specific network is conserved in **human cancers**, we performed multi-cancer TCGA analyses comparing *KRAS^{high}/SCRIB^{low}* tumors with normal tissues. Heatmaps generated using TCGA patient data revealed an overall trend of increased expression of WNT, DNA damage/apoptosis, Hippo, and JNK pathway-associated genes, suggesting increased activity of the tumor specific network pathways in patient tumors. Additionally, immunohistochemical analyses of human patient tissue arrays demonstrated increased and mislocalized expression of Wnt- and YAP-regulated proteins in tumor biopsies, consistent with the observations in the ***Drosophila*** model. These results provide the mechanisms driving **KRAS-driven epithelial cancers** and create a cross-species paradigm for identifying conserved therapeutic nodes. Here, we present our findings about novel insights into how Yki and Wg cooperatively regulate tumor growth through crosstalk with JNK signaling.

231T Genetic Mapping of 6e.29, 6d.44, and a.21 Cell Growth Mutations in the Fly-CURE

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The Fly-CURE is a Course-based Undergraduate Research Experience that utilizes an EMS genetic screen with the Flp/FRT system in Drosophila melanogaster to generate mutations that disrupt cell growth and development in the eye. Mutations on chromosome 2R are mapped through standard complementation to identify the causative gene. The current work describes the phenotypic characterization and genetic mapping of three Fly-CURE mutations: a.2.1, 6d.44, and 6e.29. The EMS mutagenesis for the a.2.1 mutation was conducted in a background of blocked apoptosis. ;FRT42D,Dark82,mw+,a.2.1 /CyO were crossed to w;FRT,Dark82;Ey>Flp to generate homozygous mutant clones (-/-;red) in the eyes. The eyes of a.2.1 flies displayed significant red>white pigmentation and scarring. Mapping experiments with a.2.1 demonstrated failure to complement to BDSC lines 8044, 9683, and 24339. Subsequent complementation data narrowed down the candidate gene region to nucleotide positions 6,181,745…6,245,428 on 2R. Interestingly, previous whole genome sequencing of this mutant identified a whole priority SNP candidate in the SCAP gene locus, which falls just outside of this candidate gene region. Further genetic work will need to be done to determine the causative mutation of the observed a.2.1 phenotype. The 6e.29 and 6d.44 EMS mutagenesis were conducted in a background of normal apoptotic activity. In these crosses, ;FRT42D,6e.29/CyO and ;FRT42D,6d.44/CyO and were crossed to Ey>Flp;FRT42D,GFP, mw+ to generate homozygous mutant clones (-/-; white) in the eyes. Both the 6e.29 and 6d.44 mutation displayed white over red overgrowth phenotypes. 6e.29 failed to complement when crossed to BDSC 8910, which narrows the candidate region to 11197412…11665391. Crosses to subdeficiencies in this chromosomal region will narrow down the candidate gene loci for the 6e.29 mutant. The 6d.44 mutant was crossed to > deficiency stocks on chromosome 2R and none of the stocks resulted in a failure to complement. Whole genome sequencing of 6d.44 heterozygotes demonstrates high priority SNPs at multiple loci, including the ars2 gene locus. Future work will crossing 6d.44 to ars2 mutant stocks and, if necessary, crossing 6d.44 to any deficiency stocks that have not yet been covered.

232T Increasing mitotic retention of GAGA Factor in early embryo has limited impact on transcriptional identity

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Development depends on the faithful maintenance of gene-expression programs as cells proliferate. During mitosis, chromatin condenses, transcription arrests, and most transcription factors (TFs) dissociate from the genome. It remains unclear how, despite these substantial disruptions, the cell rapidly and robustly re-establishes the transcriptional program to maintain cell identity. A subset of TFs remains associated with mitotic chromosomes, suggesting that they may function to preserve transcriptional memory. Due to technical challenges to specifically disrupt protein activity during mitosis, the functional relevance of mitotic retention of TFs remains unresolved. To address this, we are investigating GAGA factor (GAF), a TF essential for promoting gene expression in the early *Drosophila* embryo. GAF is retained on chromatin throughout the 13 rapid, synchronous mitotic divisions of early development, but mitotic retention is lost at gastrulation. To understand the impact of mitotic retention on development and gene expression, we engineered two gain-of-function GAF mutants. By extending the C-terminus or mutating phosphoserines in the DNA-binding domain, we increased GAF mitotic retention at both heterochromatin and euchromatin and prolonged its mitotic association into gastrulation. These data may indicate that chromatin affinity promotes mitotic retention and chromatin maturation limits it. Despite dramatic changes in mitotic retention, the GAF phosphoserine mutant occupies largely the same genomic loci as wildtype, and embryos develop normally. However, quantitative image analysis of labeled GAF molecules demonstrated increased mitotic retention results in greater nuclear concentration and altered binding kinetics as compared to wildtype. To determine the impact on gene expression, we compared the transcriptomes of both gain-of-function GAF mutants to wildtype controls. Contrary to the current model suggesting mitotic retention promotes transcription of cell-identity genes, we did not identify increased GAF-target gene expression. Ongoing live imaging experiments will determine the effects of increased GAF mitotic retention on transcriptional reactivation following mitosis. Our findings demonstrate that increased GAF mitotic retention does not influence target gene expression or inhibit development, challenging the prevailing assumption that mitotic retention reflects a function in mitotic memory.

233T Investigating the Roles of Specific Cyclins in the Dissemination of *Ras*^{V12}-Driven Tumor Cells in *Drosophila melanogaster*

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Tumor cell dissemination is the initial step of cancer metastasis, which is responsible for the majority of cancer-related deaths. However, the molecular mechanisms that drive dissemination remain poorly understood. Through a candidate-based RNAi screening of kinases and phosphatases utilizing the *Drosophila melanogaster Ras*^{V12} intestinal tumor model, we found that knockdown of some cyclins blocks dissemination, whereas knockdown of others does not. Cyclins, which pair with Cyclin-dependent kinases (Cdk), are best known for their regulation of the cell cycle, but they can also regulate other processes, such as transcription. Here, we investigate the role of both canonical cell cycle cyclins and transcriptional cyclins in tumor cell dissemination. We found that knockdown of cell cycle cyclins, such as Cyclin D, had no observable effect on tumor cell dissemination. However, knockdown of some transcriptional control cyclins, like Cyclin T, reduced cell dissemination as well as resulted in a rounder morphology and were more densely packed. These results suggest that transcriptional cyclins play a distinct and underappreciated role in tumor cell dissemination and can provide potential targets for metastasis prevention therapeutic strategies.

234T Mechanisms of Mitotic DNA repair by DNA Polymerase Theta

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Cells must protect their genome from DNA damage by endogenous and exogenous factors. DNA double-strand breaks (DSBs) are particularly deleterious if they persist into mitosis. DSBs can generate acentric DNA fragments lacking canonical kinetochore-spindle attachments, posing risks for chromosome mis-segregation and micronuclei, aberrant nuclear structures linked to genome instability. However, we previously showed that *Drosophila melanogaster* hindgut papillar cells accurately segregate acentric fragments and prevent micronuclei. We further found that early-acting DNA repair factors are recruited to DSBs and are resolved during mitosis, and that micronucleus prevention depends upon the alternative end-joining (Alt-EJ) protein DNA polymerase Theta (Pol Theta). Based on our findings, we propose that Alt-EJ displaces early-acting repair factors from broken DNA ends and then anneals the ends, forming a “tether” by which acentric DNA segregates. In support of this model, we recently discovered that both the helicase and polymerase functions of Pol Theta are required for papillar organogenesis upon DSB induction. To further understand Pol Theta mitotic function at single nucleotide resolution, we have developed a site-specific DSB system to sequence Pol Theta-mediated mitotic repair events. We are also examining the localization of a fluorescently tagged Pol Theta during mitosis. Finally, we are defining a larger molecular network involved in mitotic DNA damage responses. To do so, we are conducting epistasis studies between Pol Theta mutants and knockdown of hits from a candidate screen that are required for papillar cell DSB sensitivity. Our ongoing studies will unveil novel regulation of mitotic DNA damage tolerance.

235T Metazoan Nup98 and Nup96 control entry into S phase

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To ensure complete and accurate replication of the genome in S phase, cells must execute a transcriptional program in G1 phase to guarantee sufficient replication and regulatory proteins will be available during S phase. Progression from G1 to S phase is regulated by the E2F-dependent transcriptional activation of key cell cycle genes, including *Cyclin E*. While the E2F transcriptional program has been well-characterized across many systems, there are likely many unknown factors that participate with E2F to promote entry into S phase. We have found that depletion of Nup98-96, a component of nuclear pore complexes, prevents Drosophila cells from entering S phase. *Nup98-96* encodes a single polypeptide that undergoes autoproteolysis and generates two proteins with distinct functions and localizations. We conducted an RNA-interference screen of all metazoan nuclear pore proteins and found that lack of entry into S phase is not generic to all nucleoporins. Rather, lack of S phase entry is specific to depletion of only a few nucleoporins and Nup98-96 depletion showed the strongest effect. Given that Nup98 functions in transcriptional regulation, we measured transcript levels in Nup98-96-depleted cells. We found that Nup98-96 regulates many E2F-dependent genes necessary for progression into S phase. Critically, S phase entry can be rescued in Nup98-96-depleted cells by co-depleting the Cyclin E inhibitor, Dacapo. Taken together, we have identified Nup98-96 as a critical regulator of entry into S phase, likely through transcriptional regulation of *Cyclin E* as part of the E2F transcriptional program. Here, we investigate the mechanism by which Nup98 and Nup96 regulate entry into S phase.

236T *Drosophila Blm* Mutant Males Show Increased Meiotic Nondisjunction

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In humans, Bloom syndrome, caused by a lack of functional BLM protein, manifests as short stature, skin abnormalities, a high predisposition to cancer, and reduced fertility. Previous research in *Drosophila melanogaster* identified *Blm* DNA helicase as crucial for proper chromosome segregation during female meiosis, with *Blm* females showing increased meiotic nondisjunction (ndj). The role for Blm during female meiosis was shown to be necessary for proper crossover patterning. *Drosophila* males do not undergo meiotic crossing over, but it is not known whether Blm plays a role in suppressing meiotic ndj, perhaps by another mechanism. Our lab assayed sex chromosome ndj in male *Blm* mutants and found an approximately 3-fold elevated rate of ndj compared to *Blm* heterozygous males. The lack of clustering among aneuploid progeny suggests the ndj events occur primarily during meiosis, although these may result from pre-meiotic chromosome damage caused by aberrant mitotic recombination in *Blm* males. These findings indicate a conserved role for *Blm* in meiotic genome stability across sexes and highlight *Drosophila* as a valuable model for understanding Bloom syndrome's underlying mechanisms.

237T Examining Regulation of Bloom Syndrome Helicase Regions Conserved Among Closely Related *Drosophila* Species Provides New Insights on Function

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Bloom Syndrome helicase (Blm) is a RecQ helicase with roles in homology-directed DNA double strand break repair (HDR), cell-cycle progression, and development. Pathogenic mutants in human *BLM* cause the autosomal recessive disorder Bloom Syndrome, with affected individuals predisposed to many different types of cancer. Previous work using *Drosophila Blm* mutants lacking helicase activity have shown defects in repairing DNA double-strand breaks (DSBs) by HDR, improper crossover patterning and segregation of chromosomes in meiosis, and compromised embryonic development due to cell-cycle progression defects. While Blm orthologs have a well conserved and structured RecQ helicase domain, much of the protein does not have a well characterized function and is poorly conserved across metazoa. Because of this, we compared closely related *Drosophila* species to identify regions of conservation that could suggest important functions. Two of these *Drosophila*-conserved regions in the N-terminus were deleted in *D. melanogaster* using CRISPR/Cas9 gene editing and Blm functions were then assessed. Deletion of conserved region 1 (CR1) or conserved region 2 (CR2) led to various effects on Blm roles in development, HDR, and meiosis, including some compromised functions. We then examined the role of regulation in these regions by mutating predicted ATR/ATM kinase sites to mimic or prevent phosphorylation. Preliminary results suggest that for CR2, mimicking phosphorylation may serve to rescue defects observed when this region is deleted, while preventing phosphorylation does not rescue. These results provide novel insights into functions of the N-terminal region of Blm helicase and suggest important and distinct functional roles and potential regulation of previously uncharacterized Blm regions.

238T Condensin I function requires a putative interaction domain of the E3 ligase CRL4^{Cdt2}

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The segregation of acentric DNA fragments resulting from double stranded breaks (DSBs) that persist into mitosis is crucial to preventing the formation of micronuclei, which can lead to catastrophic genome shattering known as chromothripsis and overall genomic instability. Previously, we have shown that the rectal papillar cells in Drosophila are prone to mitotic DNA damage yet are able to prevent micronuclei by segregating acentric DNA fragments. Using this model, we identified the Cullin-RING ubiquitin ligase 4 complex (CRL4^{Cdt2}) to be a crucial regulator in responding to mitotic DSBs. We found that CRL4^{Cdt2} acts in the same pathway as DNA polymerase theta (PolQ), which we have previously shown to be required for acentric DNA segregation in order to repair DNA during mitosis. To uncover possible targets of CRL4^{Cdt2} that act in the context of DNA damage, we then immunoprecipitated Cdt2-bound proteins that are specific to S2 cells with DNA damage. In addition to PCNA, which is required as a scaffold for substrates to bind to before being ubiquitinated by CRL4^{Cdt2}, we identified several DNA damage-specific Cdt2 interactors.

From these interactors, we discovered that multiple subunits of the Condensin I complex, specifically the HEAT-subunits CAP-D2 and CAP-G contain PCNA-interacting protein domains (PIP boxes) that may enable their interaction with CRL4^{Cdt2}. In order to better understand the role of these putative PIP boxes in Condensin I subunits, we generated novel Cap-D2 alleles- a premature truncation allele (null) and a CRISPR deletion of the putative PIP box. We find that both CapD2 alleles disrupt *Drosophila* development, and FLP/FRT clones of both alleles compromise clonal cell expansion in multiple tissues. However, the CapD2 PIP box deletion allele exhibits distinct (weaker) phenotypes from the null allele, highlighting novel roles for this putative CRL4^{Cdt2} interacting sequence. Given these findings, we are currently testing the DNA damage specific role of CapD2 lacking the putative PIP sequence. Our findings are revealing new mechanistic insight into Condensin I and CRL4^{Cdt2} function in the contexts of development, mitotic division, and DNA damage.

239F Investigating the molecular mechanisms of wound-induced mitotic inhibition

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Healing a wound requires complex repatterning of cellular behavior. Both migration and proliferation contribute to this repatterning to form a concerted tissue response to close a wound. In the *Drosophila* epithelial pupal notum we can live-image cell movement and mitotic events after laser wounding using a GFP tagged alpha tubulin. Although the pupal notum is a mitotically active diploid tissue, our preliminary data reveals a distinct zone of mitotic inhibition in cells closest to the wound during the 4-5 hour closure period. This response is apparent in wounds both with and without plasma and nuclear membrane damage. To investigate the molecular mechanisms behind the cellular switch between proliferation and migration after wounding, we are planning to perform a candidate screen, conditionally knocking down genes in one half of the wound bed, with the goal of identifying genes responsible for both the inhibition and the reactivation of mitosis after wounding.

240F Creating a CRISPR-engineered Polo-like kinase 4 mutant sensitized to small molecule inhibitors in *Drosophila*

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An essential protein that regulates the centrosome is the serine-threonine kinase Polo-like-kinase 4 (Plk4). Plk4 is the master regulator of centrosome biogenesis. It has been proposed that Plk4 has functions beyond centrosome biogenesis, however, the study of these in *Drosophila* is a challenge as Plk4 mutants lack centrosomes to observe. We aimed to characterize novel roles for Plk4 in centrosome dynamics using small molecule inhibitors to acutely perturb Plk4 function, while centrosomes are still present. However, we found *Drosophila* Plk4 is not susceptible to Centrinone, a commercially available Plk4 inhibitor. From the cells treated with centrinone (n=73) 98.6% had 2 or more centrosomes; cytokinesis failure, an off-target effect, was also observed in 56.2% of treated cells. Therefore, we created a gatekeeper mutant (*plk4*^{as1}) which changes the shape of the ATP binding site, sensitizing the kinase to inhibition by PP1 analogs . Unfortunately, we found that without inhibitor present the gatekeeper mutation caused a loss of Plk4 function; this was quantified by the absence of centrosomes in 97.7% of the mutant cells (n=174) compared to 1.2% of wild type cells (n=82). This was further displayed by decreased survivability of the mutants; *plk4*^{as1} homozygous pupae eclosed but died shortly after. Through comparison to other analog sensitive kinases by sequence alignment, we identified a secondary mutation which could rescue kinase function (*plk4*^{as2}). Excitingly, 100% of the *plk4*^{as2} cells measured (n=76) possessed 2 centrosomes. When the *plk4*^{as2} line was treated with a PP1 analog inhibitor, the cells displayed a reduction in centrosome number, while the wild-type cells maintained 2 centrosomes. Thus, we have successfully created a Plk4 mutant sensitized to small molecule inhibition. This tool will be used to probe Plk4 functions beyond centrosome biogenesis in *Drosophila*, and its role in asymmetric cell division.

241F Investigating the protein-protein binding domains of Mute: a novel negative regulator of replication-dependent histone gene expression in D. melanogaster

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Histones are proteins that package DNA, thereby regulating access to the information encoded in the genome. During S-phase, coordinating DNA replication with histone production is necessary to maintain genome stability as cells proliferate. Replication-dependent (RD) histone genes are expressed only during S-phase, and their level of expression must be tuned to the rate of DNA synthesis to ensure accurate genome duplication and chromosome segregation during cell proliferation. Histone locus bodies (HLBs) are phase-separated nuclear structures located at the RD-histone genes in metazoans, such as Drosophila and humans, facilitating histone transcription and mRNA processing. In Drosophila, the proteins MXC and FLASH (homologous to human NPAT and FLASH) are essential for HLB formation and histone gene expression. Mute, an HLB component and homologue of human Gon4L/YARP, was recently identified as a possible negative regulator of histone gene expression. We have shown that the loss of Mute in stage 14 Drosophila embryos leads to an increase in cells expressing replication-dependent histone mRNAs in the ventral nerve cord compared to wild-type. We hypothesize that Mute represses histone gene expression through protein-protein interactions mediated by hydrophobic amino acids in Mute's two PAH domains, similar to other repressive hub proteins, such as Sin3a. To test this hypothesis, we generated plasmid constructs of full-length Mute N-terminally tagged with mVenus and containing deletions of PAH1, PAH2, or both domains, as well as phenylalanine to alanine point mutations altering four key hydrophobic residues in each PAH domain and the linker region between the two. These constructs were transfected into Drosophila S2 cells, and immunofluorescence with confocal microscopy was used to assess the localization of Mute to HLBs. All single-domain deletions and point mutants produced stably tagged Mute protein that localized to HLBs, although at lower levels than wild-type tagged Mute. Interestingly, mutation of two amino acids in the linker region, which interact with both PAH domains, led to aggregation of Mute at the nuclear periphery. This result suggests that individual PAH domains are not required for Mute's localization to the HLB but may be required for its function as a negative regulator of RD histone gene expression. We are currently engineering these Mute mutations into the endogenous mute gene of D. melanogaster to evaluate the role of the PAH domains in regulating histone transcription and their larger impact on development and viability.

242F Understanding the mechanisms of disrupted cell signaling using a fruit fly epithelial tumor model

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Disrupted cell-cell communication causes uncontrolled cell proliferation, leading to cancer. The Wnt/Wg signaling plays a crucial role in cell-cell communication during biological development. Wnt signaling is implicated in multiple human cancers, yet mechanisms that allow Wnt signaling to drive tumorigenesis remain unclear. We use fruit fly (Drosophila melanogaster) as a model organism to investigate the role of Wg signaling in tumorigenesis. Previous experiments show that levels of Wg are upregulated in tumors, and downregulation of Wg signaling decreases tumor size. Interestingly, tumor cells also have elevated levels of the Drosophila glypican, Dally-like protein (Dlp). In normal tissue, Dlp on the cell surface distributes Wg away from Wg-producing cells to cells that receive Wg. Additionally, a fraction of Dlp can be cleaved by proteases like Matrix Metalloproteinase 2 (Mmp2), causing Dlp to be internalized along with the bound Wg ligand. Thus, the cleavage of Dlp by Mmp2 provides a molecular break on extracellular Wg distribution. Interestingly, Mmps are also required in tumor cells for growth and might be involved in Dlp cleavage in tumor cells. Based on these observations, we hypothesize that tumor cells maintain higher Wg levels on the cell surface by maintaining higher levels of Dlp on the cell surface, allowing Wg levels to promote excessive Wg signaling in tumor cells. We further hypothesize that in tumor cells, cleaved Dlp is retained on the cell surface in tumor cells due to defects in endocytosis, which typically internalizes Dlp from the cell surface. In summary, our study will elucidate how the Dlp-Mmp2 signaling axis is co-opted by tumor cells to facilitate elevated Wg signaling in tumor cells.

243F Examination of fancm mutants in repair of CRISPR/Cas9 double strand breaks reveals increased usage of theta-mediated end joining

Hannah E Duncan, Bryson E Vaughan, Aiyana Williams, Evan B Dewey Biology, Winthrop University

For multicellular life to survive, genome stability must be maintained. DNA is exposed to tens of thousands of sources of damage each day, including ten to fifty double strand breaks per cell cycle, and it must combat this damage to prevent genome instability and cancer. Often, our cells use Homology Directed Repair (HDR) to repair these breaks. To do so, the undamaged sister chromatid is used as a template. HDR must be appropriately regulated to ensure use of the identical sister chromatid, and not the non-identical homologous chromosome. This is to prevent crossovers between non-identical homologs, which can potentially lead to cancer and disease. It is known that CRISPR/Cas9 induces double-strand breaks (DSBs), but little is known about the role of HDR in repair stemming from a Cas9 DSB and also in how key HDR genes are regulated in this process. Our research focuses on one such gene, fancm, and the roles it may play in regulating HDR for CRISPR-induced breaks. To do this, we mutated fancm in the Drosophila melanogaster model and cut a target homologous chromosome with CRISPR/Cas9 in developing male germline cells to watch how the cells repaired the broken target using an unbroken template. Interestingly, rather than the expected HDR products, we observed products of a backup pathway, Polymerase Theta-Mediated End Joining (TMEJ). This process involves the annealing of DNA microhomologies by DNA polymerase Theta, which causes small deletions. The use of this pathway suggests that when fancm is mutated, cells may be prevented from invading the template and performing HDR, instead using the backup TMEJ pathway. Further investigation is required to fully understand fancm's role in HDR.

244F Studying Non-Crossovers in *fancm* mutants using CRISPR/Cas9

Aiyana Williams, Hannah Duncan, Bryson Vaughan, Evan B Dewey Biology, Winthrop University

DNA is subject to tens of thousands of damage every day. Repair of this damage is crucial to maintaining genome stability, but genes involved in the repair process are at risk of mutations of their own. Homology Directed Repair (HDR) is responsible for precisely repairing double strand breaks using homologous chromosomes as a template. During HDR, two products can result: a crossover, when large swaths of DNA are exchanged between homologs, and non-crossovers (NCOs), when only the minimal amount of DNA needed for repair is swapped between homologs. Misregulated HDR leading to COs can lead to genome instability and cancer. Our research examined NCOs in Drosophila with a fancm gene knockout. Fancm is responsible for starting the Fanconi Anemia (FA) pathway which removes Interstrand Crosslinks (ICLs), creating a double strand break (DSB) to be repaired by HDR. To study HDR products lacking fancm, we made fancm mutant males and induced CRISPR/Cas9 double strand breaks in their germline cells at specified locations. This is done in one allele of the rosy (ry) gene (target homolog), but not the other (template homolog), via a single nucleotide polymorphism (SNP). These males are then crossed with females containing a deletion in the ry gene to recover repair products. The paternal chromosome, housing potential HDR repair products in resulting progeny, can then be selectively amplified via PCR using primers directed to the deleted ry region. If a NCO occurred in the parental male's germline it can be detected and reconstructed in the amplified products from the mixed presence of target and template SNPs. We see varying lengths of template DNA in NCOs in fancm mutant males which may suggest compromised repair synthesis. More analysis is needed to fully understand the effects of fancm loss on HDR.

245F Testing the capacity of the *Chlamydia* effector Tarp to inhibit cell death by targeting the host Hippo pathway

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As the most prevalent bacterial STI in the United States and leading cause of infection-related blindness, *Chlamydia trachomatis* (*Ct*) poses a significant threat to public health. As an obligate intracellular bacterium, *Ct* injects specialized proteins called early effectors into the host cell to drive *Ct* entry. The early effector Tarp (translocated actin recruiting protein) nucleates and bundles F-actin to promote invasion, mediated via Tarp's C-terminal region. Although the C-terminus is well characterized, the function of the N-terminus of Tarp (N-Tarp) is not as well known. Using *Drosophila melanogaster* as an *in vivo* model to discover and study effector function, we previously described a novel function of the N-terminal region of Tarp — inducing the transcriptional activity of the Hippo pathway, a conserved signaling pathway that controls cell proliferation and survival. Host cell viability is crucial for the intracellular development of *Chlamydia* and it has been observed that *Chlamydia*-infected cells are resistant to apoptotic stimuli, though the mechanism remains unclear. Interestingly, many downstream targets of the Hippo pathway are also involved in aspects of apoptosis, including *bantam* (a regulator of pro-apoptotic protein Hid), and *Diap1* (an inhibitor of *Drosophila* initiator caspase Dronc). Therefore, we hypothesize that the N-terminal region of Tarp promotes cell survival by targeting Hippo signaling. To test this, we will determine whether N-Tarp activity is sufficient to inhibit or reduce a *Drosophila* model of induced cell death (via expression of pro-apoptotic genes, *reaper* or *eiger*) in the wing and eye imaginal discs. We will also identify N-Tarp molecular interactions using IP/MS proteomics approach in S2 cells. Together, we aim to describe a novel molecular function of the N-terminal region of Tarp, informing how host cell viability is maintained during *Ct* infection.

246F Endocrine ERK dynamics control adult size and metamorphosis independently

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In mammals, adult size is determined at the conclusion of puberty with the transition from adolescence to adulthood. Similarly, in *Drosophila*, this occurs just before metamorphosis, when larvae transition into reproductively competent adults. The onset of metamorphosis and the cessation of growth is regulated by the secretion of steroid hormones from endocrine glands, such as the prothoracic gland (PG). Previous studies have implicated the ERK/MAPK pathway and multiple receptor tyrosine kinases in this process, yet how ERK signaling integrates these inputs to govern developmental timing remains unclear.

We investigated the temporal dynamics of ERK signaling in the PG and found that ERK is activated during both the first (L1) and third (L3) larval instars. To dissect its functional role, we utilized optogenetics to control ERK activity in the absence of EGFR activity. This allowed us to rescue the EGFR mediated ERK activity specifically and dynamically.

While ERK activity is most prominent directly prior to metamorphosis, we find that early ERK is essential for triggering metamorphosis. Whereas late-stage ERK activation merely accelerates the timing of the event, reducing adult sizes. These findings challenge the prevailing view that the more dramatic late ERK pulse is the primary trigger for metamorphic initiation. Our data suggest that early ERK signaling establishes a permissive state required for later hormonal cues to execute the transition.

This study highlights the importance of understanding ERK temporal dynamics in coordinating developmental checkpoints and provides new insight into how neuroendocrine signals are integrated to regulate body size and metamorphosis in Drosophila.

247F Investigating the relationship between active Polo kinase localization and B chromosome drive during female meiosis

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B chromosomes are supernumerary, non-essential chromosomes found in thousands of eukaryotic species. These chromosomes are often maintained in a population over several generations due their ability to be inherited at a higher than Mendelian frequency, a phenomenon referred to as drive. Recently, B chromosomes were found in a laboratory stock of *Drosophila melanogaster* and are subject to drive when only one functional copy of *matrimony* (*mtrm*) is present. Mtrm is a critical regulator of Polo kinase during female meiosis, and its reduction leads to the frequent missegregation of non-crossover chromosomes and the mislocalization of Polo on the meiotic spindle. Under normal conditions, active Polo mostly localizes to the spindle, but a reduction in Mtrm leads to its increased localization on the oocyte DNA. To investigate the relationship between Polo kinase localization and B chromosome transmission, we are currently using immunofluorescence to detect where active Polo is localizing in prometaphase oocytes. We are testing several genotypes that have been shown to exhibit high, medium, and no B chromosome drive, and our hypothesis is that the active Polo localization will become more abnormal in genetic backgrounds where the B chromosome drive is stronger. We anticipate that our data will continue to illuminate the role of Mtrm and Polo kinase in the biased segregation of the B chromosomes, which will ultimately contribute to our understanding of chromosome dynamics and the cellular mechanisms that ensure faithful chromosome segregation during female meiosis.

248F Mutant *C.3.3* identified from a conditional Flp/FRT EMS screen harbors lethal mutations in *Rpe* and *Nup75*

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A conditional Flp/FRT EMS mutagenesis screen was conducted in the *D. melanogaster* adult mosaic eye to look for regulators of the cell cycle, cell division, and tissue development. The starting chromosome for this screen harbored an allele of *Dark*^{82} that blocks the canonical apoptosis pathway in the mosaic mutant tissue. The screen identified conditional mutations that disrupted the cell growth and developmental patterning in the adult eye. One mutant from the screen, *C.3.3*, was characterized by having a reduction in the ratio of mutant tissue to wild type tissue in the mosaic eye. This mutant was genetically mapped by undergraduates in the Fly-CURE consortium at Loyola Marymount University Nevada State University, and the United States Airforce Academy. This group identified two distinct lethal mutations on the right side of chromosome 2. The lethal mutations were further mapped to specific genes, *Rpe* and *Nup75*. Using homologous recombination, we identified that *Rpe* and *Dark*^{82}, had a similar phenotype to *C.3.3* overall (*Dark*^{82}, *Nup75*, and *Rpe*) suggesting that *Rpe* is driving the conditional mosaic eye phenotype. Interestingly, the *Nup75*, *Dark*^{82} phenotype was more severe than the overall *C.3.3* mutant phenotype, suggesting *Rpe* may have a role in suppression of the Nup75 mutant phenotype. Overall, our findings provide a more thorough understanding of the interplay between multiple mutants in the developing *Drosophila* eye.

249F Differential meiotic regulation of the X-Chromosome in the Synaptonemal Complex mutants

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Aneuploidy is the leading cause of infertility and miscarriages, arising from errors in meiotic cell division. During meiosis, chromosome segregation requires high fidelity to ensure proper recombination and genetic diversity. The synaptonemal complex (SC) is a key multi-protein structure that facilitates homologous chromosome pairing and converts DNA double-strand breaks (DSB) into crossovers. Analysis of partial loss of function SC mutants in *Drosophila melanogaster* reveals a reduction in X chromosome crossover frequency compared to autosomes. We aim to build on these observations by elucidating if meiotic events such as SC assembly and DSB formation occur differently on the X chromosome and autosomes. To achieve this goal, we will employ complementary cytological approaches, including chromosome-specific oligopaints, DNA FISH, and immunofluorescence. Additionally, we are examining the relationship between the X chromosome and the nucleolus, which may influence meiotic chromosome behavior since the X chromosome harbors the rDNA. By determining the roles of these components and the SC, we can gain a better understanding of chromosome behavior and the segregation defects underlying reproductive errors.

250F A progeroid mutation exposes a regulatory role for BAF during mitotic broken chromosome rescue

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Dividing cells must surround all their chromosomes in a single nuclear envelope or risk forming micronuclei. However, the formation of a single nucleus is challenged when cells divide with broken chromosomes, which remain separate from daughter nuclei at the time of nuclear envelope formation. During divisions with intact chromosomes, the DNA-bridging, nuclear envelope protein barrier-to-autointegration factor (BAF) plays an important role in grouping chromosomes into a single nucleus. Here, we analyzed BAF's role in capturing broken chromosomes into telophase nuclei in *Drosophila*. We found that BAF enriched on protein-coated DNA tethers originating from both the broken and telomere ends of chromosome fragments at different kinetics than global BAF localization to chromosomes. While some of these tethers promote the incorporation of fragments into daughter nuclei, all others must be efficiently resolved. Using an allele of BAF orthologous to the mutation responsible for the human disease Néstor-Guillermo progeria syndrome, we found that progeroid BAF was preferentially decreased on tethers that would otherwise promote micronucleation. Decreased BAF was correlated with a weaker tether. In addition, progeroid BAF was decreased on the chromosome fragments themselves as well as the "core" regions of the reforming nuclear envelope. Remarkably, flies heterozygous for progeroid *baf* experienced an increased rate of broken chromosome rescue compared to flies with wild-type *baf* or heterozygous for a null allele of *baf*. Collectively, these results suggest a mechanism in which the telophase regulation of BAF plays an important role in promoting the formation of euploid daughter nuclei in response to broken chromosomes.

251F How do RING proteins affect double strand break formation/resolution throughout synaptonemal complex progression in *Drosophila melanogaster*?

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Meiosis is a form of cell division that creates sex cells with half the number of chromosomes. Accurate chromosome segregation and distribution is essential to the creation of a viable embryo. Aneuploidy, a meiotic event resulting in a cell with an incorrect number of chromosomes, is responsible for approximately half of all miscarriages. A key step during meiosis is the formation of a multi-protein structure, called the synaptonemal complex (SC), which links chromosomes together and facilitates recombination. In *Drosophila melanogaster*, the SC promotes the formation of double strand breaks (DSBs) and their resolution into either crossovers (COs) or non-crossovers. Defects in SC assembly can result in errors early in meiosis and further downstream effects. Our lab is interested in investigating the key mechanisms that facilitate successful meiotic progression, to provide key insight on infertility and miscarriage.

In order to further investigate the role of the SC in establishing the recombination landscape, we are examining its relationship with a set of RING protein pro-crossover factors, Narya, Nenya, and Vilya. These are known to have a role in both DSB formation and CO resolution. Additionally, a key central region SC protein, Corolla, is known to interact with Vilya through yeast 2-hybrid experiments, which further implicates a significant role of RING proteins interactions facilitating SC dynamics. To assess the interplay of the SC and RING proteins, we will take a two-prong approach examining both protein-protein interactions and colocalization of RING proteins in SC mutants using a combination of genetic and microscopy approaches. Together these studies will determine the role of RING proteins and the SC in establishing a normal CO landscape during meiosis.

252S Investigating the spatial regulation of Hippo pathway activator Kibra in asymmetrically dividing neuroblasts

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The Hippo signaling pathway is a conserved signaling pathway that restricts tissue growth across species, including Drosophila and humans. Components of the pathway have also been shown to be required for the asymmetric cell division of Drosophila neural stem cells, the neuroblasts. During neuroblast division, the Par complex, consisting of atypical protein kinase C (aPKC), Par-6, and Baz, localizes to the apical cortex and drives the basal localization of cell-fate determinants, ensuring both self- renewal and differentiation into a daughter ganglion mother cell. Although this polarity network is well defined, how Hippo pathway components are localized and activated in neuroblasts remains unknown. Here we show that the localization of Kibra, an upstream activator of the Hippo pathway, is coupled to neuroblast division in that it polarizes to the apical cortex during prophase. We have found that F-actin dynamics and aPKC synergize to promote Kibra polarization. Although Kibra can bind to aPKC, our results show that the aPKC binding domain is neither necessary nor sufficient for Kibra's polarization, suggesting that aPKC does not physically tether Kibra to the apical cortex. In investigating whether aPKC kinase activity regulates the actin flows that drive Kibra polarization, we found that inhibiting aPKC kinase activity results in membrane blebbing and reduced apically directed actin flows and accumulation. These observed defects suggest that aPKC's role in Kibra polarization involves, at least in part, actin dynamics. However, the exact mechanism driving Kibra's polarization remains unclear. Currently, we are examining the possibility that interactions with other polarity proteins might contribute to Kibra's localization to the apical cell cortex. Together, these experiments provide mechanistic insight into how Kibra, and by extension Hippo pathway activity, is spatially regulated by apical polarity and the actin cytoskeleton in mitotic neuroblasts.

253S Impact of Sex on Germline DSB Repair in *Drosophila melanogaster*

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DNA double-strand breaks (DSBs) are a particularly serious form of DNA damage that can lead to large-scale genome instability and diseases, such as cancer. DSBs can be repaired by non-homologous end joining (NHEJ), single strand annealing (SSA), or homologous recombination (HR). A previous study in *Drosophila melanogaster* found a statistically significant decrease in HR repair in the female germline compared to males when DSBs were induced in 0-3 day old embryos and larvae. To understand the role of sex on DSB repair patterns across different developmental stages, an *I-SceI* transgene with a *ubiquitin* promoter (*Ubi-I-SceI*) or an *I-SceI* transgene with a *Bam* promoter (*Bam-I-SceI*) were used to induce DSBs continuously starting at fertilization and in only the pre-meiotic germline, respectively. A Direct Repeat of *white* (DR-*white*) assay was used to determine the proportion of DSB repair by HR. It was found that HR repair was not statistically different in the female germline compared to the male germline when DSBs were induced constitutively using *Ubi-I-SceI*. However, using the *Bam-I-SceI* assay, HR repair was higher in the male premeiotic germline compared to its female counterpart. These findings suggest that HR repair is, on average across developmental stages, equally as common in the female germline compared to the male germline, but is less common in the female pre-meiotic germline and 0-3 day old germlines. Future investigations will determine the role of p53-mediated apoptosis on HR repair in the pre-meiotic germline using *Bam-I-SceI* in *p53* mutants.

254S Elucidating the Relationship Between CtIP and Blm Helicase in Single-Stranded Annealing

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The human genome is vulnerable to damage by exogenous and endogenous sources. Both sources have the potential to induce double-stranded breaks (DSBs), a particularly severe form of DNA damage in which both strands of the double helix are broken. If left unrepaired, or repaired insufficiently, these breaks can lead to genomic instability—a hallmark of cancer. Understanding the mechanisms by which DSBs are repaired, as well as the drivers of sufficient DSB repair, is therefore critically important.

One pathway DNA DSBs may be repaired if the break occurs between repetitive sequences is single-stranded annealing (SSA). SSA requires sufficient end-resection, a process where the 3' end of the DNA end is removed, revealing complementary sequences. These complementary sequences can anneal, resulting in repair of the DSB with a loss of one of the repeats and any intervening sequence. Previous studies in mammalian systems have identified C-terminal binding protein-interacting protein (CtIP) and Bloom syndrome helicase (BLM) as key proteins within this pathway. When phosphorylated, CtIP is recruited to the break site and works with other proteins to initiate end-resection. This process results in a 3' overhang, allowing BLM to promote extensive end-resection via other exonucleases (DNA2, EXO1), generating the long stretches of single-stranded DNA required for SSA.

Given both CtIP and BLM's role in promoting SSA, this study investigates whether *Drosophila*. *melanogaster CtIP-Blm* double mutants exhibit more defects in SSA than single mutants. Through this, we aim to elucidate the interactions between Drosophila CtIP and Blm within the SSA pathway.

255S Investigating the effect of mild cold temperature on Drosophila female germline stem cells

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Drosophila melanogaster has a longer lifespan at lower temperatures while it exhibits accelerated aging at higher temperatures, and recent work from our lab has also shown specific effects of temperature on adult oogenesis. In particular, Drosophila female germline stem cells (GSCs) and oocyte quality are better maintained at a mild cold temperature of 18°, and GSCs at 18° appear to have a prolonged S phase. My research project aims at understanding how the GSC cell cycle is regulated at 18° and whether the altered GSC cell cycle is connected to the improved maintenance of GSCs at 18°. I am currently using the fluorescence ubiquitin cell cycle indicator (FUCCI) system along with other cell cycle markers to characterize the cell cycle of GSCs at 18° compared to 25°. A detailed characterization of the GSC cell cycle at 18° will provide a solid foundation for addressing my future research questions.

256S Modeling the impact of unscheduled endocycling on tissues and tumors

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The endocycle is a variant cell cycle consisting of repeating synthesis (S) and growth (G) phases without a Mitotic (M) phase, resulting in large polyploid cells. Developmental endocycling cells (devECs) naturally occur in many tissues and are important for supporting tissue growth. However, cells can also enter unscheduled endocycles in response to conditional signals such as stress, wounding, aging, and cancer. These unscheduled switches can be detrimental to tissue growth and function, such as in the case of Polyploid Giant Cancer Cells (PGCCs). PGCCs are often therapy resistant and associated with poor patient prognosis. We have been modeling PGCCs in *Drosophila* using the UAS/GAL4 system to genetically inhibit mitosis and induce unscheduled endocycles. Our previous work showed that induced endocycling cells (IEC's) are resistant to apoptosis and undergo a JNK-dependent senescent arrest at various ploidies. The IEC's also exhibit transcriptional changes and release molecules associated with a Senescence Associated Secretory Phenotype (SASP) with both cell autonomous and non-autonomous effects. Unlike most devECs, these induced endocycling cells can also return to error prone division, generating aneuploid daughter cells and leading to increased genetic instability. Our recent findings indicate that IEC's can synergize with oncogenes such as *Ras* and y*orkie* to bypass senescence and further promote genome instability and neoplastic tumor growth. I will discuss how our results in *Drosophila* are providing new insights into the ways PGCCs contribute to human cancer.

257S Investigating the role of aPKC-dependent phosphorylation on Kibra's subcellular localization and function

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The Hippo pathway is a well-studied regulator of tissue growth composed of a kinase cascade (Tao, Hippo, and Warts), a transcriptional co-activator (Yorkie) and several upstream regulators (Crumbs, Expanded, Merlin, and Kibra). We have focused on Kibra because of its importance in assembling pathway components into a signaling complex at the cell cortex. Kibra is found in distinct medial and junctional pools at the apical cortex in imaginal epithelial cells, and appears to be trafficked between these pools. Interestingly, medial Kibra activates Hippo signaling more efficiently than junctionally localized Kibra. We have previously shown that Kibra's localization is regulated in part by antagonistic interactions between actomyosin flows, which bring Kibra toward the medial domain, and the apical polarity protein atypical protein kinase C (aPKC), which tethers Kibra to the junctional cortex. Kibra contains a well-conserved aPKC substrate recognition sequence in its C-terminal region, but whether Kibra is a substrate for aPKC has not been well-studied. Here, we address two questions: 1) Does aPKC phosphorylate Kibra, and 2) Is this phosphorylation important for Kibra's function?

We asked if Kibra is a substrate for aPKC in S2 cells using Phos-Tag gel shift assays and found that aPKC promotes Kibra phosphorylation. To investigate the functional significance of this phosphorylation, we generated Kibra expression constructs and transgenes containing mutations at this site. We also mutated an adjacent site thought to be necessary for aPKC to bind to its substrates. We found that these mutations affect Kibra's ability to associate with aPKC in S2 cells and change Kibra's subcellular localization in tissues. Additionally, we examined the effects of ectopic expression of these mutant Kibra transgenes on Hippo pathway activity by assessing their effect on wing growth. Taken together, these data confirm that interaction with aPKC at the junctional cortex limits Kibra's ability to localize medially. However, the relationship between the subcellular localization of these mutant forms of Kibra and their effect on wing growth is less clear, suggesting that aPKC phosphorylation might impact more than just Kibra's localization. This work paves the way for a better understanding of how Kibra's localization is regulated and how its interaction with aPKC is important for its activity in the Hippo pathway.

258S Neural stem cells regulate intracellular calcium to control cell identity and function

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Brain development requires relatively few asymmetrically dividing neural stem cells that expand to form numerous diverse cell types. Calcium (Ca2+) is widely recognized as one of the most important factors in nearly every aspect of cellular life and death, but how Ca2+ is involved in processes such as cell fate decisions and neuronal diversity is not well understood. Ca2+ is capable of orchestrating diverse cellular processes across cell types due to the unique expression levels and structural differences between cell types. This enables cells to respond differently to changes in Ca2+ concentration, and changing Ca2+ dynamics can promote specific behaviors or elicit transcriptional changes. We seek to define the mechanisms of Ca2+ dynamics in Asymmetric Cell Division (ACD) which is how our bodies create trillions of diverse cells. Mutations in Ca2+ signaling are related to neurodevelopmental disorders such as schizophrenia, bipolar disorder, and autism spectrum disorders. To study these processes, we utilize Drosophila melanogaster neuroblasts (NB), which are the neural stem cells of the fly brain. We have found that NB lineages possess Ca2+ gradients associated with the degree of cellular differentiation. Despite the wide implications of Ca2+ in cellular physiology, the functional relevance of this Ca2+ gradient to neural development is completely unknown. To further investigate this, we knocked down the sarcoendoplasmic reticulum Ca2+ ATPase (SERCA) in NB, which induced high cytoplasmic Ca2+ concentrations and depleted ER luminal Ca2+ concentrations, and found that this was sufficient to transform type II NB lineages into a "type I-like" NB lineage fate. Interestingly, incubating larval brains in high Ca2+ conditions was sufficient to elevate cytoplasmic Ca2+ levels which reduced proliferation and promoted differentiation, but did not transform the type II NB lineage identity. Our findings implicate distinct functions for cytosolic and ER luminal Ca2+ levels within type II NB. We propose NB keep Ca2+ levels low to facilitate proliferation, whereas high Ca2+ promotes differentiation. We hypothesize that ER Ca2+ levels are required for proper Notch processing and trafficking to the plasma membrane. This work will generate novel insight into how cell types regulate Ca2+ activity to enact unique and specific physiology in the brain and has implications for understanding how disruptions in Ca2+ signaling cause developmental disorder and disease.

259S Metabolic reprogramming and signaling imbalance drive collective invasion and heterogeneity in a Drosophila epithelial tumor model.

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Tumor cells often exhibit differences in their behavior and metabolism, but the mechanisms underlying these differences and their contribution to tumor malignancy are not fully understood. Using a *Drosophila* wing imaginal disc tumor model combining a neoplastic tumor suppressor gene mutation (*lgl* or *scrib*) and an oncogenic form of Ras (*RasV12*), we explored how metabolic and signaling changes shape tumor heterogeneity. To investigate the heterogeneity, we performed single-cell RNA sequencing on this tumor model. This scRNA-seq analysis revealed that a group of cells within the invasive clone clusters (highly expressing MMP1) show strong upregulation of lactate dehydrogenase (*LDH*, encoded by *ImpL3*) and a marked reduction in Wingless (Wg) expression. These heterogeneous expression patterns were not observed in the benign tumor clusters growing at the apical side of the epithelial layer. This pattern suggests a shift toward glycolytic metabolism consistent with the Warburg effect, where tumor cells rely on lactate production to sustain growth and survival under stress. Imaging analysis on this tumor model confirmed their heterogeneous expressions within the invasive tumor clones. Furthermore, we observed the LDH-high and Wg-low clones made a cluster in the actual tumors. Previous studies in *Drosophila* and mammals link LDH activity and lactate to epigenetic regulation through metabolites like L-2-hydroxyglutarate and histone lactylation. Building on these findings, our study provides in vivo evidence that metabolic reprogramming and reduced Wg signaling together create functional heterogeneity within tumors. This work highlights a conserved mechanism through which metabolic adaptation and signaling imbalance may cooperate to drive collective invasion and tumor progression.

260S Cloning and Characterizing a negative regulator of larval tissue-specific growth

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Development in humans and most animals, is characterized by differential growth in which individual organs and tissues grow at different rates relative to each other. This is referred to as allometric growth, but the molecular mechanisms that control it are poorly understood. Larval tracheal growth in *Drosophila melanogaster* is an excellent model to investigate allometric growth. The trachea is the primary gas exchange organ in the fly. Its development is genetically controlled during embryogenesis, and it grows allometrically during the larval stages. In larva, its growth is characterized by cellular growth with no cell division. Previous work has identified specific genes required for larval (rather than embryonic) tracheal growth. Specifically, Uninflatable (Uif) and Matrix metalloproteinase 1 (Mmp1) are positive regulators of tracheal growth, where mutations result in excessively short and broken trachea. We conducted a genetic screen of more than 250 larval lethal mutations and identified 8 mutations that resulted in altered larval tracheal growth. Several of these mutations display reduced tracheal growth similar to *uif* and *Mmp1* mutations, but one mutation resulted in long and highly convoluted trachea in 3rd instar larvae. The purpose of this project is to clone and characterize this novel larval lethal mutation (known as *l(3)12265*). We are currently examining the relative trachea length of mutant larvae by measuring the ratio of the tracheal dorsal trunk length from the transverse connective in the fourth abdominal segment to the posterior spiracles versus total body length. We are also analyzing the size and shape of tracheal cells in mutant larvae and quantifying the expression of Uif and Mmp1 in these mutant trachea. Finally, to identify the gene responsible for this mutation, we have conducted whole genome sequencing and will be testing candidate genes with RNAi to see if it will recapitulate the tracheal growth defects.

261S Impact of Caloric Density on Meiotic Recombination Rate

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Meiotic recombination is a crucial part of meiosis that generates novel combinations of alleles and ensures proper chromosomal segregation. The rate of recombination has been shown to be affected by environmental perturbations and genetic background, known as recombination rate plasticity. One hypothesis in the literature is that organisms under stressful conditions increase recombination as a mechanism to improve fitness. This is known as Fitness-Associated Recombination (FAR) and predicts a negative relationship between individual fitness and recombination. In this study, we manipulated caloric density and measured its impact on both survival and recombination. Previous experiments in our lab have shown that higher recombination rates correspond to increased DNA damage during oogenesis; however, we did not directly measure fitness. Here, we reared two outcrossed genotypes of *Drosophila melanogaster* from the Drosophila Genetic Reference Panel on standard Bloomington media modified with varying caloric densities (0.5x, 1x, and 2x calories). We measured fecundity through egg counts, fertility through adult counts, and survival through comparing egg counts with adult counts. We also measured adult body mass. In addition to these measurements, individuals were scored on seven phenotypic markers across the X chromosome to measure meiotic recombination rates. Increasing caloric density had a statistically significant effect in increasing fecundity and adult body mass. However, higher caloric density caused a statistically significant decrease in egg-to-adult survival. Recombination rates were significantly affected by caloric density and genetic background. While caloric density altered fitness and recombination, these responses did not show the expected negative correlation hypothesized. These results suggest a more complex relationship between fitness and recombination. Future studies should focus on other mechanistic hypotheses for explaining plasticity in recombination.

262S Employing single-cell genomics to dissect the role of E2F/Rbf during early development of the *Drosophila* flight muscles

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The *Retinoblastoma* tumor-suppressor, *Rbf*, encodes a protein that suppresses the activity of E2F transcription factors, thereby inhibiting the G1/S transition during cell cycle progression. Additionally, E2F and Rbf control myogenic transcriptional programs during *Drosophila* muscle development. The E2F family members, E2F1 and E2F2, require dimerization with the Dp subunit to bind DNA; hence, both are inactive in *Dp* mutants. To investigate the role of E2F/Rbf during differentiation at the onset of flight muscle development, we employed single cell RNA sequencing (scRNA-seq). First, we built a high-resolution atlas of ~30k wild-type adult muscle precursor cells (AMPs) derived from the wing imaginal discs of third instar larvae. Then, to further resolve cell clusters across modalities, we performed chromatin accessibility assay (scATAC-seq). We identified novel clusters representing various states of differentiation for direct and indirect flight muscles: one characterized by the expression of *Sp1* and another by co-expression of *Sp1* with *Enhancer-of-split* genes. We will present pseudotime trajectory analysis that models the progression of myoblast differentiation across clusters, and *in vivo* validation using immunofluorescence and cell-tracing experiments. With this atlas for reference, we explored transcriptomic changes following the inactivation of E2F and Rbf in asynchronous AMPs. The expression of *Dp* and *Rbf* was knocked down using a muscle-specific driver, *Mef2-GAL4*, and the *UAS-Dp-RNAi* and *UAS-Rbf-RNAi* transgenes. Cells were profiled by both scRNA-seq and scATAC-seq, and data were integrated and compared to the reference atlas. Differential expression of cell cycle phase-specific marker genes revealed that muscle-specific *Dp* depletion increased the number of G1 phase AMPs across all clusters, while *Rbf* depletion decreased it. These findings were validated by Fluorescence-Activated Cell Sorting (FACS) that confirmed this cell cycle dysregulation consistent with the canonical roles of E2F and Rbf. We also identified a cluster unique to the *Mef2>Rbf-RNAi* genotype that highly expressed the pro-apoptotic gene, *hid*. Further differential gene expression and chromatin accessibility analysis will be presented to elucidate the effects of E2F inactivation on other transcriptomic programs. Collectively, our results illustrate the power of single-cell genomics to dissect the role of E2F/Rbf during AMP proliferation and early muscle differentiation.

263S From Phosphorylation to Activation: A Drosophila Perspective on APC/C activation in female meiosis

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The Anaphase-Promoting Complex/Cyclosome (APC/C) is a multi-subunit ubiquitin ligase that ensures timely degradation of cell cycle regulators. While its role in mitosis is well characterized, APC/C regulation during meiosis remains poorly understood. We investigated how Cyclin B3 (CycB3), activates the APC/C. We previously showed that CycB3, in complex with Cdk1 (Cyclin-dependent Kinase), phosphorylates APC3, a core subunit of APC/C that has been implicated in its regulation. Here, we test a phospho-relay model in which phosphorylation of APC3 by the CycB3-Cdk1-CKS (Cdk subunit) complex promotes subsequent phosphorylation of a disordered loop of another subunit of APC/C, APC1, enabling recruitment of the APC/C, co-activators, Fizzy (Fzy)/Cdc20 and Cort, (a meiosis specific co-activator) leading to subsequent APC/C activation. To test this model, we generated a non-phosphorylatable APC3 mutant and performed rescue experiments in a *APC3*^{RNAi} background. The mutant partially fails to rescue, and immunofluorescence analysis revealed defects in polar body formation and in the syncytial mitotic divisions. We also generated a *Flag-APC1*^{DL}, deletion loop mutant. The overexpression of this mutant, but not *Flag-APC1*^{wt}, leads to female sterility and drives the early activation of APC/C. Moreover, genetic epistasis tests expressing *Flag-APC1*^{DL}*;CycB3* double mutants indicate that CyB3 initiates the phosphorelay, whereas APC1 disordered loop functions downstream to activate APC/C in meiosis, as predicted by phosphorelay model. Our findings not only clarify a novel regulatory pathway controlling meiotic progression but also provide broader insights into how cyclins and phosphorylation events regulate the cell cycle machinery in meiosis.

264T Drosophila GCN1 is essential for general neuronal activity independent of the canonical GCN1–GCN2 pathway.

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General Control Nonderepressible 1 (GCN1) is a factor best known for initiating the integrated stress response (ISR) through GCN2 activated upon amino acid deprivation. Recent biochemical studies indicate that GCN1 recognizes collided ribosomes (disomes) during translation elongation, which appear more frequently in response to amino acid starvation. Because disomes interfere with proper mRNA translation, they are cleared in healthy cells through GCN2-mediated ISR or the ribosome-associated quality control (RQC) pathways. Although GCN1 functions have been characterized extensively at the biochemical level in cultured cells, it's *in vivo* roles remain poorly understood.

To address this, we generated a *gcn1* loss-of-function mutant in *Drosophila* using CRISPR/Cas9. While GCN1 knockouts cause embryonic lethality in mice, GCN1-deficient flies survived to adulthood. The mutant adults exhibited severe neuronal phenotypes, including slow locomotion, impaired climbing ability, and pronounced bang-sensitive seizure behavior in heat-shock paralysis assays. These phenotypes were also recapitulated by pan-neuronal *gcn1* knockdown using *elav-GAL4*, suggesting a disruption in the regulation of neuronal activity. Notably, these phenotypes were absent in *gcn2* mutants and pan-neuronal *gcn2* knockdown, indicating that neuronal GCN1 functions independently of the canonical ISR pathway.

To identify neuronal subtypes responsible for these phenotypes, we inhibited *gcn1* in specific excitatory (cholinergic, glutamatergic, monoaminergic) or inhibitory (GABAergic) populations. However, *gcn1* KD in individual subtypes did not produce comparable phenotypes observed in the mutant or in pan-neuronal *gcn1* knockdown. These findings suggest that global neuronal GCN1 function, rather than activity within a single subtype, is required for maintaining circuit-level signaling that prevents seizure induction.

Together, our results reveal that GCN1 regulates neuronal activity through a pathway independent of canonical GCN2 signaling. These insights highlight a previously unrecognized, organism-level role of GCN1 in neural circuit.

265T Stress Responsive ATF4 Directs Leader-Follower Organization in Collective Cell Migration

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Collective cell migration is a crucial feature of development and is utilized by cancer cells to invade as cohesive groups. As the cells migrate, they experience mechanical and physiological stress, including endoplasmic reticulum (ER) stress, which is caused by misfolded proteins. The integrated stress response (ISR) resolves this stress by inducing the expression of transcription factors like ATF4. How the ISR pathway regulates normal migration *in vivo* remains unclear. To better understand the role of the ISR pathway in collective cell migration, we use the *Drosophila* border cell model. In the ovary, 6 to 8 follicle cells form the border cell cluster, which moves collectively between large nurse cells towards the oocyte. This enables us to visualize and quantify the effect of ISR on collective cell movement. We recently found that either overexpression or reduction of ATF4 inhibits border cell migration. However, the mechanism by which ATF4 regulates collective cell migration remains unknown. **We hypothesize that ATF4 must be maintained at an optimal level to support coordinated collective cell movement.** To test this, we generated mosaic clusters in which one or two border cells either overexpressed or downregulated ATF4, but the rest of the cells were wild type. Time-lapse live imaging revealed that ATF4-altered cells consistently shifted to the rear of the migrating cluster. Using the TrackMate plugin in ImageJ, we quantified overall cluster velocity and the spatial position of ATF4-altered cells relative to wild-type neighbors. ATF4-altered cells showed a marked reduction in leader occupancy without affecting their speed, indicating a positional bias rather than a motility defect. These findings highlight the significance of optimal ATF4 expression in facilitating spatial organization within migrating collectives. ATF4 here acts as a rheostat, not just a direct switch. Both under and overactivation may disrupt adhesion remodeling, actomyosin contractility, and/or guidance cue reception, so cells lose the leader competition and sort to the rear. This study provides the first evidence that an ATF4-dependent stress recovery program can drive leader-follower dynamics in collective cell migration. Together, these data provide a stress-driven mechanism for coordinated invasion, highlighting that ATF4 may serve as a potential target to limit tumor spread and better understand developmental defects.

266T Molecular Mechanisms Governing Nurse Cell Death During Drosophila Oogenesis

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Cell death and clearance of cellular debris are essential to the development and homeostasis maintenance of an organism. These phenomena in *Drosophila* oogenesis play critical roles in ensuring the proper development of the female reproductive system and the formation of functional eggs. In the *Drosophila* ovary, germline-derived nurse cells (NCs) undergo non-apoptotic regulated cell death as part of normal development. We have found that NC death is controlled largely non-autonomously by the surrounding somatic stretch follicle cells (FC), and that the phagocytic machinery is required for the final steps of NC death: acidification, nuclear membrane breakdown, and DNA fragmentation. Lysosomal machinery acting in the surrounding FCs non-autonomously promotes the acidification and DNA fragmentation of nurse cells. To gain a comprehensive view of how the stretch follicle cells carry out NC death non-autonomously, we have examined how the cells' endoplasmic reticulum and plasma membrane are eliminated. In addition, we aimed to elucidate the fate of ring canals, specialized structures of the membrane, during NCs' elimination. We find that the NC membrane begins to break down prior to acidification, and we hypothesize that the NC plasma membrane undergoes trogocytosis by the neighboring FCs. Moreover, ring canals are eliminated subsequent to the NC PM removal mechanism. In addition, we show that Draper, a phagocytic receptor, is essential to the proper removal of NC plasma membrane and NC nuclei. By observing the interaction between the plasma membranes of FCs and NCs during late-stage oogenesis, NC death and clearance will be better understood. This work sheds light on mechanisms of phagocyte-dependent cell death.

267T The *Drosophila* CEBPG homolog Irbp18 is essential for crc (ATF4)-induced Integrated Stress Response (ISR) in degenerative disease models

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Cells adapt to diverse conditions of stress in part by activating the Integrated Stress Response (ISR). Two bZIP transcription factors, Xrp1 and crc (ATF4 homolog), are induced to regulate a gene expression program during ISR in *Drosophila*. These bZIP factors need to form heterodimers to induce target genes, and the CEBPG homolog, Irbp18, is Xrp1's established dimerization partner. Previous in vitro binding studies reported that crc can dimerize with Irbp18 and slbo (another CEBP family protein), but whether these dimerization partners mediate ISR in vivo remains unknown. Here, we report an essential role of *Irbp18* in crc-mediated ISR. Specifically, *Irbp18* was required for most transcriptional changes in a *Drosophila* model for Retinitis Pigmentosa, in which the *ninaE*^{G69D} *allele* activates ISR in photoreceptors. In the *Irbp18* loss-of-function background, crc failed to bind most target DNAs as revealed through a photoreceptor-specific CUT&RUN. Moreover, photoreceptor-specific gene expression profiling revealed that *crc* is unable to induce target transcripts when overexpressed in the *Irbp18* mutant background. By contrast, loss of *slbo* had a relatively modest effect on the induction of crc target genes. *Irbp18* loss accelerated retinal degeneration. In addition, loss of *Irbp18* suppressed the lethality of *Parkin* mutants, a model of Parkinson's Disease with excessive ISR activity. These results indicate that *Irbp18* serves as an essential node in ISR signaling, affecting pathological outcomes in *Drosophila* disease models.

268T EMS mutagenesis-based forward genetic screen to explore mechanisms of the newly identified cell death erebosis in the *Drosophila* midgut

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In the *Drosophila* midgut, enterocytes are essential for nutrient absorption and preserving the integrity of the gut barrier. These cells are constantly replaced through homeostatic turnover, during which intestinal stem cells divide and differentiate to generate new enterocytes. We recently identified a novel mode of enterocyte death, distinct from established cell death mechanisms, which may play a role in physiological cell turnover. This process has been named erebosis. Erebosis is characterized by the accumulation of Angiotensin-converting enzyme (Ance) in enterocytes and a progressive loss of various proteins. Erebotic cells exhibit a loss of organelles, cytoskeletal elements, and cell adhesion structures, underscoring the unique characteristics of this form of cell death. While these features have been characterized, how erebosis is regulated at the molecular level remains to be determined. In this study, we are using EMS mutagenesis as a genetic screening strategy to identify key genes that suppress erebosis, specifically targeting the X chromosome in hemizygous males. We have identified a mutant that exhibits suppression of erebosis in the midgut. We performed whole-genome sequencing to identify the mutations and the corresponding gene in the suppression mutant. We are presently investigating the mutant's morphological characteristics and the mechanisms underlying erebosis suppression.

Keywords: Erebosis, *Drosophila* midgut, enterocytes, EMS screening, X-chromosome

269T A molecular mechanism that promotes the survival of severely Rhodopsin-deficient photoreceptors

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Vitamin A deprivation (VAD) leads to night blindness and is the leading cause of preventable childhood blindness. Chronic VAD has been shown to severely damage the photoreceptors, but the molecular impacts remain poorly understood. Like humans, *Drosophila* need vitamin A to synthesize light-sensing pigments such as Rhodopsin 1 (Rh1). In vitamin A-deprived flies, the light-sensing compartments (rhabdomeres) of the photoreceptors are underdeveloped, and immature Rh1 accumulates in the cell body. Previously, we discovered a novel transmembrane protein, Major photoreceptor stabilizer (Mps), which is expressed during early photoreceptor development in wild type. Mps is maintained into adulthood in vitA-/Rh1-deficient flies and stabilizes the damaged rhabdomeres. However, the molecular mechanism that mediates this stabilization remains unclear.

A yeast-two-hybrid screen with the cytoplasmic tail of Mps as a bait yielded InaD, a photoreceptor-specific scaffolding protein, as a potential interactor. We validated the Mps-InaD interaction *in vivo* by performing co-immunoprecipitation (co-IP) with CRISPR-engineered *mps-V5* flies and a transgenic *GMR>inaD-GFP* line. In addition, we validated the Mps-InaD specifically in photoreceptors *in vivo* by performing proximity labeling. To this end, we expressed a TbID-V5::Mps fusion protein with the photoreceptor-specific *rh1* promoter to biotinylate Mps's interaction partners and performed streptavidin pulldown. On immunoblots, we obtained the expected ~75 kDa InaD signal, while no (negative control) signal was obtained for Arrestin 2, an abundant rhabdomeric phototransduction protein. Taken together, the yeast-two-hybrid, co-IP, and proximity labeling results strongly support the model that transmembrane protein Mps stabilizes Rhodopsin-deficient rhabdomeres through a physical interaction with the scaffolding protein InaD.

In conclusion, this study provides insights into a novel molecular mechanism that stabilizes damaged light-sensing compartments of severely Rhodopsin-deficient photoreceptors. This will enhance our understanding of how sensory neurons adapt to sensory receptor deficiency on the molecular level and promote their survival.

270F The Hippo signalling pathway regulates the timing of epithelial cell replacement during *Drosophila* abdominal morphogenesis

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Developmental apoptosis plays an important role in shaping the organs of developing animals. Such a process occurs during the development of the adult fly abdomen, in which the apoptosis of larval epidermal cells (LECs) is delicately balanced with the proliferation of the presumptive adult epithelial cells. How the correct timing of LEC clearance is achieved during pupal development, and the signalling pathways involved in the coordinated regulation of this process, are at present poorly understood. Through a series of live imaging experiments, we have discovered that the Hippo signalling pathway plays a role in regulating the timing of LEC clearance during abdominal development. Downregulating Hippo pathway activity promotes LEC survival into adulthood, whereas upregulating Hippo signalling induces premature LEC removal from the epithelium. Crucially, this effect was found to be dependent upon Hippo pathway-mediated transcription, with increases in the expression of pro-survival genes and increased nuclear localization of the Hippo effector Yorkie occurring in LECs during early pupal development. These findings illustrate a novel role for Hippo signalling in abdominal morphogenesis and highlight a biologically relevant example of the pathway's function as a regulator of developmental apoptosis.

271F The Effect of the Western Diet on Ferroptosis of Kidney Like Tissue

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Age and diet represent primary factors contributing to the development of chronic diseases, including Chronic Kidney Disease (CKD) which is characterized by progressive degeneration of renal tissue. Components of the modern Western diet, specifically high fat, salt, and sugar content, have been established as major contributors to the development of CKD. These diets have demonstrated the capability to induce renal oxidative stress. Ferroptosis, a regulated form of cell death categorized by iron-dependent lipid peroxidation, has been implicated in renal oxidative stress. However, the development of CKD due to dietary nutrient overload in an aging population remains inadequately understood, so we investigated how aging and diet interact in the onset of chronic disease. Special high fat, high salt, and high sugar food was prepared for multiple drosophila populations by mixing additional coconut fat, sodium chloride, and sucrose into normal liquid Drosophila media before cooling. The Drosophila were exposed to the special diets after reaching maturity and were left on those diets until their death. Here we show that exposure to high salt and high fat diets causes damage in dissected Drosophila kidney-like tissue (Malpighian Tubules, MT) via ferroptosis in aging populations. We found individuals exposed to diets of as low as 3% fat and 0.6% salt exhibit significantly decreased longevity and accelerated onset of renal ferroptosis, however low and high sugar concentrations showed no significant change. Higher concentrations demonstrate faster degeneration, individuals only 1 week old on 20% fat or 1.8% salt diets demonstrate comparable ferroptosis levels to subjects multiple weeks old but on a standard Drosophila media. Our results demonstrate how much the rate at which oxidative stress of renal tissue is accelerated by components of the Western diet. We expect this research to act as a foundation for future genetic and mechanistic studies aimed at identifying pathways that drive the development of ferroptosis and CKD, with potential implications for advancement of novel therapies targeting ferroptosis and improving healthspan and lifespan.

272F Investigating the Role of Caspases in Tumor Growth

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Tumor cells have a robust survival machinery that helps them to evade processes that try to eradicate them. One such strategy is evasion of cell death. However, this mechanism has many missing links. *Drosophila*, the common fruit fly, has been extensively used to develop tumor models and study the interaction between tumor cells and wild type cells. Previous studies have shown that Dronc, the initiator caspase in *Drosophila*, can initiate a non-apoptotic signaling cascade in tumor cells and help them compete out the wild type cells. Factors such as crosstalk with other signaling pathways, signal amplification loops, exocytosis, and intercellular interactions like apoptosis-induced proliferation are the driving forces behind the survival advantage of tumor cells over neighboring wild type cells. How the tumor cells switch Dronc to a non-apoptotic pathway, how tumor-bordering wild type cells undergo cell death, and how the intercellular communication happens in this niche remain unanswered questions and warrant further investigations. Here, we present our findings based on immunohistochemistry, morphological assays and immunoblotting, to illustrate the function of Dronc in tumor growth and progression.

Keywords: Dronc; cell competition; apoptosis-induced proliferation, tumor proliferation; immunohistochemistry; immunoblotting

273F Polyploid cells exhibit enhanced resistance to heat stress in *Drosophila* larval tissues

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Polyploidization, or whole-genome doubling, is a common feature of many solid cancers and has been implicated in therapy resistance and cancer recurrence. Polyploid cancer cells (PCCs) often survive cytotoxic stress such as irradiation or chemotherapy, but the cellular mechanisms that underlie this resilience remain poorly understood. While PCCs are known to resist radiation-induced apoptosis, it is unclear whether their tolerance extends to other types of stress and whether distinct molecular mechanisms are involved. To address this question, we used the *Drosophila* larval tissues as an in vivo model to examine how polyploid cells respond to heat stress. Third-instar larvae were subjected to acute heat shock, and cell death was assessed by immunostaining for activated Dcp1 and other markers of apoptosis and necrosis. Preliminary results show that polyploid cells exhibit significantly fewer apoptotic and necrotic signals compared to diploid cells under the same stress conditions, indicating a strong resistance to heat-induced cell death. These findings suggest that polyploid cells possess a broad stress tolerance beyond radiation, potentially through stress-specific regulatory pathways. Ongoing work is focused on identifying molecular mechanisms responsible for this heat stress resilience, including candidate factors implicated by our RNA-seq data such as JAK/STAT signaling pathway. Together, this study highlights the utility of Drosophila epithelial models for dissecting stress response mechanisms in polyploid cells and may provide insight into how PCCs contribute to cancer recurrence through enhanced stress adaptability.

274F Evaluating cell competition genes in Drosophila wing discs

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Diamond Blackfan Anemia (DBA) is a genetic form of anemia that results from haploinsufficiencies in Rp genes, for example Rps19. DBA is characterized by erythropoietic defects and by congenital anomalies. Interestingly, DBA patients also experience higher cancer rates. Mutations in Rp genes also occur in sporadic human cancers. In Drosophila, Rp gene mutations lead to the 'Minute' phenotype of developmental delay and thin adult bristles. In mosaics, cells that are haploinsufficient for an Rp gene are outcompeted by wildtype cells through an active process of apoptosis that affects Rp haplo-insufficient cells at close distances. One function of cell competition is to select against aneuploid cells that arise spontaneously. Because 80 eukaryotic Rp genes are scattered throughout the genome, aneuploidy often results in Rp gene haploinsufficiency. Cell competition depends on expression of the transcription factor Xrp1 in the loser cells, suggesting that the Rp haploinsufficient cells may be recognized and targeted for elimination by one or more transcriptional targets of Xrp1. To explore the mechanism, we use a novel method that exploits FLP-FRT recombination to generate clones of aneuploid, Rp haploinsufficient cells in wing discs, we will use Gal4-driven gene expression to selectively elevate or knock down gene expression of Xrp1 targets specifically in aneuploid clones. If a gene is **up**regulated in Minute cells by Xrp1, we will **down**regulate this gene to see whether cell competition is disrupted. If a gene is **down**regulated in cell competition, we will **up**regulate that gene in Minute cells to see whether cell competition is prevented. In addition to assessing transcriptional targets of Xrp1 for their roles in cell competition, we can also evaluate pathways that have already been implicated in cell competition by other studies, such as flower, azot and Sparc, innate immunity genes, and Dpp and Jnk signaling pathways. Because most cancer cells are aneuploid, it is possible that mechanisms of cell competition that are conserved might be important in the development or prevention of human cancer.

275S Stress in Ribosomal Protein Mutants

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Cancer is characterized by aneuploidy, or the gain or loss of chromosomes. Aneuploidy frequently affects ribosomal protein (Rp) copy number because Rp genes are dispersed randomly throughout the genome. Consequentially, the gene dose of Rp genes are frequently affected in cancer genomes. In *Drosophila melanogaster,* heterozygous Rp mutants activate Xrp1, a transcription factor that also mediates part of the transcriptional response of p53. Accordingly, DNA damage and other stresses also activate Xrp1. In Rp mutant imaginal discs, Xrp1 activates genes involved in DNA repair and cell cycle arrest. It is unknown why this response occurs. We hypothesize that Xrp1 adapts cells to some stress caused by Rp haploinsufficiency and aim to identify the stress that Rp haploinsufficiency produces. We hypothesize that Xrp1 RpS3 mutant *Drosophila*, lacking the adaptation provided by Xrp1 expression, may reveal a stress caused by RpS3 haploinsufficiency. We will describe the sensitivity of Xrp1 RpS3 mutants to stress-inducers such as heat or the drugs cisplatin, which causes DNA crosslinks, paraquat, sodium arsenite, and acetaminophen, which cause oxidative stress, or 5-fluorouracil and hydroxyurea, which cause replication stress. We will investigate the metabolic differences in Xrp1 Rps3 mutants using Liquid Chromatography-Mass Spectrometry (LC-MS) of *Drosophila* third-instar larvae wing imaginal discs and compare them to those of wild-type larvae wing imaginal discs. If Rp haploinsufficient strains are experiencing stress due to Rp gene haploinsufficiency, they may accumulate genetic modifications over time. We are generating new Rp haploinsufficient strains isolated in the presence of rescuing genomic transgenes so that the unmodified Rp gene mutant phenotype can be obtained at any time using a simple genetic cross to remove the rescue transgene. Better understanding of the effects of Rp haploinsufficiency may help understand cancer and Diamond Blackfan Anemia, a ribosomopathy often resulting from Rp gene haploinsufficiency.

276S Using Drosophila to investigate interactions between mammalian regulators of apoptosis Bax and Bcl-xL, and the mitochondrial porin VDAC

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Major regulators of apoptosis in mammalian cells are the Bcl-2 family proteins. These include the pro-apoptotic Bax and the anti-apoptotic Bcl-2 and Bcl-xL proteins. When activated, Bax forms complexes on the mitochondrial outer membrane, causing the release of Cytochrome c, triggering apoptosis. Bcl-2 and Bcl-xL inhibit apoptosis by binding to Bax and blocking its activity. The activities of Bax and other Bcl-2 family proteins also influenced by the mitochondrial porin called Voltage-dependent ion channel (VDAC) found in the mitochondrial outer membrane; however, the nature of their interactions is poorly understood. Murine Bax is a potent inducer of cell death when expressed in *Drosophila*. Expression of Bax in wing or eye induces cell death, causing loss of wing tissue and rough eye phenotype. We are developing *Drosophila* as a model system for investigating interactions of mammalian Bcl-2 family members and VDAC. We show that co-expression human Bcl-xL with Bax completely blocks Bax activity, restoring normal wing development. We constructed transgenic lines expressing mouse *Vdac1* and *Vdac2*. We observe that *Vdac1* can rescue the lethal phenotype of mutant *Drosophila* VDAC ortholog *porin*. Using these phenotypes as an output we are investigating the interactions between Bax, Bcl-xL, and VDAC. We are currently testing whether observed similarities in amino acid sequence between BH3 domains in Bcl-2 family proteins and the N-terminal domain of VDAC are sites of protein interactions that regulate apoptosis.

277S scRNA-Seq Reveals Tumor- and Stress-Induced Changes in Drosophila Follicle Cells

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Efficient cell death and clearance are essential for maintaining tissue homeostasis during development, as persistent apoptotic debris can disrupt morphogenesis and promote disease. While specialized professional phagocytes handle most clearance events, epithelial neighbors often serve as auxiliary non-professional phagocytes (NPPs) to ensure complete debris removal. The Drosophila melanogaster ovary provides a powerful in vivo model for dissecting NPP biology during developmental germline cell death. Recently, we discovered that overactivation of the Notch signaling pathway (via NICD overexpression) can prematurely drive the transformation of follicle cells into NPPs and halt oogenesis at mid-stages. While such events naturally occur under stress conditions such as nutrient deprivation, NICD overexpression strongly reinforces this phenomenon more efficiently, providing direct confirmation of its establishment. These findings open a new window into understanding the regulation of non-professional phagocytosis and its developmental consequences. Using single-cell RNA sequencing of wild-type and tumor host ovaries, we identified transcriptional states and gene networks specifically enriched or depleted in NPPs under tumor-bearing conditions. Candidate genes such as Hnf4, Fer2LCH, Dor and many more were selected due to their differential expression and putative roles in cytoskeletal remodeling, metabolic adaptation, and engulfment. Analysis revealed diverse clearance defects, including persistence of apoptotic nurse cells, disrupted epithelial organization, no efficient membrane expansion, and impaired engulfment machinery. These findings highlight the molecular diversity of NPP programs and uncover tumor-induced vulnerabilities in their clearance machinery, offering new insight into how tissue-resident phagocytes integrate apoptotic recognition, metabolic reprogramming, and cytoskeletal control to preserve tissue integrity.

278S Fundamental role of iPLA2 in mitochondrial quality control

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The Parkinson's disease (PD) genes *pink1* and *parkin*, which respectively encode a mitochondrially targeted protein kinase, and an E3 ubiquitin ligase, participate in a key pathway of mitochondrial quality control that eliminates damaged mitochondria. However, how Parkin precisely and effectively responds to PINK1 activation on damaged mitochondria remains poorly understood. In this study, we established a genetic screen to identify PINK1-dependent Parkin receptor, through a PINK1/Parkin-induced cell death model in *Drosophila*, and found that knocking down *iPLA2-VIA*, encoding iPLA2, an enzyme that catalyze the hydrolysis of phospholipids (PLs), rescued severe degeneration of retina cells induced by co-expression of PINK1 and Parkin. Here, we demonstrated that mitochondrial located iPLA2 remodels mitochondrial membrane by controlling the ratio of PLs to lysophospholipids (LPLs). Mechanistically, we found that PINK1 activates iPLA2 through phosphorylation to catalyze PLs to LPLs in damaged mitochondrial membrane. Subsequently, iPLA2 improved ubiquitin binding to mitochondria by increasing mitochondrial membrane fluidity, promoting Parkin mediated proteasomal degradation of mitochondrial proteins. Remarkably, we found that both in *Drosophila* and mammalian cell, iPLA2 is essential for Parkin inducing mitophagy rapidly and therefore to maintain mitochondrial homeostasis, which can be suppressed by PD related mutation and proximal phosphorylation mutations.

279T Using deep learning sequence models to understand nucleosome and 3D genome organization in the early *Drosophila* embryo

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Transcriptional regulation is encoded in the DNA sequence, which specifies transcription factor binding, chromatin accessibility, enhancer activation and target gene expression. A poorly understood element is how DNA sequence encodes the three-dimensional (3D) genome organization and how these sequence features impact gene regulation. While individual DNA motifs have been implicated in shaping genome architecture, the broader, combinatorial sequence rules linking 3D genome organization and gene expression remain elusive. To address this gap, we trained a BPNet model (as part of the BPReveal package) on MNase-nucleosome data from the early *Drosophila* embryo from DNA sequence, extracted the learned motifs and analyzed the role of insulator motifs. Moreover, we trained Akita to predict the pairwise contacts of Micro-C data in the early Drosophila and conducted perturbation tests to understand the role of insulator motifs. We found that some motifs show a similar perturbation response in the nucleosome model and the Micro-C model, raising the possibility that nucleosomes play an underappreciated role in the 3D chromatin organization. We are currently probing the models to understand how insulators cooperate in nucleosome organization and TAD boundary formation.

280T Phosphorylation as a modulator of HP1a function

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The Heterochromatin Protein 1 (HP1) protein family was named due to its association with heterochromatin. Three decades of studies have shown in multiple models that while typically, each species has a family member that contributes to heterochromatin formation and maintenance, HP1 proteins can have a range of functions in the nucleus, including in DNA repair, gene regulation, and replication. HP1a in *D. melanogaster* is best known for its role in heterochromatin, but it also has multiple functions. For example, it is both a repressor and an activator of transcription. How HP1a carries out multiple distinct functions is poorly understood, but its post-translational modifications, particularly phosphorylation might play a role. We generated two new HP1a alleles, replacing serines S89, S90, and S91 either with glutamic acid (E) or alanine (A). The SA allele blocks phosphorylation at these sites, while the SE allele mimics the charge of phosphorylation. Homozygous phospho-block SA/SA animals died as embryos, while homozygous phospho-mimic SE/SE animals survived to adulthood. In all mutants, HP1a mRNA is stable and present at levels comparable to wildtype control strains. HP1a protein levels differ between the mutant and control strains, which generally show similar levels of phosphorylation overall. Both phospho-mimic SE and phospho-block SA alleles modify position effect variegation with the impact depending on the specific reporter. Lifespan assays suggest that the SE allele has beneficial effects, particularly in females, while the presence of an SA allele decreased lifespan in males compared to controls. In some of the groups, the positive effect on lifespan is accompanied by increased physical activity early in life. SE homozygous animals also show genetic-background-dependent sterility. Finally, transcriptome analysis confirms that the SA and SE alleles cause large-scale changes to the transcriptome, with the SA allele causing stronger dysregulation. The types of genes affected are diverse, with one prominent category being linked to reproduction. On-going studies investigate the chromatin changes precipitated by the SA and SE alleles. Together, our studies to date demonstrate that regulation of phosphorylation at S89, S90, and S91 is important for the multifunctionality of HP1a, particularly its role in silencing and gene regulatory functions.

281T Auxin-mediated knockdown of cohesin subunits in *Drosophila* embryos

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The cohesin protein complex is an essential regulator of chromatin organization and transcription that is conserved across all eukaryotes. The cohesin complex consists of a tripartite ring of SMC1, SMC3, and Rad21 that associates with chromatin through Nipped-B, is localized to regions of interest through SA1, and is released from chromatin by Wapl. Cohesin encircles chromatin *in cis* to form DNA loops throughout interphase, regulating chromatin architecture and bringing together distal regulatory elements to facilitate transcription. Cohesin additionally encircles sister chromatids *in trans* to ensure the proper segregation of chromosomes during mitosis. In mice and zebrafish, cohesin has been shown to help facilitate transcription in early embryogenesis during zygotic genome activation (ZGA). However, previous works have not been able to achieve a complete knockdown of cohesin in a developing organism without causing fatal mitotic defects that obscure the resulting phenotype. To investigate how cohesin functions during ZGA in the Drosophila melanogaster embryo, we used CRISPR/Cas9 genome editing to introduce an auxin inducible degron (AID)-GFP tag to the C-terminus of the cohesin subunits Rad21, SMC1, SA1, Nipped-B, and Wapl. We have quantified the nuclear expression levels of each of these proteins in the pre-ZGA (nuclear cycles 11-13) and ZGA (nuclear cycle 14) Drosophila embryo by imaging the AID-GFP tag alongside a fluorescent histone marker using confocal microscopy. We have optimized the AID system to achieve ≥90% depletion of Rad21, SMC1, and SA1 during nuclear cycle 14 with minimal mitotic defects during prior cleavages. High doses (50 mM) of auxin in PBS are sufficient to permeate the vitelline membrane, and treatment beginning at mitosis 10 yields protein knockdown with half lives ranging from 13-25 minutes. Rad21-depleted embryos cellularize and initiate gastrulation normally, but display widespread defects during zygotic resumption of the mitotic program. The AID system is a powerful tool to perform a conditional knockdown of cohesin, allowing us to separate its role in transcription from its role mediating the cell cycle.

282T Leveraging genetic divergence to uncover mechanisms of genomic organization

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Gene expression is governed by the organization of the genome within the nucleus into active and inactive compartments. Active regions are gene rich and accessible to transcription factors. By contrast, repetitive DNA is localized to inactive nuclear compartments. These repetitive elements must be silenced through the formation of constitutive heterochromatin, which clusters in the nucleus to form chromocenters. This nuclear organization helps ensure robust silencing of repeats through cell divisions. Failure to properly establish this chromatin architecture leads to developmental defects and cancer. Nonetheless, the mechanism by which the subnuclear regions are established to direct gene expression and development remains unclear. To provide fundamental insights into how repetitive sequences are regulated, we focus on the early stages of embryo development when genome organization is established. During the maternal-to-zygotic transition, the transcription factor GAGA Factor (GAF) binds and silences GA-rich satellite repeats. Simultaneously, GAF activates transcription of genes located in active compartments. Reflecting this dual role, GAF is non-uniformly distributed in the nucleus – present in diffuse populations in active compartments or concentrated foci in inactive compartments. Yet how a protein performs opposing functions in both active and inactive regions of the same nucleus remains poorly understood. We hypothesize that to simultaneously direct gene expression and heterochromatin formation GAF levels are precisely balanced with the number of binding sites. We aim to test this by altering the ratio between repetitive regions and the transcription factor that binds them and determining the effects on silencing. To accomplish this, we leveraged the evolutionary divergence between two Drosophilids as a tool to endogenously lower repeat abundance, while maintaining GAF levels. We use high-throughput sequencing to test the effect of reducing satellite abundance on chromatin accessibility (ATAC-seq) and gene expression (total RNA-seq). We find that perturbing the ratio of GAF to the satellite it binds results in increased chromatin accessibility over transposable elements that are normally silenced. By combining evolutionary, genetic and biochemical approaches, this work will provide fundamental insights into mechanisms by which repetitive elements are silenced and how this contributes to nuclear organization.

283T Histone Writer and Residue Mutations Reveal an H3K36me2/me3 Transcriptional Switch

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Animal cells employ histone post-translational modifications (PTMs) to regulate fundamental developmental processes. Direct attribution of these functions to specific histone tail residues has been difficult due to redundancies within histone multi-gene families, and their variants. Further complicating interpretation, most chromatin-modifying enzymes have non-histone substrates. To parse the relative contribution of histone tail residues and their cognate writer enzymes, we have developed a histone gene replacement system. Histone H3 lysine-36 trimethylation (H3K36me3), deposited co-transcriptionally by the lysine methyltransferase (KMT) Set2, marks gene bodies. Our previous work showed that X-chromosome gene expression changes due to H3K36 and Set2 mutation are context-dependent and often in apposition (hereafter called "switch" genes). Here, we employ genomic approaches in combined *H3.3*^{K36R}*H3.2*^{K36R} and *Set2*Δ mutants to gain insight into the mechanism mediating opposing transcriptomic changes in these mutants.

Genome-wide total RNA-seq analysis of *Set2*Δ and *H3.3*^{K36R}*H3.2*^{K36R} mutants shows that "switch" genes are present on all chromosomes, not only the X. Intriguingly, analysis of a Set2KD S2 cell dataset suggests that H3K36me2 may act in opposition to H3K36me3 at these genes. Several indirect readouts of RNA polymerase II processivity suggest that *Set2*Δ and combined *H3.3*^{K36R}*H3.2*^{K36R} mutations may exert opposite effects on this metric. A number of H3K36-methyl readers have potential to regulate effects on transcript abundance, including proteins that regulate histone acetylation (Mrg15 in the context of NuA4) and deacetylation (Rpd3). Analyses of publicly available chromatin profiling data in conjunction with our RNA-Seq, reveal that Rpd3 and Mrg15 are most enriched at one cluster of switch genes, comprised of highly acetylated, high expression, low complexity genes. Cut and Tag chromatin profiling demonstrates that the distribution of H3K36me2 is shifted towards the 3' end in *Set2*Δ mutants and eliminated in the *H3K36R* mutants. Surprisingly, we observe global hypo-acetylation of the H4 tail in both *Set2*Δ *and H3.3*^{K36R}*H3.2*^{K36R} with a much more severe effect in the combined H3K36R mutant, revealing that H3K36me2 and me3 are critical for maintaining genome acetylation. These data suggest that transcriptomic changes at switch genes may be mediated by a mechanism other than H4 acetylation.

284T Identification of RNA modifications and their implications in alternative splicing and polyadenylation

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Currently, over 170 RNA modifications have been identified in RNA- far exceeding the number - discovered on DNAs and proteins. Some of these modifications are regulated by a group of proteins called "writers", "readers" and "erasers". The writers catalyze the modification, while readers interpret the functional consequence, and erasers remove the modifications. Our analyses focus on the transcriptome wide detection of these modifications and the biological roles of N^6 methyladenosine (m6A), 5-methlycytosine (m5C) and Pseudouridylation (Psi) on *Drosophila melanogaster* mRNA. This is because, m6A is the most the abundant and common modification in eukaryotic transcripts and occurs on all RNA species. The modification is mediated by the writer, methyltransferase complex which is composed of the central catalytic subunit- methyltransferase-like 3/14 (Mettl3 and 14), and its associated proteins such as WTAP, and nito, vir, fl(2)d genes. m5C is a DNA/RNA modification and is regulated by the writer, RNA m5C methyltransferase such as the NSUN family proteins and DNA methyltransferase 2. The modification is mediated by the readers ALYREF and Y-box binding protein 1 and can be reversed by Ten-eleven translocation family protein. And the irreversible conversion of uridine to Pseudouridylation(Ψ) by Pseudouridine Synthases (PUS) have been implicated in many cellular processes in other organisms. However, traditional transcriptome analyses based on high throughput cDNA library constrains the ability to detect RNA base modifications and to directly associated them with alternative polyadenylation an splicing events. This limitation, along with the lack of genetic perturbation models such as knockout, has restricted comprehensive analysis of the regulatory roles of m6A, m5C and Psi. To address this, we generated transcriptome wide maps of these three modifications using direct RNA sequencing from one-week-old Drosophila head for *FR113N*, *w1118* and *Mettl3* knockout samples. Our analyses identified novel modification sites across all datasets and revealed their roles in alternative splicing and polyadenylation. The most modified genes detected after differential methylation analysis varied across all datasets: in *w1118* replicates, the top m6A-modified genes were *Mhc*, *Mlc2* and *Ald1*; for m5C, *ninaE*, *Mlc2* and *Adhr*, and for Psi, *Ald1*, *Mhc* and *SERCA*. In *FR113N* replicates, the top m6A modified genes were *IA-2*, *ninaE*, and *cpx*; m5C modified genes were *ninE*, *Arr1* and *Adhr*; and Psi modified genes were *cpx*, *IA-2* and *ninaC*. Our results demonstrate the broad distribution and regulatory potential of m6A, m5C and Psi in Drosophila, particularly in relation to splicing and alternative polyadenylation.

285T The genetic basis of mitotic lethality of the *359-bp* satellite in *Drosophila* hybrids

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The *359-bp* satellite repeat (also called the 1.688 gm/cm^3 repeat) is widespread among the *melanogaster* group fruit flies. It forms a large pericentric satellite on the X chromosome of *D. melanogaster* but is nearly absent in *D. simulans*. This satellite is the primary cause of embryonic lethality in F1 female hybrids of *D. simulans* ♀ X *D. melanogaster* ♂, with lethal daughters displaying failed mitotic segregation and anaphase bridges containing the 359-bp repeat. Maternal factors of *D. simulans* are also required for this hybrid lethality phenotype and previous studies have found substantial variation among *D. simulans* strains for their degree of hybrid embryonic lethality. However, identifying these maternal factors has been challenging because hybridization rarely occurs in this cross, rendering QTL mapping prohibitive. Here, we use a highly recombined pool of *D. simulans* formed by intercrossing two strains with opposite levels of hybrid lethality for over 50 generations. We were able to increase the hybridization rate with environmental stimuli and sequenced 183 recombinant individuals associated with either no lethality or full lethality in their hybrid female offspring. QTL mapping with these recombinants reveals a major locus on chromosome 3L. In addition, two loci on 2L and 3R are associated with weaker lethality effects. Because only individuals with extreme phenotypes were sequenced, there is likely additional modifiers to this phenotype but below our detection power. Our results therefore suggest that hybrid lethality is a polygenic trait in *D. simulans*. Currently, we are performing transgenic allele-swap between parental strains to test for causal genes near the major QTL loci.

286T Establishing the functional landscape of the D. melanogaster B chromosome centromere

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Most models of karyotype evolution are inferred from comparative genomics of chromosomal rearrangements between species separated by millions of years of evolution, obscuring the context of how chromosomes change. Chromosome fusion is a structural rearrangement that plays a major role in shaping karyotypes but can lead to the formation of dicentric chromosomes that are prone to chromosome instability. Evidence of the successful stabilization and maintenance of dicentric chromosomes exists in nature, but research into the mechanism(s) behind dicentric chromosome stabilization is still ongoing. The supernumerary, non-essential B chromosomes that arose less than 25 years ago in *Drosophila melanogaster* are a newly established model system that has the potential to provide valuable insights into how new chromosomes form and stabilize. Our current model predicts that the B chromosomes arose from a fusion of two centric fragments that originated from chromosome 4, leading to a dicentric chromosome that is not prone to chromosome instability. To test this model, we performed fluorescent *in situ* hybridization (FISH) on metaphase chromosome spreads using probes that bind specifically to the chromosome 4 centromeric island *Lampedusa*. Our preliminary data indicates that *Lampedusa* is enriched on the B chromosomes compared to chromosome 4, which is consistent with our model. We are currently exploring if both centromeric islands are capable of forming active centromeres by examining the location and enrichment of an active centromere marker, CENP-C, on the B chromosomes as compared to the essential chromosomes. Together, this work will provide the foundation for using the B chromosomes as a model system for the empirical study of *de novo* chromosome formation and dicentric chromosome stabilization, ultimately leading to a deeper understanding of how new chromosomes arise and how chromosome stability is maintained after fusion.

287T Family Matters: Understanding expression of histone sibling genes

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Replication-dependent histones are some of the most highly conserved proteins across eukaryotes. Their regulatory patterns are also largely conserved: the expression of histone genes dramatically increases during S phase and expression outside of S phase is a phenotype of diseases in humans. Although the amino sequence and cell cycle regulatory patterns of histones are similar between eukaryotes, the DNA coding sequence, cis regulatory elements, and genomic organization of the histone genes have diverged widely. How the precise cell cycle expression patterns of histone genes are maintained in spite of diverse genomic organization and regulatory mechanisms remains a mystery.

Eukaryotic genomes often carry many copies of the histone genes to compensate for the extreme transcriptional demands required to produce enough histone protein products. While histone gene copy number varies widely, there are commonalities in general histone gene organization across the genomes of numerous organisms, including fruit flies and humans. One common gene organization is the clustering of histone genes, suggesting that clustering contributes to regulation. I aim to determine how clustering contributes to histone gene expression throughout development.

There are several assumptions about histone gene regulation within and between clusters, as the identical nature of histone coding sequences is prohibitive in probing expression patterns. One assumption is that the histone mRNA production is proportional to the number of genes within a cluster. To tackle this assumption I took advantage of the model system Drosophila virilis, which has two asymmetrical histone loci: a major locus of ~175 histone genes, and a minor locus with ~30 histone genes. A useful natural feature within the Drosophila virilis genome is that there are single nucleotide variations between the genes at the major and minor histone loci. To determine histone mRNA expression throughout early embryonic development, I performed droplet digital PCR with Taqman probes specific to either locus. I determined that histone transcription is relatively equal between the dramatically asymmetrical loci in the first four hours of embryonic development. This result refutes the assumption that the number of histone genes within a cluster determines the transcriptional output.

288T SET8 Contributes to DNA Replication During Early Drosophila Development Independently of H4K20me1

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A key aspect of animal development is variation in cell cycle modalities that involve shortening or lengthening of different phases. Several major metazoan phyla contain species in which early embryogenesis is characterized by rapid cleavage cycles that lack gap phases and dramatically reduce the length of S phase, including to as short as 3 minutes during the first few cycles of *Drosophila* embryogenesis. These cycles require substantial alterations to the DNA replication program, but the mechanisms behind these changes remain incompletely understood. Monomethylation of lysine 20 of histone 4 (H4K20me1) has been implicated in regulating DNA replication, primarily through perturbations of the enzyme that establishes this mark, SET8. However, SET8 has non-catalytic functions and non-histone substrates, so phenotypes observed from SET8 perturbation cannot be solely attributed to the loss of H4K20me1. Indeed, flies engineered to express only the unmodifiable H4K20A mutant histone survive to adulthood whereas homozygous SET8 null mutant animals are not viable. This phenotypic difference suggests that SET8 has developmental functions beyond its role as an H4K20 lysine methyltransferase. We CRISPR-engineered an allele of *Set8* encoding a single Arg to Pro substitution in the catalytic domain of SET8 that is predicted to eliminate methyltransferase activity. Unlike *Set8* null mutants, homozygous *Set8*^{RP} mutant flies are viable. However, eggs laid by *Set8*^{RP} females never hatch regardless of the paternal genotype, indicating a defect in early embryogenesis, which is governed by maternally deposited gene products. The rapid S-M cleavage cycles are defective in embryos from *Set8*^{RP} females: there is a high incidence of anaphase bridges and lagging chromosomes during mitosis, abnormal nuclear distribution, and variable degrees of chromatin compaction, as revealed by EdU labeling and live imaging. In these embryos, SET8^{RP} protein levels and localization are similar to wild type SET8, suggesting that intact methyltransferase activity of SET8 is required for replication during the cleavage cycles. Surprisingly, nuclei in embryos from *Set8*^{RP} mothers that fail to replicate still contain abundant amounts of H4K20me1, suggesting there are mechanisms other than SET8 for depositing and/or maintaining H4K20me1 in the early embryo. We propose that SET8 has substrates other than H4K20 needed for the rapid S phases of early development.

289T Y-linked variation in *D. melanogaster* acts as a regulator of the epigenome

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Heterochromatic regions of eukaryotic genomes are gene poor, yet they exert broad trans-acting effects on gene regulation through competition for shared DNA-binding proteins and regulating factors. Studies with model organisms have revealed numerous ways in which heterochromatin abundance and organization shape cellular function, including regulation of transcription, timing of zygotic genome activation, and modulation of the immune system. Building on this foundation, we test a targeted sink hypothesis, positing that specific heterochromatic repeats sequester regulatory proteins by means of shared binding motifs. We generated a series of genetically identical fly lines varying only by their Y chromosomes taken from around the world to capture global Y chromosome diversity (Y-replacement lines). Quantifying the repetitive content across the Y-replacement lines we observe over 10 million base pairs of satellite sequence lost or gained, and RNA-seq of male heads identifies more than 600 differentially expressed genes. To identify candidate proteins under the influence of a sink effect, we applied CUT&RUN against various TFs and histone modifications to quantify the epigenetic variation across these Y-replacement lines. For example, the GAGA factor (GAF) appears to be sequestered by a subset of Y-linked satellites, which appears to attenuate the CUT&RUN signal on GAF-regulated autosomal genes. These findings suggest that specific Y-linked heterochromatic repeats have trans-acting regulatory effects on specific subsets of genes that share binding motifs. The potential evolutionary consequences of this have not escaped our attention.

290T dAnp32 functions with Jabba to promote embryonic development

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During early embryonic development, the chromatin landscape undergoes a major transformation which alters transcriptional activity. This transformation is in part regulated by the exchange of canonical histones (*e.g.*, H2A) with histone variants (*e.g.*, H2A.Z), mediated via numerous histone chaperones. In vertebrates, one such chaperone is Anp32e. In zebrafish embryos, loss of Anp32e leads to premature accumulation of H2A.Z at developmentally regulated promoters. Several mechanisms have been proposed for Anp32e function: *In vitro* studies demonstrated that Anp32e can evict H2A.Z from the nucleosome; in cultured cells, Anp32e was shown to protect H2A.Z in the cytoplasm. Yet it remains unclear how Anp32e regulates H2A.Z during development. To address this gap, we use *Drosophila* Anp32 (dAnp32) as a model. In early embryos, dAnp32 is present both in the cytoplasm and nuclei and shuttles between them, as assessed with multiple YFP/GFP trap lines. Using a proximity ligation assay, we found positive signal for dAnp32-H2A.Z in both the cytoplasm and nucleus, suggesting that dAnp32 interacts with H2A.Z in both compartments. These findings are consistent with models in which dAnp32 functions as an H2A.Z evictor in the nucleus or to cytoplasmically sequester H2A.Z or to shuttle H2A.Z between compartments. To determine what role dAnp32 plays during early development, we generated *dAnp32-null* flies. Homozygous stocks are viable and fertile; embryos from these stocks display no obvious defects in early embryogenesis and hatch at wild-type rates. However, flies lacking both dAnp32 and the cytoplasmic H2A.Z chaperone Jabba are almost entirely sterile, with hardly any embryos hatching and many displaying severe morphological defects by blastoderm stages. Jabba is known to protect H2A.Z from degradation (by anchoring it to lipid droplets), thus providing an H2A.Z reservoir for the early embryo. Because *Jabba* single mutants still retain a small amount of cytoplasmic H2A.Z, the double mutant phenotype is consistent with dAnp32 and Jabba working synergistically to protect distinct pools of H2A.Z. We will test this model by measuring H2A.Z levels in *dAnp32* embryos, both globally and specifically in the cytoplasm. We will also determine if lack of dAnp32 affects the rate of H2A.Z import into nuclei or the amount of H2A.Z present in chromatin.

291T Understanding the Role of Nuclear Envelope Transmembrane (NET) Proteins in Chromatin Organization

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Nuclear Envelope Transmembrane (NET) proteins are components of the Nuclear Envelope (NE) proteome. They are known to anchor heterochromatin to the nuclear periphery, maintain nuclear structure, regulate gene expression, position chromosomes and regulate the process of myogenesis. Maintaining heterochromatin at the nuclear periphery specifically, is important for ensuring proper gene silencing and genome organization, yet the precise NET tethers of chromatin are not fully known. Some NET proteins have been explored in mammalian models and have been implicated in human diseases. NET39 is a protein that plays a conserved role in proper muscle development. NET39 is downregulated in patients with Emery-Dreifuss muscular dystrophy (EDMD), and genetic knockdown of NET39 leads to severe defects in muscle growth and functions were observed in mice. In our study, we utilized Drosophila as a model to study NET proteins in larval body wall muscle. Our work recently confirmed that NET39 plays a similar role in Drosophila myogenesis. To investigate the role of NET proteins in chromatin organization, we used RNA interference to knock down several NET proteins and analyzed their impact on different markers that are associated with heterochromatin via immunostaining.Our preliminary results show that depletion of NET39 led to a drastic reduction in Heterochromatin Protein 1 (HP1) accumulation in the nucleus. On the other hand, methylation of lysine 9 of histone H3 (H3K9me2/3) levels appear to be normal. HP1 normally recognizes and binds H3K9me2/3, which allows for the assembly of heterochromatin. We hypothesize that binding of HP1 to H3K9me2/3 may be disrupted in NET39 knockdown conditions. We also observed lethality at the early pupal stage, further suggesting that NET39 is important for progression to adulthood. We currently plan to assess markers of gene activation and carry out expression analysis to confirm that gene silencing is impacted. Further studies include deciphering the mechanism by which knocking down NET39 impacts HP1 binding and by utilizing DNA FISH and ChIP-seq to determine the targets of NET39. Overall, our preliminary results point to a new NET that functions as a tether and stabilizer of heterochromatin at the nuclear periphery.

292T Interrogating the role of histone acetylation in zygotic genome activation

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During development, a single precursor generates multitudes of cells with specialized forms and functions via the differential regulation of gene expression. Epigenetic mechanisms help to ensure proper gene regulation without altering the DNA sequence by creating functionally specialized chromatin domains. Within these domains, specific patterns of histone post-translational modifications (PTMs) are proposed to facilitate or repress transcription across successive cell divisions. Despite the tight correlation with different gene expression states, the precise role of histone PTMs in epigenetic gene regulation remains incompletely understood. One challenge to experimental investigation of histone PTM function is the existence of pre-established epigenetic states in cells whose developmental identities have already been established. The early *Drosophila* embryo presents a unique opportunity in this regard because cell identities have not yet been determined and most histone PTMs are initially absent. During the nuclear cycles surrounding zygotic genome activation, histone PTMs appear in a stereotypical sequence along with the emergence of transcription. This time-resolved elaboration of the histone PTM landscape coupled with transcription makes the early *Drosophila* embryo an excellent experimental system to study how specialized chromatin states are established *de novo*. Acetylation of histone H3 lysine 18 (H3K18ac) and 27 (H3K27ac) are amongst the earliest histone PTMs to appear in chromatin and are enriched at the *cis*-regulatory elements of the earliest expressed genes. However, whether H3K18ac or H3K27ac contributes to gene activation in the embryo is not known. Here, we utilize an RNAi based tool to deplete the maternal pool of histones that are deposited into the egg prior to fertilization and replace them with transgenic histones engineered to encode non-modifiable mutant residues. We are examining the requirement of H3K18 and H3K27 alone or in combination through developmental phenotyping, live imaging of nascent transcription, and genomic profiling. This work will help to elucidate the specific roles of H3K18 and H3K27 modification in gene activation and the establishment of specialized chromatin states.

293T Rethinking the basis of dosage compensation of the Drosophila X chromosome

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A popular model to explain X chromosome dosage compensation involves the Male Specific Lethal (MSL) complex that binds the male X bringing along a histone acetyltransferase (MOF) that enriches H4Lys16Ac, a typical mark of gene activation, to double the expression of the single X. Mutations in members of the complex were claimed to reduce the expression of the male X by about half but all these studies, wittingly or unwittingly, normalize the X expression to the autosomes making it ambiguous whether the X is reduced or the autosomes increased. Indeed, examination of those data or experiments determining the absolute expression indicates that the autosomes are inversely increased in expression and the X is not reduced (in general). Trisomy for the X chromosome in flies (metafemales) also shows dosage compensation for most genes and the major effect on the autosomes is a reduced amount with a peak at the inverse level (~67%). These results reflect the phenomenon of the inverse dosage effect of transcription factors in aneuploidy studies, documented now in maize, Drosophila, Arabidopsis, yeast, mouse, human and others. The claim that the male X expression is reduced by half in *msl* mutants without modulating expression across the whole genome is unlikely, given the dosage sensitivity of transcription factors. When the MSL complex is disrupted, there is a redistribution of H4Lys16Ac to be uniform across chromosomes (Cytology: Birchler lab, 1999; ChIP-seq: Akhtar lab, 2021). Such an increase of the histone modification on the autosomes is consistent with the increased expression. Ectopic expression of MSL2 in females organizes the MSL complex on the X's with increased H4Lys16Ac, but there is no increase in expression, while targeting MOF alone to transgenes will increase expression in females without the MSL complex, indicating some component overrides the effect of H4Lys16Ac. Mutations in nucleoporin Megator (Capelson lab) have overexpression of the male X suggesting that they, and potentially other genes, influence the override process. In the context of the results of gene expression in aneuploids across the phyla, it is hypothesized that males and metafemales capitalize upon an inverse dosage effect of transcription factors to bring about dosage compensation of the X chromosome with the MSL complex in males preventing overexpression of the X and indirectly muting an inverse effect on the autosomes by sequestering away MOF.

294F H3K27me3 resolves unique, spatially distributed patterning states in *Drosophila* embryogenesis

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The production of discrete structures that define the body plan during development requires the establishment of positional identity. During zygotic genome activation (ZGA), stable patterns of spatially restricted hox genes are instituted through transient zygotic patterning networks. Hox gene patterns are then maintained over the lifespan of the fly by Polycomb group (PcG) proteins, which prevent ectopic expression by epigenetically silencing genes in the appropriate cells. Although there are clear requirements for PcG proteins in the long-term maintenance of hox gene patterns, how transient patterning networks direct PcG proteins to establish spatially restricted silencing is unclear. A challenge in studying this problem is the embryo is a mixture of many cell states. To deconvolve the role of PcG proteins in spatially organized patterning, we utilize mutant *bcd osk tsl Tl*^{RM9} embryos with reduced developmental patterning. We find that by HCR and RNA-seq, these mutant embryos develop uniformly with a single, neuroectodermal identity. These embryos allow us to resolve the unique regulatory state of hox genes at the level of PcG activity as well as within the neurogenic program. PcG proteins are recruited to developmental genes through the binding activity of Pho, which results in the trimethylation of Histone H3 lysine 27 (H3K27me3), a repressive chromatin modification which is epigenetically inherited. PcG proteins are also responsible for the ubiquitylation of Histone 2A (H2Aub), which aids in the establishment of H3K27me3. We observe in our mutants that ChIP-seq of H3K27me3 reflects the "off" states of patterning genes from the onset of ZGA. We have identified that patterns of H3K27me3 are dynamic; gain and loss of H3K27me3 is surprisingly rapid as cells undergo differentiation. Neurogenic genes are initially decorated with H3K27me3 at ZGA, but are rapidly demethylated when neurogenesis begins. Many of these neurogenic genes depleted of H3K27me3 show a concurrent increase in H3K27 acetylation, a modification associated with active transcription. In contrast, H2Aub and Pho binding are retained at these regions during ZGA and differentiation, suggesting that these regions remain competent to PcG silencing, and that additional factors are required to license H3K27me3 deposition. This study provides a basis to determine the regulatory logic for the establishment and maintenance of H3K27me3 as a function of developmental patterning cues.

295F Sex-dependent Dietary Impacts on the Epigenetic Silencing of Transposable Elements

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Transposable elements (TEs) are “selfish” genetic elements that can copy themselves and “jump” to other locations within the genome. To combat the selfish replication of TEs, eukaryotic hosts deposit repressive epigenetic marks to prevent their transcription and movement. Interestingly, past studies indicate specific dietary conditions can have a profound influence on the epigenetic regulation of the genome, because metabolites derived from diet can act as cofactors, inhibitors, or substrates of histone-modifying enzymes, influencing the addition or removal of epigenetic modifications. Yet, our understanding of how diet may influence the epigenetic silencing of TEs has remained limited. To address this knowledge gap, I used our lab-developed transgenic reporter strains to efficiently quantify the strength of epigenetic silencing of TEs. In these trains, an mCherry fluorescent marker is placed adjacent to a TE insertion to measure the degree of TE epigenetic silencing. The repressive epigenetic marks enriched at the TE can spread into the adjacent mCherry gene, impacting the Drosophila mCherry expression. By rearing these strains under standard, turmeric, and ketogenic dietary conditions, I found that while females exhibited weakened TE epigenetic silencing on both ketogenic and turmeric diets, males surprisingly showed the opposite effect. In this study, we aim to investigate whether the sex differences in TE epigenetic silencing on altered diets between sexes are due to the effect of the Y chromosome, which is known to influence the chromatin environment. We leverage the fact that, in flies, sex is determined by the dosage between the X chromosomes and autosomes, and used attached X^X chromosome stocks to generate females with a Y chromosome (XXY) or males without a Y chromosome (XO). Our results surprisingly showed that both XXY females and XO males exhibit reduced epigenetic silencing of TEs on ketogenic and turmeric diets, suggesting that sex-dependent effect on TEs epigenetic silencing is likely mediated by the Y chromosome, but only in the male tissue environment. Currently, we are working on a series of crosses to analyze how the presence of the Y chromosome affects TEs epigenetic silencing between brothers (XY or XO) and sisters (XX or XXY), which better controls for the overall genetic background. These efforts will expand our understanding of the role of the Y chromosome in mediating sex-difference dietary responses of TE epigenetic silencing.

296F Mechanisms of Rif1-mediated regulation of DNA replication in *Drosophila melanogaster*

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During the early nuclear cycles of Drosophila embryogenesis, the entire genome is replicated at essentially the same time to facilitate rapid divisions. As the embryo develops, this disordered replication program gives way to an organized pattern of replication in which different regions of the genome replicate at distinct points in S phase in a process known as replication timing. Establishment of replication timing during embryogenesis is characterized by the onset of late replication at heterochromatin and the prolongation of S phase, both of which are dependent on Rif1. Rif1 controls replication timing from yeast to humans and localizes to heterochromatin. How Rif1 is recruited to heterochromatin to delay replication, however, is unknown. Here, we investigate the mechanisms of metazoan Rif1 recruitment to chromatin using the Drosophila early embryo as a model system. We performed live imaging of embryos expressing GFP-tagged Rif1 fragments to define the function of each Rif1 domain in chromatin recruitment and replication timing. Rif1 contains N-terminal HEAT repeats, an intrinsically disordered region (IDR), and a C-terminal domain. Surprisingly, we find that the HEAT repeats act as a chromatin-binding domain, while the IDR is neither necessary nor sufficient for localization to chromatin. The IDR, which contains several CDK and DDK motifs, regulates the ability of the HEAT repeats to bind chromatin in a phosphorylation-dependent manner. Furthermore, we show that the C-terminal domain of Rif1 promotes multimerization. In addition to defining how each domain functions in localization, we will also characterize their function in the control of replication timing. By defining how Rif1 associates with chromatin, we aim to provide mechanistic insight into how replication timing is established during development.

297F Investigating the Megator-dependent dosage compensation repressive pathway

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Dosage compensation is a process by which one sex of a gonochoristic organism equalizes sex chromosome expression to match the other. The epigenetic mechanism utilized by Drosophila melanogaster males remains incompletely understood. The male-specific lethal (MSL) complex binds to most of the single X chromosome, resulting in its two-fold upregulation to match the female’s two X chromosomes. However, how this precise level of upregulation is maintained remains unclear. Our previous research identified the nuclear pore protein Megator (Mtor, Tpr in mammals), a component of the nuclear pore basket, as a factor involved in maintaining this transcriptional precision. Depletion of Mtor resulted in aberrant hyperactivation of the male X chromosome.

My research expanded on this discovery through two aims. First, an RT-qPCR screen identified co-functioning partners of Mtor that similarly regulate this pathway. **RNAi knockdowns of Su(Var)3-3 and Rrp6 caused significant increases in X-linked dosage compensation gene expression, classifying them as putative “hits”.** Xmas2 was also identified as a partial hit. RNA-sequencing of Su(Var)3-3 and Rrp6 knockdowns will further assess genome-wide transcriptional effects and clarify whether histone demethylases and the RNA processing exosome complex modulate the restriction mechanism controlling male X activation.

Second, **analysis of histone marks in Mtor and Su(Var)3-3 knockdowns revealed decreased H3K9Me2 signal at both chromocenters and chromosome arms**, consistent with Su(Var)3-3’s role in promoting heterochromatin formation. H3K4Me1 levels rose in Su(Var)3-3 KDs but decreased in Mtor KDs, while H3K27Me3 increased in both. While the precise mechanism remains under investigation, these results implicate histone methylation and exosome function in fine-tuning dosage compensation and provide broader insight into Mtor’s epigenetic role in development.

298F Chromosomes in conflict: Uncovering how the B chromosome disrupts chromosome 4 segregation during female meiosis

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The supernumerary B chromosomes recently discovered in *Drosophila melanogaster* are non-essential, heterochromatin-rich elements that are believed to have originated from chromosome 4. Although dispensable, B chromosomes disrupt normal female meiosis by inducing high levels of chromosome 4 missegregation. To investigate the relationship between B chromosome and chromosome 4, we genetically analyzed their co-segregation patterns during female meiosis. The transmission frequency of B chromosomes was assessed in progeny that inherited zero, one, or two copies of chromosome 4 from the female parent. We discovered an inverse relationship between chromosome 4 inheritance and B chromosome transmission: when both chromosome 4 homologs were inherited, the frequency of B chromosome transmission was low, and vice versa. This result indicates that the segregation of chromosome 4 and the B chromosomes are not independent from one another, suggesting that they may rely on similar mechanisms for their segregation during female meiosis. We then investigated if modulating the number of B chromosomes would affect chromosome 4 segregation during female meiosis by measuring chromosome 4 nondisjunction as a function of parental B chromosome copy number. Our results clearly demonstrate a positive correlation between B chromosome copy number and chromosome 4 missegregation, consistent with our previous result demonstrating these chromosomes do not segregate independently. We hypothesize that chromosome 4 and the B chromosomes are interacting during late oogenesis through the establishment of aberrant non-homologous heterochromatic threads. These threads normally arise between non-crossover chromosomes to establish a transient connection that provides the necessary tension for proper segregation in the first meiotic division. If multiple B chromosomes are present, they may potentially interfere with the thread formation between chromosome 4 homologs, leading to aberrant chromosome 4 segregation. To test this, we plan to examine the cytological interaction between B chromosomes and chromosome 4 by visualizing heterochromatic threads during late oogenesis. Overall, our research aims to enhance our understanding of how supernumerary, nonessential chromosomes influence the segregation of essential chromosomes during female meiosis.

299F Transgenerational inheritance of odor experience alters gene expression and behavior in offspring

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Organisms are continuously exposed to volatile compounds from their food and environment. Drosophila larvae burrow into rotting fruit and encounter numerous volatile compounds, particularly from yeast fermentation. A few of these dissolved volatile compounds can directly alter gene expression by inhibiting histone deacetylase (HDACi) and increasing histone acetylation. Since chromatin marks are often retained as a form of molecular memory in cells, we wanted to understand the effect across metamorphosis. As expected, larvae exposed to a low concentration of one such HDACi odorant from yeast showed changes in gene expression. Surprisingly, the larval-specific exposure also left a profound effect on gene expression in the adult brain. Many of the differentially expressed genes (DEGs) in the brain were predicted to play a role in behavioral processes. To further understand the mechanism, we are assessing alterations in chromatin accessibility and acetylation patterns to evaluate whether epigenetic modifications established in larval cells persist into adulthood. Adult flies also spend a significant time on fermenting fruits and exposing them to the odorants also induced transcriptomic changes in various organs. Apart from the antennae and fat body, a particularly interesting observation was changes in the ovaries. Remarkably, gene expression changes were seen in the offspring and persisted to at least the next generation. Moreover, the brain DEGs in the offspring were enriched for behaviors such as locomotion, memory, and circadian rhythm. To understand the underlying epigenetic changes in the ovaries that may help propagate this effect, we evaluated various small and long non-coding RNAs (lncRNAs) and identified a suite of lncRNAs that potentially executed the transgenerational effects. Together, these findings suggest that exposure to specific HDACi microbial volatiles can influence the epigenetic state, gene expression, and ultimately behavior across development and generations. These findings open the door for future studies in humans as well, since these HDACi volatiles are prevalent in various foods, beverages, cigarette smoke, and even emitted by the skin microbiome.

300F NASP functions in the cytoplasm to prevent histone H3 aggregation during early embryogenesis

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From their molecular birth until their incorporation into chromatin, histones are bound by specific chaperones that serve unique functions in histone trafficking, stability, and chromatin deposition. The H3-specific chaperone NASP binds directly to H3 and is required to prevent degradation of soluble H3 in vivo. Where NASP functions and how NASP affects H3 dynamics and stability is unknown. Using the Drosophila early embryo as a model system to understand NASP function in vivo, we show that NASP does not directly affect H3 nuclear import or export rates. Rather, reduced H3 levels in NASP-deficient embryos indirectly affect nuclear import and the amount of H3 deposited into chromatin. Crucially, we find that cytoplasmic NASP prevents H3 aggregation in vivo and that H3 aggregation and degradation are developmentally separable events. Thus, we propose the main function of NASP in vivo is to prevent H3 aggregation, thereby indirectly protecting H3 from degradation.

301F Characterizing PR-DUB Function in Stochastic Cell Fate Decisions during Drosophila Eye Development

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Cells with identical genomes take on distinct fates due to specialized patterns of gene expression. While developmental mechanisms often lead to highly reproducible cell fate decisions, in some cases, stochastic gene expression leads to random patterns of cell types that are unique to each individual. The mechanisms controlling stochastic fate decisions are not well understood. Within the Drosophila eye, two R7 photoreceptor subtypes are characterized by the expression of either Rhodopsin 3 (Rh3) or Rhodopsin 4 (Rh4). These subtypes are distributed in a random pattern across the retina. Despite this random spatial pattern, Rh4 is consistently expressed in 67% of R7s in wild type flies.

The specification of these R7 subtypes is controlled by the transcription factor Spineless (Ss). When Ss is present, Rh4 is expressed. In its absence, Rh3 is expressed. *Ss* expression is controlled by dynamic regulation of transcription and chromatin during development. An early-acting enhancer opens chromatin at the *ss* locus in precursor cells. In differentiating cells, the *ss* locus stochastically remains open or recompacts. In terminally differentiating R7s in which the *ss* locus is open, a late-acting enhancer reactivates *ss* expression. In contrast, in cells where the *ss* locus is closed, *ss* remains repressed.

We conducted an RNAi screen of candidate chromatin regulators of the *ss* locus. I identified Calypso, the catalytic component of the Polycomb Repressive Deubiquitinase (PR-DUB) complex, as a regulator of *ss*. When Calypso is knocked down, the proportion of SsON R7s increased significantly, suggesting that Calypso plays a role in regulating the stochastic expression of *ss*.

Here, we utilize DNA FISH, RNA FISH, clonal analysis, and somatic CRISPR to observe the impact of knocking out Calypso on the expression of *ss* at the DNA, RNA, and protein levels across different timepoints during development. These experiments will provide insight into how Calypso regulates the *ss* locus, and further our understanding of the mechanisms by which stochastic cell fate decisions occur. Gaining insight into these mechanisms will contribute to a more complete understanding of how cell fate decisions are regulated during development.

302F *Drosophila* Tet deregulation in Glia Disrupts ECM Integrity and Drives VNC Elongation in Larval Brains

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Ten-eleven translocation (TET) proteins are conserved epigenetic regulators that control DNA methylation and transcription, playing essential roles in development. Aberrant TET activity is implicated in cancer, as TET proteins display either oncogenic or tumor-suppressive roles. *Drosophila melanogaster* has only one homolog of this gene (*dTet*), which is highly expressed in the central nervous system (CNS) during development. Previous studies showed that dTet knockdown leads to lethality, locomotor defects, and abnormal axon patterning in the larval ventral nerve cord (VNC). Here, we examined the consequences of glia-specific dTet deregulation. Both overexpression and knockdown of dTet in glia altered adult lifespan, locomotor behavior, and circadian rhythms. dTet overexpression produced an elongated VNC characterized by disrupted midline glia and loss of axon midline crossing. Transcriptomic profiling revealed downregulation of genes involved in VNC condensation, chromatin organization, and extracellular matrix (ECM) remodeling. Proteomic analysis revealed the downregulation of transcription regulators and key developmental pathways, including Hippo, Notch, and Wnt associated with cytoskeletal disorganization and altered glial patterning. Remarkably, analysis of glioblastoma (GBM) patient RNA-seq data revealed a coordinated upregulation of TET and ECM genes, mirroring the glial dTet phenotypes observed in *Drosophila*. Together, these findings identify glial dTet as a key transcriptional regulator of VNC architecture and neuron-glia communication, and suggest a conserved mechanism of TET-mediated CNS tissue remodeling across species.

303F Transcriptional state of the sex chromosome territory in spermatocytes of *Drosophila* pseudoobscura

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The evolution of heteromorphic sex chromosomes, such as the XY system in Drosophila and mammals, has generated profound differences between the sexes, driving the emergence of regulatory mechanisms like Meiotic Sex Chromosome Inactivation (MSCI). A key unresolved question is whether MSCI arises as an intrinsic and immediate consequence of chromosomal context or evolves gradually through selective pressures. Drosophila pseudoobscura, a member of the obscura group, is particularly informative because of its history of sex chromosome–autosome fusion events that produced younger sex chromosomes. Specifically, the autosomal Muller D fused with Muller A approximately 15 million years ago, forming the metacentric XL/XR chromosome. In this configuration, XR corresponds to the intermediate-age X arm (Muller D), whereas XL retains the ancestral X (Muller A). The homologous Muller D underwent extensive degeneration, giving rise to the heterochromatic Y chromosome (YD). The XL/XR system therefore represents an excellent model for testing whether the intermediate-age X arm experiences inactivation and whether such regulation is shaped by its linkage to the ancestral, repressed XL territory. Because chromosome territories in primary spermatocytes form diffuse chromatin without distinct cytogenetic landmarks, identifying them requires DNA probes. As no such probes currently exist for D. pseudoobscura, we first tested the cross-species applicability of our oligopaint libraries developed for its sister species D. miranda. Fluorescence in situ hybridization (FISH) on larval neuroblast chromosome spreads confirmed the specificity of these libraries, showing no off-target hybridization. Subsequent FISH on primary spermatocytes revealed four chromosome territories, one corresponding to the sex territory (XL/XR and YD) and the remaining three to autosomes. Using immunofluorescence against RNA Polymerase II-Ser2p, a marker of active transcription, our preliminary results indicate a reduction in transcriptional activity on the YD chromosome compared to the autosomes. This is an unprecedented observation, as the Y chromosome in Drosophila melanogaster is known to be highly active in primary spermatocytes.

304F Investigating The Role of Histone Lysine Demethylase Proteins in Drosophila Dosage Compensation

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In heterogametic organisms, the genetic phenomenon of dosage compensation (DC) corrects the imbalanced expression of sex chromosomes. In Drosophila, this process occurs via two-fold upregulation of the single male X chromosome – an activity that is mediated by histone post-translational modifications (PTMs). Previous studies suggested that tri-methylation of histone H3 lysine-36 (H3K36me3) was necessary for male viability; however, its requirement for DC is controversial. Other lines of investigation suggest that mono-methylation of histone H4 lysine-20 (H4K20me1) could also play a role in this process. In this study, we explore the hypothesis that a group of histone lysine demethylases (aka “eraser” proteins) may be involved. We therefore carried out a genetic screen that measures the viability and sex ratio of animals that express reduced amounts of lysine demethylase (KDM) proteins in both wild-type and DC-sensitized genetic backgrounds. The results suggest that several of these candidates either support or antagonize male lethality in the context of DC. Although the precise mechanisms for involvement of these KDM genes in dosage compensation remains to be determined with future experiments, the current results broaden our understanding of how organisms balance the expression of X chromosomes between different sexes.

305F Using genetic complementation analysis within and between multigene families to crack the histone code

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Complementation analysis is a cornerstone of classical genetics, used to predict whether mutations that produce similar phenotypes lie within the same gene or biochemical pathway. Historically, this approach could not be employed across multigene families like those encoding metazoan histone genes. To overcome this limitation, we developed a *Drosophila* genetic platform wherein endogenous replication-dependent histone genes can be deleted and replaced with transgenic arrays containing at least 12 tandemly arrayed histone gene repeats. By crossing animals bearing different 12-copy arrays (each with distinct missense mutations), we can generate 24-copy progeny for genetic interaction tests. These tests can be performed within the same histone subtype (intragenic complementation; e.g. H3 × H3) or between different ones (intergenic epistasis; H3 × H4). This approach enables systematic dissection of histone residue relationships that would be impossible to carry out in other metazoan model systems.

Here, we generated a pairwise complementation matrix between five different H3.2 alleles (WT, K4R, K9R, K27R, and K36R), revealing a full range of outcomes. The data show that certain H3.2 residues share common pathways or mechanisms necessary for proper adult development whereas others do not. Next, we carried out intragenic complementation tests between two H4 residues (K16 and K20) involved in X chromosome dosage compensation (DC). Notably, K16R/K20A mutants display strong male-specific lethality, whereas control crosses do not. These findings support the notion that H4K20 plays a crucial role in DC. To test whether they function redundantly or synergistically within this pathway, we carried out male-rescue experiments using 6x H4K16 missense alleles (WT, K16R, K16Q and K16A) crossed into 12x K16R or K20A backgrounds. Animals expressing a fraction (33%) of acetylation-mimicking K16Q can partially rescue both K16R and K20A male lethality, indicating that the functional requirement for H4K20 in DC is to acetylate H4K16.

These proof-of-principle results motivate us to propose a large-scale project, HistENCODE, to decipher functional interactions among histone PTMs by integrating comprehensive complementation analyses with multi-scale phenotyping, chromatin profiling, mass spectrometry and developmental transcriptomics. Details of this initiative will be discussed.

306F Home is where the histones are: mechanisms of nuclear body localization

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Histone proteins are the backbone of genomic architecture. They organize and compact the genome and any deviance in their regulation results in genomic instability and embryonic lethality. The histone loci recruit and are regulated by a suite of conserved factors that form a phase separated nuclear body known as the histone locus body (HLB). Many HLB specific factors are conserved across eukaryotes, yet it is unclear how the HLB is targeted to the histone locus. We seek to understand how these factors locate the histone locus based on characteristics other than DNA sequence. *Drosophila* serve as an ideal model to study this as the five canonical histone genes are organized into a 5kb array that is tandemly repeated ~100 times at a single locus. The critical HLB protein multi sex combs (Mxc), known as NPAT in humans, lacks sequence-specific DNA binding activity, yet it exclusively localizes to this locus and scaffolds the HLB. To determine novel protein interactions that contribute to Mxc localization, we performed a yeast two-hybrid screen using a region of Mxc important for HLB localization as bait. Our screen identified as prey a domain of the mRNA splicing factor Prp40, recently shown to localize to the HLB. We show the interaction between Mxc and Prp40 is unique to the histone locus in the early embryo and have engineered tagged versions of Mxc and Prp40 to further investigate their interaction. We aim to elucidate the mechanism in which this essential HLB scaffolding factor locates the locus, informing how locus-specific nuclear bodies find their genomic targets.

307F Characterizing the Formation of R-loops at the Centromeres of *Drosophila Melanogaster*

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The centromere is an essential chromosomal locus that serves as the site of kinetochore assembly, mediating accurate chromosome segregation during cell division. The centromere is epigenetically marked by the presence of the conserved centromere-specific histone H3 variant, CENP-A, which recruits inner kinetochore components, including CENP-C. Despite this essential and conserved function of centromeres across eukaryotes, centromeric DNA sequences are highly diverse across species, highlighting a major gap in understanding how such rapidly evolving DNA contributes to the maintenance of centromere identity.

One possible model suggests that non-canonical DNA structures, contribute to centromere identity. Our previous work in *Drosophila* identified non-canonical nucleic acid structures, such as G-quadruplexes and R-loops, at the centromere using prediction algorithms. Here, we investigate the presence of R-loops, three-stranded DNA:RNA hybrids commonly associated with active transcription and with transcription and replication conflicts, in centromere structure and function. Initial immunofluorescence staining of *Drosophila* brain tissue suggests that R-loops are present and show enrichment, notably at Centromere 2. To validate these preliminary findings and overcome concerns regarding antibody specificity, we have engineered a transgenic fly line expressing the R-loop sensor RHINO. RHINO consists of three catalytically inactive domains of RNaseH1 fused to mNeonGreen and has previously been demonstrated *in vitro* to bind R-loops with high specificity. We will utilize the RHINO sensor *in vivo* to cytologically confirm R-loop localization and enrichment. Future studies will employ RHINO in immunoprecipitation assays to precisely map R-loop localization and conduct co-localization studies to track R-loop interactions with key centromeric components. Collectively, these findings aim to elucidate the structural contribution of R-loops and their potential role in ensuring faithful chromosome segregation during mitosis.

308S Ulp1: A Clock for Heterochromatin Repair

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Pericentromeric Heterochromatin occupies ~30% of fly and human genomes and is mostly composed of highly repeated DNA sequences prone to aberrant recombination. And yet, repair mechanisms in heterochromatin remain poorly understood. Our previous studies in Drosophila cells showed that 'safe' homologous recombination (HR) repair of heterochromatic double-strand breaks (DSBs) requires the movement of repair sites to the nuclear periphery, where the strand invasion protein Rad51 is recruited to continue repair. This likely prevents aberrant recombination by isolating broken sites and their homologous templates away from ectopic (non-allelic) repair templates before strand invasion. Relocalization and exclusion of Rad51 inside the heterochromatin domain require the nucleoplasmic function of Nup98 nucleoporin, including its phase separation properties. The post-translational modification SUMOylation is also essential for this pathway, as it blocks Rad51 recruitment and HR progression inside the heterochromatin domain, thus preventing aberrant recombination. The SUMO protease Ulp1 is enriched at the nuclear pores through tethering by the Mtor/TRP subunit of the nuclear pore basket. Here we show that loss of the SUMO protease Ulp1 results in defective Rad51 recruitment to damage foci associated with the nuclear periphery in Drosophila cells, thereby demonstrating the importance of Ulp1 in HR restart after relocalization. This function is dependent on the SUMO protease activity of Ulp1. Importantly, Ulp1 release from the nuclear periphery through overexpression, mutation of the nuclear pore anchoring domain, or Mtor/TPR depletion, results in defective relocalization of DSBs, abnormal formation of Rad51 foci inside the heterochromatin domain, and heterochromatin instability. This reveals the importance of Ulp1 tethering to the nuclear pores for compartmentalizing the restart of heterochromatin repair in this location, and safe HR completion. We are now investigating Ulp1 targets and conserved pathways in mammalian cells. Together, these studies identified a new function of Ulp1 in the spatial and temporal regulator of HR repair of heterochromatin through its tethering to the nuclear periphery.

309S Identification of Novel Genes Involved in Transposable Element-Mediated Spreading of Repressive Marks in *Drosophila melanogaster*

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Transposable elements (TEs) are selfish genetic parasites that replicate and move themselves across nearly all eukaryotic genomes, including humans. The rapid accumulation of TEs imposes significant impacts on host fitness. This is especially apparent in euchromatin, the transcriptionally active, gene-rich regions of the genome, where TE movement can disrupt functional genes. To combat their selfish replication, hosts have evolved RNA-based mechanisms that enrich TE sequences with repressive epigenetic marks, such as H3K9me2/3. Interestingly, these marks are characteristic of heterochromatin, the transcriptionally inactive, gene-poor regions of the genome. Despite being evolved to benefit the host, these repressive epigenetic marks have been observed to spread for more than 20 kb from the TE insertion site, altering the epigenetic states of neighboring genes, a phenomenon we termed "TE epigenetic effects." This project investigates the genetic basis of TE epigenetic effects using extreme-QTL mapping (X-QTL), a powerful method in which individuals displaying extreme phenotypes from synthetic multiparent populations are pool-sequenced to identify causal genetic variants. Using our mapping population and a set of transgenic reporter lines whose phenotype proxies the extent of repressive mark spreading at candidate euchromatic TEs, we employed X-QTL methods to accurately and powerfully identify genetic variants contributing to varying TE epigenetic effects. We use null mutants of identified candidate genes to determine their effect in altering TE-mediated spreading of repressive marks by assessing a significant change in mCh fluorescence intensity. In addition, we are investigating whether these candidate genes also influence classic position effect variegation (PEV) effect loci, an approach that will help determine if identified candidate genes are general regulators of heterochromatin spreading, or TE-specific. Our experimental validation of these candidate genes will advance our understanding of how TEs are regulated and help determine the extent of TE-mediated spreading of repressive marks, which ultimately determines the associated functional consequences and thus host fitness.

310S Extensive pairing of homologs in single nuclei during zygotic genome activation

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Individual chromosomes largely occupy discrete areas within the nucleus leading to the formation of independent chromosome territories (CTs). In *Drosophila,* homologous maternal and paternal chromosomes have been shown to pair in 3D space with significant bearing on gene regulation. While such intricate chromosome packaging and interactions have been observed by population-based approaches, such as derivative of chromosome conformation capture, and single-cell imaging, studies investigating cell-type variability of CTs and homolog pairing during development remain limited. Here, we employed a customized Oligopaints-based imaging approach to visualize spatial heterogeneity of CTs and homolog pairing in developing embryos during the onset of zygotic genome activation (ZGA). We reveal changes in genome folding at the whole-chromosome and chromosome-arm scale. Moreover, at the whole-chromosome scale, parental homologs show extensive pairing in single nuclei, while pairing at the chromosome-arm level becomes less precise, suggesting spatial variability in chromosome conformations. We put our pairing model from single-nucleus high-resolution microscopy in the context of previously suggested structures of somatic homolog pairing. Together, these findings provide an enhanced framework for our understanding of global parental genome folding and regulation in single nuclei during early embryogenesis.

311S Diet-mediated alternation of the epigenetic regulation of transposable elements in Drosophila melanogaster

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As society advances, people are increasingly aware of the importance of diet for health. Recent studies have shown that diet not only affects metabolism but also influences gene expression through epigenetic regulation, thereby impacting disease risk and development. Transposable elements (TEs) constitute nearly 50% of the human genome, and their activity is tightly regulated by epigenetic mechanisms. For example, H3K9me2/3, histone modification, plays a key role in repressing TE activity. Reduced DNA methylation or repressive histone marks can activate TEs, causing mutations, which may disrupt essential genes and lead to disease. However, how dietary factors influence the epigenetic regulation of TEs remains largely unknown. Therefore, understanding how diet modulates TEs through epigenetic mechanisms and their potential harmful consequences is of great importance. To identify dietary factors influencing TE regulation, we designed a reporter-based system in Drosophila melanogaster,. This system leverages the biological phenomenon that repressive epigenetic marks silencing TEs can spread to neighboring genes, resulting in alterations in the expression of these neighboring genes. Therefore, we placed the reporter gene adjacent to a TE, and differences in reporter fluorescence intensity under different dietary conditions serve as an indicator of how diet affects the strength of epigenetic silencing of TEs. For the screening strategy of dietary factors, we focused on three categories: (1) natural antioxidants that act as histone deacetylase (HDAC) inhibitors; (2) key nutritional macronutrients previously reported to affect epigenetic regulation; and (3) microelements that function as cofactors for histone demethylases (KDMs) or HDACs. Our preliminary results showed that resveratrol, an HDAC inhibitor, reduced TE silencing without significantly affecting development. In contrast, a high-sugar diet enhanced TE silencing and resulted in developmental delay. Next, we are conducting genome-wide transcriptomic and epigenomic analyses to further elucidate the global effects of diet on TE silencing. Overall, these findings emphasize the critical role of diet in regulating TEs and suggest that dietary modulation of TE activity may have significant implications for organismal health.

312S JIL-1-mediated phosphorylation of the Su(var)3-9 methyltransferase impacts H3S10ph and H3K9me2 chromosomal distribution patterns

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Chromatin structure and gene expression in *Drosophila melanogaster* are dynamically regulated by post-translational histone modifications that either promote or repress transcription. The JIL-1 kinase plays a key role in this regulation by phosphorylating histone H3 at Serine 10 (H3S10ph) during interphase, a modification associated with opening chromatin and the prevention of heterochromatin spreading. In contrast, the histone methyltransferase Su(var)3-9 establishes and maintains heterochromatin by catalyzing dimethylation of histone H3 at lysine 9 (H3K9me2), a repressive chromatin mark linked to gene silencing. Previous studies identified that JIL-1 kinase interacted with Su(var)3-9 by phosphorylating it on the Serine 191 residue, but the functional consequence of this modification on Su(var)3-9 enzymatic activity remains unclear.

To investigate the interplay between these enzymes, we employed a LacI-LacO tethering system to target Su(var)3-9 and JIL-1 to specific chromatin loci in *Drosophila* salivary gland polytene chromosomes. This approach allowed us to visualize the chromatin modifications through immunostaining and fluorescence microscopy. By expressing the LacI-JIL-1 construct, enrichment of H3S10ph was detected at the targeted chromatin sites, confirming that JIL-1 can effectively induce phosphorylation and euchromatic structure at these loci. In contrast, tethering Su(var)3-9 through the LacI-Su(var)3-9 construct led to the spreading of the repressive H3K9me2 mark along the chromosome arms, while H3S10ph redistributed to the chromocenter. This suggests that Su(var)3-9 activity can potentially override euchromatic marks and promote heterochromatin expansion. Immunohistochemistry of chromosomes from the Su(var)3-9 S191A mutant, which prevents phosphorylation by the JIL-1 kinase, reveals that H3K9me2 re-localizes to the centromeric regions, while H3S10ph is restored on the chromosome arms. These findings indicate that JIL-1-mediated phosphorylation of Su(var)3-9 may directly modulate its activity. Overall, these findings reveal a dynamic antagonistic relationship between JIL-1 kinase and Su(var)3-9 in establishing chromatin boundaries and maintaining genome integrity.

313S Investigating the role of H3.3S31 in the *Drosophila melanogaster* chromatin landscape

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Histone proteins are the building blocks of nucleosomes, which control genome organization and function. Histone proteins can be organized into two main categories: canonical histones whose synthesis is coupled with DNA replication during S phase of the cell cycle, and variant histones that are expressed throughout the cell cycle and deposited into chromatin using different chaperone complexes than the canonical histones. Both categories of histone proteins contain N-terminal tails that undergo post-translational modifications (PTMs), such as acetylation, phosphorylation, and methylation, which contribute to the regulation of gene expression. The N-terminal tail of variant histone H3.3 differs from that of canonical histone H3.2 by a single amino acid: H3.3 contains a serine at residue 31 whereas H3.2 contains alanine at this position. Recent studies in different organisms and cell types have demonstrated that phosphorylation of H3.3S31 (H3.3S31ph) influences the level of other histone PTMs, including H3K27 acetylation (H3K27ac) in mouse embryonic STEM cells and *Xenopus* oocytes, H3K27 trimethylation (H3K27me3) in mouse skeletal muscle, and H3K36 trimethylation (H3K36me3) in mouse macrophages. H3.3S31ph modulation of these histone PTMs is thought to be critical for proper gene expression, stress responses, and immune responses. However, it remains unknown if H3.3S31 is phosphorylated in *Drosophila* and whether this modification is essential for proper PTM accumulation and cellular function. To determine the role of H3.3S31ph, we generated H3.3S31A mutant *Drosophila* using CRISPR and measured the levels of H3 PTMs using western blot assays. We found no change in the accumulation of H3K9 dimethylation nor H3K27 trimethylation in H3.3 mutant flies. Interestingly, we observe reduced levels of H3K27ac in H3.3S31A flies compared to wild-type, suggesting crosstalk between H3.3S31 and H3K27 modification. We attempted to detect H3.3S31ph directly using western blot assays and found that a commercially available anti-H3.3S31ph antibody exhibits nonspecific binding to H3.2, indicating caution should be employed when interpreting results using such commercial antibodies. As previous literature suggests that H3.3S31ph is required for the immune response in mice, we are currently developing an immune challenge assay with our H3.3S31A mutant *Drosophila* to test functional roles of H3.3S31 in the innate immune response.

314S Interplay Between Nuclear Organization and Dosage Compensation in *Drosophila melanogaster*

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Dosage compensation in *Drosophila melanogaster* males achieves increased expression of their single X chromosome to match the expression of both female X chromosomes. This process is mediated by the Male Specific Lethal (MSL) complex, which is recruited to X-linked genes. The complex is recruited to the Chromatin Entry Sites (CES), and spreads to nearby active genes to increase the expression. How complex selectively binds the X chromosome is poorly understood. Our lab has shown that a family of AT-rich 1.688^{X} satellite repeats enriched on the X chromosome enables recruitment of the MSL complex by nearby genes, but by a mechanism distinct from the CES. How CES and 1.688^{X} repeats cooperate to identify the X chromosome is still unclear. We hypothesize that nuclear organization participates in recruitment of the MSL complex. We screened genes involved in chromosome architecture and nuclear organization to identify factors that influence X recognition. Knockdown or mutation of several insulator proteins reduced survival of males with mutations that impair X localization of the MSL complex. These factors are now being tested for roles in recruitment by CES and 1.688^{X} satellites using a reporter system developed by our lab. Our findings suggest selective X recognition involves CES and 1.688^{X} satellites engaging different aspects of nuclear organization.

315S Cohesin and condensin contribute to MSL-mediated dosage compensation in *Drosophila melanogaster*

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Dosage compensation in *Drosophila melanogaster* males equalizes X-linked gene expression with that of females by doubling transcription from the single male X chromosome. This process involves the Male Specific Lethal (MSL) complex, which selectively binds the X chromosome to upregulate expression. The mechanisms governing this selective binding remain poorly understood. Previous work has shown that GA-rich Chromatin Entry Sites (CES) recruit the MSL complex through the CLAMP adapter protein but AT-rich 1.688^{X} satellite repeats promote recruitment by a distinct, siRNA-dependent pathway. The coordination between these two recruiting elements is not understood. My research focuses on how higher-order chromosome architecture, particularly the cohesin and condensin complexes, contributes to X-chromosome recognition. Both complexes are central to chromatin organization and nuclear topology, suggesting they may facilitate spatial proximity between CES or 1.688^{X} repeats and target genes. Knockdown or mutation of cohesin and condensin subunits reduces male viability in dosage compensation mutants, implicating these complexes in MSL recruitment or spreading. Using a luciferase-based reporter system developed in our lab, I am testing how condensin and cohesin influence recruitment by CES and 1.688^{X} satellites. This work aims to uncover how architectural protein complexes interface with chromatin entry and satellite elements to ensure faithful X recognition and regulation.

316S Breaking the repeats: Targeted disruption of satellite DNA

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Substantial fractions of eukaryotic genomes consist of satellite DNA (satDNA): large blocks of tandemly-repeated sequences in heterochromatic genome regions. SatDNAs are dynamic in their location and abundance across species, perhaps driven by high mutation rates and selfish evolution. However, some satDNAs comprise genomic regions with essential functions like centromeres and telomeres, and have effects on organismal phenotypes, raising intriguing questions about their potential functional significance. Whether the specific sequence, the size, or structural organization of satDNA arrays contribute to their functional effects remains elusive. Due to their highly repetitive nature, satDNA arrays are difficult to study using genetic and genomic approaches. Previous studies of satDNA function have relied on large deletions that remove chromosomal segments containing satDNA repeats, among other sequences. To attribute effects specifically to satDNA requires a more targeted approach. Recent advances in long-read sequencing provide new insights into satDNA structural organization and precise genetic manipulations of satDNA. Here we successfully use CRISPR/Cas9 to manipulate an autosomal pericentromeric satDNA locus called Responder (Rsp), located on chromosome 2R in Drosophila melanogaster. We selected Rsp as our focal satDNA because of its effects on spermatogenesis: Rsp is the target of a sperm-killing male meiotic driver called Segregation Distorter (SD). The sensitivity to sperm killing by SD correlates positively with Rsp copy number, making it an ideal system to explore satDNA functional effects. We used a sequential CRISPR/Cas9 approach to generate an allelic series of Rsp deletion and expansion variants in two genetic backgrounds. We characterized these variants using whole genome sequencing, fluorescent in situ hybridization (FISH), and genetic crosses to assess drive strength. To determine if satDNA variants affect organismal fitness, we assayed egg-to-adult viability, fecundity, hatch rate, larval viability, and pupal viability. We find that our CRISPR-mediated satDNA mutations have phenotypic effects that correlate with copy numbers. We detect effects on fitness that may correspond to variation in satDNA structural features, context, or genetic background. This work establishes a new framework for investigating satDNA, and offers new insights into the evolutionary and functional role of satDNA in genome evolution and organization.

317S Distinct DNA-binding specificity of the N-terminal zinc fingers of Zelda revealed by DAP-seq

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Pioneer transcription factors are a class of proteins capable of engaging compact chromatin and making it accessible to other regulators. In Drosophila, the pioneer factor Zelda activates the zygotic genome by interacting with numerous regulatory elements. DNA binding is mediated by the C-terminal domain with four zinc fingers (ZnF3–6) that recognize CAGGTAG motifs. Zelda also contains two N-terminal zinc fingers whose DNA-binding properties remain poorly characterized. To test whether Zld-N can recognize alternative motifs, we performed a protein binding microarray analysis. The top PBM motif for Zld-N was AAGGGT. To determine how Zld-N recognizes motifs in the context of genomic DNA, we performed DNA Affinity Purification Sequencing (DAP-seq), an in vitro DNA binding method used to map protein–DNA interactions genome-wide, with Zld-N (ZnF1–2) and Zld-C (ZnF3–6). As expected, the top 1000 Zelda-C peaks were strongly enriched for the canonical CAGGTAG motif known from embryonic ChIP-seq. In contrast, the top 1000 Zelda-N peaks revealed a distinct AA(G/A)GGT motif, closely matching the PBM result, confirming specific and reproducible DNA-binding activity of Zld-N. We also performed an overlap analysis of Zelda-N and Zelda-C DAP-seq peaks and found that only 2% of them overlap, which supports the independence of binding preferences between the two fragments. In addition, we have currently comparing overlaps between DAP-seq peaks and Zld ChIP-seq peaks from published datasets. Together, these data show that the N-terminal zinc fingers of Zelda possess their own specificity for the AA(G/A)GGT motif, distinct from that of the C-terminal domain.

318S Distinguishing Functional Roles of Nup93 Paralogs in Tissue Identity and Growth

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The nuclear pore complex (NPC) is a highly conserved macromolecular structure which spans the nuclear lipid bilayer membrane. The NPC comprises 30 different types of proteins called nucleoporins (Nups), which are involved in nucleocytoplasmic transport, chromatin organization, gene expression regulation, DNA repair, RNA processing and quality control, and cell cycle control. Interestingly, *Drosophila* contains two orthologs of human Nup93, Nup93-1 and Nup93-2, suggesting that flies have evolved distinct functions for each paralog. Understanding the paralog's unique roles will provide greater insight into diverse functions of human Nup93, which has been linked to cancer and kidney disease. Nup93 is currently known for its essential role in NPC formation and association with chromatin, including at Polycomb-silenced domains. The differences between Nup93-1 and Nup93-2 in *Drosophila* are yet to be fully established or characterized. Our preliminary evidence suggests that while both contribute to NPC architecture, they differ in their roles in signaling pathways and gene expression. We found that the loss of Nup93-2 exhibits large, tumor-like structures and irregularities in larval wing discs. Consistently, the RNA sequencing data revealed an upregulation of the JAK/STAT and Wingless signaling pathways specifically in Nup93-2 RNAi-depleted tissue. The tumor-like phenotype and the hyperactivation of the JAK/STAT pathway suggests that Nup93-2 plays a unique role in signaling or gene silencing, relative to Nup93-1. We used RNAi-mediated knockdowns in vitro and in vivo to assess how downregulation of each paralog affects NPC stability and whether upregulation of the JAK/STAT pathway is independent of NPC levels and the structural roles of Nup93. Using CRISPR-tagged Nup93-1-HA and Nup93-2-FLAG, we also analyzed sub-nuclear localization of Nup93 paralogs in different tissues via immunofluorescence and quantitative colocalization analysis, to determine whether the paralogs occupy distinct NPC structures or localize with other protein complexes. Functional differences will continue to be explored through a genetic rescue experiment to determine whether activation of the JAK/STAT pathway is directly responsible for the growth phenotypes observed in the Nup93-2 knockdown. Together, these experiments reveal how the Nup93 paralogs contribute to NPC structure, signaling, and gene regulation, and identify a unique role of Nup93-2 in repression of JAK/STAT signaling and growth control.

319S Determining The Roles of Nuclear Pore Proteins In Polycomb Mediated Gene Silencing and Chromatin Modification

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Nucleoporins, or Nups, are protein components that make up the nuclear pore complex (NPC) . Nup93 paralogues, part of the inner ring subcomplex, have been shown to associate with repressive chromatin states via ChiP-seq binding maps. Notably, Nup93 co-localizes with H3K27Me3, a histone modification deposited by Polycomb Repressive Complex (PRC) 2. Nup93 in *drosophila* has been demonstrated to physically interact with components of PRCs , specifically RYBP, E(Z) and Pc via co-immunoprecipitation. This indicates an integral role for Nup93 paralogues in the Polycomb repressive pathway. This study aims to elucidate the specific molecular mechanisms by which Nup93 paralogues and other inner ring Nups contribute to PRC mediated gene silencing using S2 cells as an *in vitro model*.

To begin to interrogate the potential for differential expression of Nup93-1 and Nup93-2, and Nup107, a RT-qPCR screen of Polycomb target genes was carried out. Target gene expression in RNAi mediated knockdowns indicated that Nup93 paralogues have a significant and independent silencing effect on Polycomb target genes. Nup107 knockdowns functioned as a negative control, demonstrating no effect on target gene expression. Data indicated that Nup93-2 displays more repressive activity than Nup93-1 and when both paralogues are knocked down concurrently, the resulting phenotype resembles the knockdown of Nup93-1.

We used western blot analysis to assess the impact knocking down Nup93-1, Nup93-2 and Nup107 on polycomb associated histone marks including H3K27me3 H2Ak119ub and the mitotic marker H3s28pho. Data collected from this set of experiments indicates there is no global change of polycomb associated histone marks during knockdown of either Nup93-1, Nup93-2 or the knockdown of both 93-1 and Nup93-2. However, the data suggests that there is a significant decrease in H3s28pho when Nup93-1 and Nup93-2 are knocked down separately, but not when knocked down together. To further explore local chromatin changes ChIP-seq will be performed under the same knockdown conditions.

A yeast 2 hybrid screen identified direct binding partners unique to Nup93-2: 14-3-3-zeta and 14-3-3-epsilon, proteins known to associate with H3s28pho. This suggests a mechanism whereby Nup93-2 anchors histones at the nuclear pore via 14-3-3 variants. These findings highlight a novel link between nuclear architecture and epigenetic regulation that is being explored further using *in silico* modeling.

Collectively the findings of this study aim to elucidate mechanisms linking NPC components to PRC mediated gene repression with implications for developmental processes and diseases associated with epigenetic dysregulation, including cancers and neurodegenerative disorders. By characterizing Nup93 interactions with polycomb pathways this study provides a foundation for understanding how nuclear architecture influences chromatin states.

320S Determining the Relationship between Kismet and Polycomb and Trithorax Group Proteins in *Drosophila* Larval Motor Behavior

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Kismet (Kis), the chromatin remodeling enzyme, is the *Drosophila* homolog of the chromodomain helicase DNA binding proteins 7 and 8 (CHD7, CHD8) found in mammals. Our lab has previously found that Kis facilitates the localization of synaptic proteins, endocytosis, and neurotransmission. Kis was first discovered as a suppressor of the Polycomb Repressive Complex (PRC) 2 and interacts cooperatively with the Trithorax Group Enzymes Absent, small, or homeotic 1 (Ash1) and Trithorax (Trx). Kis likely recruits Ash1 and Trx to chromatin but opposes the activity of Pc and E(z), components of PRC1 and PRC2, respectively. We are currently investigating whether these relationships affect larval motor behavior. Future experiments will assess whether these proteins cooperatively or antagonistically affect transcription of four target genes including *amyloid-precursor protein-like* (*appl*), *endophilin B* (*endoB*), *Neuroligin 2* (*nlg2*), and *Ras-related protein 11* (*rab11*). Each of these targets were previously found to be directly regulated by Kis. By investigating the complex relationships between Kis, Pc, E(z), Ash1, and Trx, in *Drosophila*, we can develop a better understanding of how CHD proteins regulate target genes and how dysregulation may lead to neurodevelopmental disorders.

321S The Chromatin Remodeler Kismet and the Histone Acetyltransferase CBP Cooperatively Promote Synaptic Functions Without Altering Global H3K27ac or H3K27me3 Levels

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Synaptic dysfunction is a hallmark of neurodevelopmental disorders including CHARGE syndrome and autism spectrum disorders. Epigenetic mechanisms including chromatin remodeling and histone modification play a critical role in the development and maintenance of synaptic functions. Although chromatin remodeling proteins are known to promote synaptic functions, how they interact with histone-modifying enzymes to orchestrate the transcription of genes essential for synaptic function remains largely unresolved. This study explores the potential cooperative function of Kismet, the *Drosophila* homolog of the human chromatin remodeling enzymes CHD7 and CHD8, and CREB-binding protein (CBP), a histone acetyltransferase, in regulating histone modifications associated with active chromatin states. During development, components of Polycomb Repressive Complexes 1 and 2 (PRC1 and PRC2) oppose the activity of the chromatin remodeler Kismet and the histone acetyltransferase CBP. Kismet, CBP, and the histone methyltransferase Trithorax related (Trr) promote transcriptionally active chromatin states. We found that *kismet* and *PRC1* mutants exhibited reduced levels of histone H3 lysine 27 acetylation (H3K27ac), while *PRC1* and *PRC2* mutants exhibited increased trimethylation of H3K27me3 in larval postsynaptic muscle nuclei. In contrast, none of the animals examined exhibited altered H3K27ac or H3K27me3 in presynaptic motor neurons nuclei. Additionally, several synaptic proteins were differentially affected in these same animals. *CBP*, *kismet* and *trr* mutants showed reduced EndoB levels. *CBP* mutants showed reduced Rab11. We also found reduced Amyloid Precursor Protein-Like (APPL) in *kis, PRC1,* and *PRC2* mutants and elevated Fasciclin II (FasII) in *Trr* and *PRC1* mutants. To determine how CBP and Kismet regulate synaptic EndoB, we will knockdown or overexpress CBP in *kis* mutants in both presynaptic motor neurons and postsynaptic muscle cells. These data suggest that aberrant synaptic functions in neurodevelopmental disorders may not involve global alterations in H3K27ac or H3K27me3 but that chromatin remodelers and modifiers regulate the synaptic levels of EndoB, APPL, and FasII.

322S Gene annotation of *tango* and *Elongator complex protein 6* in *Drosophila* species.

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The Genomics Education Partnership (GEP) provides undergraduates with access to research activities and genome analysis experience. In this study, we apply the GEP tools to analyze the DNA sequences of several *Drosophila* species, identify the orthologs and prepare structural models of selected genes. We focus on insulin pathway-interacting genes as its regulation is a contributing factor in obesity, a major health concern. The *Elongator complex protein 6* (*Elp6*) mediates the insulin receptor-target of rapamycin (In-TOR) signaling, affects cell growth and metabolism, including the triglyceride to protein ratio. Gene *tango/HIF-1β (tgo)* encodes a transcription factor which acts in hypoxia response in *D. melanogaster*. We identified locations of *tgo* orthologs in *D. simulans, D. obscura, D. novamexicana* and *Elp6* in *D. kikkawai, D. hydei* and *D. mojavensis* based on Genome Browser and BLAST search for corresponding amino acid sequences. *tgo* orthologs in *D. simulans* and *D. obscura* show greater similarity (99.7% and 95.1%) than *D. novamexicana* (80.2%) to *D. melanogaster*, consistent with their evolutionary relationship. Strikingly, most of the variation in the amino acid sequence occurs in the C-terminal portion of tango, with N-terminal part highly conserved. In contrast, amino acid differences are distributed more uniformly across the Elp6 protein, indicative of potential conservation of the specific protein regions. Furthermore, Elp6 sequence similarity is higher between *D. hydei* vs *D. mojavensis* compared to *D. melanogaster* and *D. kikkawai*. The single unique coding exon and two isoforms are present in all analyzed species, except *D. simulans* showing a single isoform. The genomic neighborhood of *Elp6* shows higher conservation between *D. kikkawai*, *D. hydei* and *D. mojavensis* compared to *D. melanogaster*, which contains an additional gene in the region. Interestingly, the five genes surrounding *tgo* in *D. melanogaster*, *D. simulans* and*D. novamexicana* show the same order and orientation, but one of the genes is absent in *D. obscura*. Together, this data pinpoints the differential changes affecting specific gene segments and genomic regions in related *Drosophila* species.

323T Population genomics of *Drosophila subpulchrella* and adaptive evolution of *suzukii* species complex

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While many *Drosophila* species lay eggs on overripe fruit, *D. suzukii* has evolved to oviposit on fresh and firm fruit. Because of its agricultural impact and ecological novelty, recent studies have actively investigated various aspects of evolution in *D. suzukii*, such as behavioral, physiological, morphological, and population genomic dynamics. However, despite having diverged from *D. suzukii* approximately 2–3 million years ago and being its closest relative, biological and genomic information on *D. subpulchrella* remains limited due to its restricted distribution and the difficulty of collecting this species. Our laboratory has constructed high-quality reference genomes for both *D. suzukii* and *D. subpulchrella*, providing a robust genomic foundation for comparative analyses. To understand the adaptive evolution of *D. suzukii*, it is essential to investigate the population dynamics of *D. subpulchrella*, which provides the necessary comparative framework for reconstructing the evolutionary trajectory of this lineage. Here, we generated a population genomic dataset for *D. subpulchrella* using a pool-sequencing sample consisting of a large number of isofemale lines established from wild populations collected in Japan. Chromosome-wide analyses showed that both *D. subpulchrella* and *D. suzukii* have high levels of nucleotide diversity, but *D. subpulchrella* tends to have lower nucleotide diversity than Japanese *D. suzukii* populations, with a particularly noticeable reduction on chromosome 3R. A similar pattern was observed in Tajima's D, where *D. subpulchrella* exhibits more negative values on chromosome 3. Tajima's D results and population studies also indicate that both *D. subpulchrella* and *D. suzukii* underwent population expansion. Differences between species may reflect the influence of underlying genome structural features. Because the *suzukii* species complex has larger genomes than the melanogaster species complex, we have been dissecting the contributions of gene amplification and transposable element expansion to investigate whether, and how, they contribute to lineage-specific traits. Our results provide a foundational population genomic resource for *D. subpulchrella* and highlight key genomic and population differences between the two sister species.

324T A predicted structural interactome in *Drosophila*: the roles of intrinsically disordered regions and functional associations

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Proteins function through dynamic interactions with other proteins in cells, forming complex networks fundamental to cellular processes. While high-resolution and high-throughput methods have significantly advanced our understanding of how proteins interact with each other, the molecular details of many important protein-protein interactions are still poorly characterized, especially in non-mammalian species, including *Drosophila*. Recent advancements in deep learning techniques have enabled the prediction of molecular details in various cellular pathways at the network level. In this study, we used AlphaFold2 multimer to examine and predict protein-protein interactions from both physical and functional datasets in *Drosophila*. We found that functional associations contribute significantly to high-confidence predictions. Through detailed structural analysis, we also found the importance of intrinsically disordered regions in the predicted high-confidence interactions. Our study highlights the importance of disordered regions in protein-protein interactions and demonstrates the importance of incorporating functional interactions in predicting physical interactions between proteins. We further compiled an interactive web interface to present the predictions, facilitating functional exploration, comparative analysis, and the generation of mechanistic hypotheses for future studies.

325T Centromeric targeting enables a retroelement to propagate and shape centromere evolution

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Transposable elements—"jumping genes" that copy and paste themselves throughout the genome—face a fundamental challenge: how to spread without harming their host. Although typically deleterious due to insertional mutagenesis or genome instability, TEs can also act as powerful engines of genetic innovation, introducing new regulatory and structural variation. Centromeres, marked by chromatin containing the centromere-specific histone variant CENP-A, exhibit low recombination frequencies, tolerate sequence variation without loss of function, and are transcriptionally active. These characteristics make them attractive safe zones for transposable element insertion and propagation. Turnover between satellite repeats and TEs at centromeres is a major force driving of the rapid evolution of centromere DNA.

The non-LTR retroelement *G2/Jockey-3 (Jockey-3)* shows strong centromere enrichment, with 60% of its copies localized to centromeric regions. It is conserved across all five centromeres of *D. melanogaster* and *D. simulans*, despite divergence in their centromeric satellite DNA. *Jockey-3* is also the most highly enriched repeat in CENP-A chromatin, and population analyses suggest that recent insertions preferentially occur within CENP-A-associated domains. However, the mechanisms by which this element recognizes, and targets centromeres remain unknown.

To investigate this, we generated transgenic fly lines carrying an inducible full-length *Jockey-3* transgene (UASz-*eJockey-3*) with a unique barcode to track new insertions. Upon activation, *eJockey-3* induced DNA-damage foci and developmental defects, consistent with active transposition confirmed by digital droplet PCR analyses. Immunofluorescence staining revealed that *eJockey-3*–derived DNA damage occurs preferentially at centromeres, with an over 30-fold enrichment over random expectation.

By individually tagging the open reading frames of *eJockey-3*, we found that ORF1p, but not ORF2p, mediates nuclear import and centromeric localization. Remarkably, replacing the ORF1 nuclear localization signal (NLS) into ORF2 was sufficient to confer centromeric targeting, demonstrating that this short peptide is a determinant of centromere bias. Centromeric localization of *eJockey-3* requires these ORFs to be translated in *cis* i.e., from the same transcript. Temporal analyses revealed that *eJockey-3* dissociates from chromosomes during mitosis and reforms centromeric foci in interphase, suggesting that its chromatin targeting is cell-cycle regulated.

Together, our findings define how a mobile element biases CENP-A chromatin for targeting to enhance its own spreading, reshaping the sequence landscape and evolution of centromeres.

326T Convergent Evolution of Neo-Sex Chromosomes in *Zaprionus* Species

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In many species, sex chromosomes have independently evolved from autosomes following the acquisition of sex-determining functions, leading to characteristic patterns of differentiation in which X chromosomes retain most ancestral genes while Y chromosomes undergo extensive gene loss. Studies of neo-sex chromosomes, formed by fusions between autosomes and ancestral sex chromosomes, have provided powerful natural experiments for dissecting the roles of mutation, drift, and selection in sex chromosome evolution. However, the relative contributions of these forces remain debated, in part because neo-sex chromosomes differ in their origins across species. Here, we describe two independent autosome–sex chromosome fusion events in *Zaprionus* species (subgenus *Drosophila*), dating to approximately 15 million years ago. Remarkably, both fusions involve Muller's element B, creating a unique system to compare the evolutionary trajectories of the same set of genes following independent transitions to sex linkage. We find increased expression of neo-X-linked genes in males, consistent with the evolution of dosage compensation. In addition, we observe lineage-specific amplifications of multiple genes on the Y chromosome in both systems. Together, these results suggest that aspects of sex chromosome evolution may be partially predictable based on gene function, revealing convergent evolutionary outcomes across independent lineages. This system provides a powerful framework for disentangling the contributions of key evolutionary forces, including Muller's ratchet, Hill-Robertson interference, meiotic drive, and sexual conflict, to the long-term evolution of sex chromosomes.

327T Parallel Out-Of-Ancestral range expression differentiation in the male reproductive tract of two *Drosophila* species

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The evolutionary forces shaping gene expression variation across cells, organs, populations and species remain subjects of intense investigation. One way of detecting the influence of selection is by looking for parallel patterns of gene expression variation. Drosophila species have been a classic model system for comparative transcriptomics analysis. Migrating out of their ancestral ranges, *D. melanogaster*, and its sister species *D. simulans*, have independently colonized most of the globe, facing strong novel selection pressures on traits relating to temperature, seasonality, and day-length. Previous work has focused on North African and European populations of *D. melanogaster* to characterize the Out-of-Africa gene expression effects. However, North America and Australia, both of which were colonized within the last roughly 100 years, have been largely overlooked, and little work has addressed similar issues for *D. simulans*. Here, we report the first joint analysis of transcriptomes in both *D. melanogaster* and *D. simulans*, incorporating populations from their ancestral ranges (Sub-Sahara Africa for *D. melanogaster* and Madagascar for *D. simulans*) and from derived populations in North America and Australia, with a focus on two male reproductive tissue, testis and the accessory glands. We observed inter-specific parallel Out-Of-Ancestral differential expression in testis transcriptome. Our results suggest that selection has influenced transcript abundance in similar manners in response to selection pressures relating to colonization in both species.

328T Modeling the conditions for the maintenance of parthenogenesis

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Parthenogenesis, in which unfertilized eggs develop into adults, has been observed in various lineages, including fish, reptiles, birds, and insects. In *Drosophila*, facultative parthenogenesis is often considered an alternative reproductive strategy and might be advantageous in extreme environments where finding mates is challenging. However, this reproductive strategy may come with fitness costs. For example, individuals capable of parthenogenesis often show lower fertility in sexual reproduction. In addition, producing viable unfertilized eggs can sometimes result in abnormal offspring development, which may shorten lifespan or cause other negative effects. Since both fertility and lifespan can influence the total offspring produced, we constructed an analytical model to identify parameter combinations that allow parthenogenetic individuals to remain in the population. The results showed that when there is no fitness difference, the facultative parthenogenesis is always advantageous. Even while there is a reduced fitness in parthenogenetic individuals, facultative parthenogenesis can still be maintained under certain environmental conditions. These findings suggest that facultative parthenogenesis can persist under specific ecological conditions, highlighting the potential of parthenogenic variants to increase in frequency under certain demographic conditions, such as in small populations.

329T Adaptation vs sexual selection: Exploring the genes and selective pressures underlying a pheromone difference between natural *Drosophila* populations

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In fruit flies, cuticular hydrocarbons (CHCs) act as both mating pheromones and as a protective outer layer against dry environments. CHCs are thus subject to the forces of sexual selection and environmental adaptation, forces which may be either synergistic or antagonistic towards each other. Male *Drosophila melanogaster* from West Africa have a CHC profile dominated by 7-pentacosene (7-P), whereas most other populations have males with a predominantly 7-tricosene (7-T) profile. It has been hypothesized that this difference may be a result of environmental adaptation to West Africa›s hotter environment, however, the amount of 7-T versus 7-P on a male has been shown to affect female mating preference. Initial experiments on a highly 7-P biased line from West show that it has improved desiccation resistance and stronger intra-population female preference compared to a highly 7-T biased line. Population genetic analysis has identified candidate genes for the observed 7-T/7-P ratios, which are predicted to be fatty acyl-CoA reductase and elongase genes. We are now generating strain-specific knockouts of each gene to do reciprocal hemizygosity assays to test whether these genes may have evolved in response to adaptation or sexual selection. We are also performing assays of desiccation resistance across 22 different wild-derived populations that will help determine whether desiccation resistance varies more strongly between or within geographic and environmental groups, and whether that difference correlates with levels of 7-T and 7-P.

330T Annotation of Glycogen Phosphorylase (GlyP) in *Drosophila rhopaloa and* Multiple Inositol Polyphosphate Phosphatase 2 (Mipp2) in *Drosophila miranda* in collaboration with the Genomics Education Partnership

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The Pathways Project is a Genomics Education Partnership (GEP) initiative focused on annotating and analyzing genes involved in the insulin signaling and metabolic pathways across *Drosophila* species to investigate the evolution of complex biological networks. Insulin-mediated pathways are quintessential for maintaining cellular homeostasis, and growth regulation. This research focused on the comparative annotation of Glycogen Phosphorylase (GlyP) in *Drosophila rhopaloa* (*D. rho*) and Multiple Inositol Polyphosphate Phosphatase 2 (Mipp2) in *Drosophila miranda* (*D. mir*) relative to *Drosophila melanogaster* (*D. mel*), as both genes contribute to glycogen metabolism and inositol phosphate signaling, key components of insulin-mediated metabolic regulation. Examination of the genomic neighborhoods revealed evidence of synteny for both genes. In *D. rho*, upstream genes dpr3 and CG4259 and downstream genes tho2 and CG11723 were conserved relative to *D. mel*, with CG4259 showing lower percent identity likely reflecting evolutionary divergence. In *D. mir*, upstream genes Nep1 and Ca-alpha1T and downstream genes CG4660, CG4666 and the nested gene raptor were conserved relative to *D. mel*, demonstrating preserved gene order and orientation. Tblastn searches identified robust orthologs: GlyP in D. rho (XM_017115919) revealed strong alignment on scaffold NW_025335803 with E-value of 0 and 95.51% identity and Mipp2 in *D. mir* (XM_017291126) revealed scaffold on chromosome XL with E-value 3e-72 and 73.79% identity. Manual annotation of the coding regions and subsequent protein alignment confirmed high levels of sequence conservation. Data from the BLASTp alignment of D. rho GlyP protein to *D. mel* showed 95.51% identity, while the *D. mir* Mipp2 protein displayed 73.79% identity to the reference *D. mel* sequence. These results support the hypothesis of orthology and functional conservation. Future work will include annotation of additional GlyP and Mipp2 orthologs across more distantly related *Drosophila* species and comparative analysis of evolutionary rates to understand conservation and divergence within insulin signaling and metabolic pathways.

331T Exploring transposable element invasions using historical samples

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Transposable elements are selfish genetic sequences that can ‘jump’ from one location to another within a host genome. TEs are generally deleterious, and as a result, organisms have developed strategies for silencing them using small RNAs. Once a TE is silenced, it typically begins to deteriorate until it eventually loses its function. To escape this fate, TEs can transfer horizontally into naïve host genomes. A study that documented the recent TE invasion history of *D.melanogaster* revealed a total of 11 invasions in the last 200 years, approximately one invasion every 20 years. This high rate coincides with the increased global anthropogenic activities and the spread of *D.melanogaster* from Europe & Africa to the Americas & Australia about 200 years ago. *D. simulans* is also a recent human commensal, so we sought to take a comparative approach and investigate if the same patterns are evident in the invasion history of this species. We performed a non-invasive extraction of DNA material from samples housed in the Smithsonian, including Sturtevant’s original collections of *D. Simulans*. This extends our record of TE invasions to 1915 in *D. simulans*. Our initial results suggest that non-LTR retrotransposons G6, Jockey-2, BS3, Doc, and DNA transposon Hobo invaded and spread in *D.simulans* in the 19th century. We hypothesize that *D.melanogaster* and *D.simulans* attaining the human commensal status may have increased intra- and inter-specific hybrid zones, facilitating the rapid and nearly simultaneous spread of newly invaded TEs among their genotypes.

332T Recent horizontal transfer of transposable elements in *Drosophila*

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Transposable elements (TEs) are genetic elements also known as jumping genes that increase their copy numbers within a host through various mechanisms of transposition. Their high mobility is not restricted to within a chromosome, cell, or even a single organism. Horizontal transfer, which is the movement of genetic material between distantly related species that do not interbreed, can bring unpredictable changes to species and populations, such as hybrid dysgenesis and reduced fitness.

Organisms have evolved different silencing mechanisms to suppress TEs and their movement, creating dynamic evolutionary relationships. This complex dynamic, combined with diverse potential vectors and temporal-geographical variation, adds considerable complexity to understanding TE evolution. Unlike traditional genes, TEs exhibit high mobility and rapid sequence divergence, requiring specialized computational approaches for their study. This study aims to examine horizontal transfer of TEs in *Drosophila* at a large scale to understand their movement patterns and host-TE evolutionary relationships.

Using curated TE sequences and large-scale *Drosophila* genome sequences, we employed various bioinformatics tools such as MrBayes and custom bioinformatics scripts to infer phylogenetic relationships between TEs themselves. We integrated both genotype-based phylogenetic relationships from the literature and observed phenotypic relationships such as interbreeding patterns to establish species relationships. This study reveals a broader picture of horizontal transfer events across the *Drosophila* genus.

We identified various patterns of horizontal transfer across *Drosophila* species. As anticipated, we observed majority of transfer between closely related species, with the cosmopolitan *melanogaster* group showing the highest recent transfer activity. We also observed something interesting: DNA transposons exhibited a significantly higher propensity for transfer between distantly related species compared to retrotransposons. Our phylogenetic framework advances the understanding of horizontal transfer dynamics at the species scale within *Drosophila*.

333T Population genomics of *Drosophila pseudoobscura*

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Chromosomal inversions can change the structure of genetic variation in the genome and lead to the establishment of new gene arrangements. Multiple overlapping inversion polymorphisms on the third chromosome (Muller element C) of *Drosophila pseudoobscura* have attracted substantial attention for over 88 years (since it was used as the first data set to generate an evolutionary phylogeny in 1938) and considered as an ideal system to study evolutionary forces responsible for the establishment and maintenance of inversions in natural populations. In this study, we sequenced 65 inbred lines sampled across most clines in North America. After quality filtering, we analyzed 60 strains to characterize genome-wide patterns of nucleotide variation, population structure, and differentiation among chromosomal arrangements. Genome-wide Tajima's *D* values were negative across all chromosomes, consistent with an excess of rare alleles arising from population expansion or selective sweeps. However, the magnitude of Tajima's *D* varied among chromosomes, suggesting heterogeneous evolutionary pressures. Population structure analysis revealed little to no subdivision on chromosomes 2, 4, and X, consistent with a largely panmictic species. In contrast, chromosome 3 showed strong population structure corresponding to known inversion polymorphisms, with distinct clusters representing different other arrangements. Chromosome 5 also exhibited signals of differentiation, likely reflecting its unique composition, which includes both the highly heterochromatic element F and genes translocated from the ancestral Y chromosome.

Our results provide the most comprehensive genome-wide dataset to date for *D. pseudoobscura*, doubling the number of previously sequenced strains and leveraging a chromosome-scale reference genome. These data reveal chromosome-specific patterns of diversity and structure, highlight the impact of inversions on shaping population variation, and establish a valuable resource for future studies of adaptation and speciation in this classic model system.

334T Single-cell transcriptomes reveal largely sex-coupled evolutionary changes of sexual circuits in *Drosophila*

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Sexual dimorphism in neural circuits reflects the divergent behavioral strategies employed by different sexes to achieve reproductive fitness. Meanwhile, emerging data across vertebrate and invertebrate species show substantial similarity between sexes in the gross anatomy and cell types of sexual circuits, the neural circuits that control sexual behavior. How sexual circuits evolve under sex-specific fitness consequences and a largely sex-shared circuit architecture remains unknown. In *Drosophila*, the *fruitless* (*fru*)-expressing neural circuit is essential for sexual behaviors, including rapidly evolving male courtship behaviors, and therefore represents the neural locus of sexual dimorphism and the potential "battleground" of sexual conflict in the nervous system. Here, we leverage the genetic accessibility of the adult *fru* sexual circuit and the cell type resolution provided by single-cell transcriptomics to understand cell type and gene expression evolution in adult sexual circuits between *D. melanogaster* and *D. yakuba*. We identify clear homologs of transcriptomically defined cell types between sexes and species. Sexual dimorphism in transcriptomic profiles and sex-biased gene expression is limited, cell type-specific, mostly species-specific, and highly dynamic during development. Species divergence is widespread in each sex and predominantly shared between sexes, indicating the lack of a pervasive transcriptomic footprint caused by sexual conflict. Our data further suggests that pleiotropic gene expression may constrain sex-specific gene expression evolution and prevent the full resolution of sexual conflict. Finally, we find that transcriptomic divergence between sexes decreases from pupae to adulthood, suggesting that earlier developmental stages might experience a stronger degree of sexual dimorphism and sexual conflict. Overall, our results provide insights into the organization and evolution of sexual dimorphism in sexual circuits, suggesting that an adult sexual circuit with selective dimorphic sites among a largely sex-shared architecture enables the orchestration of sex-specific behaviors while retaining evolvability.

335T Disruption of Small RNAs and Mechanistic Polymorphism in the Sperm Killing Meiotic Drive System *Segregation Distorter*

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Meiotic drivers create genetic conflict by biasing their transmission to subsequent generations at a cost to the host organism. These conflicts arise in many taxa and may influence genome evolution, but little is known about how they exploit gametogenesis. Our lab studies the mechanism of the autosomal driver, *Segregation Distorter* (*SD*), in *Drosophila melanogaster*. *SD* is a sperm killer: *SD*/+ heterozygous males transmit *SD* to 95% of their offspring, whereas *SD*/+ females transmit *SD* fairly, to 50% of offspring. *SD* chromosomes are co-adapted gene complexes consisting of a driver and multiple enhancers, held in linkage by chromosomal inversions. *SD* causes a chromatin condensation defect in wild type chromosomes during spermatogenesis through an unknown mechanism. The drive target is a large block of tandem satellite DNA repeats called *Responder* (*Rsp*). The role of the *Rsp* satellite in *SD* drive is unknown. Our earlier work showed that *Rsp* behaves as a dual-stranded piRNA cluster and is expressed early in spermatogenesis. Because piRNAs play a role in establishing silent chromatin, we hypothesized that the *Rsp*-derived piRNAs might be involved in drive. We combined cytological, genetic, and genomic approaches to test this hypothesis. We find significantly fewer *Rsp* piRNAs associated with drive in some *SD* haplotypes. However, *SD* haplotypes with different inversions affect *Rsp*-derived RNAs differently. We hypothesize that *SD* haplotypes bearing different inversions may vary in their genetic enhancers leading to variation in mechanisms of drive. To confirm that *Rsp*-derived RNAs influence *SDMad* drive, we overexpressed *Rsp* and *Rsp* piRNAs in driving *SD* backgrounds. Consistent with our RNA seq results, overexpressing *Rsp* weakens drive in an *SDMad,* but not an *SD5,* heterozygote. Therefore, we demonstrate a molecular link between drive and the *Rsp satellite* and reveal potential mechanistic variation between driving haplotypes.

336F Correlated gene copy number changes of members of the Sex Peptide Network across *Drosophila* species

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In *D. melanogaster*, the seminal fluid protein Sex Peptide regulates varied and persistent post-mating changes in female physiology and behavior through interaction with its female receptor. The persistence of the female long-term post-mating response (e.g., reduced receptivity and increased egg-laying) requires Sex Peptide binding to sperm, which is mediated by a network of seminal fluid proteins. A recent study documented substantial copy number variation of *Sex Peptide* genes in *Drosophila* species. Therefore, we hypothesized that, due to shared selective pressures and compensatory changes to maintain functionality, members of the Sex Peptide network should exhibit correlated patterns of gene duplication or loss. By similar logic, evolutionary rate covariation of amino acid sequence change has previously been observed among members of the *Drosophila* Sex Peptide seminal fluid protein network and been used to identify some network members. Here, using a computational pipeline pairing iterative protein queries to genome sequence searches with phylogenetic clustering to resolve homology relationships, we exhaustively annotated between-species copy number variation of Sex Peptide network genes. We found that these genes are ancestral to *Drosophila* and predate the origin of *Sex Peptide* itself. Further, we observed statistically significant correlations in gene duplication or loss events among network members. Using shared patterns of gene loss across the genus, we also identified and experimentally validated a new network member, demonstrating the utility of this approach for discovering additional members. Interestingly, female-derived proteins that act downstream of the Sex Peptide sperm-binding network to modulate Sex Peptide's function showed no significant correlation of gene turnover events despite coevolving in sequence with some network members.

337F Orthologs of a rapidly evolving spermatogenesis transition protein vary in their capacity to condense spermatid nuclei in *D. melanogaster*

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Atlas is an orphan gene that is required for male fertility in *Drosophila melanogaster*, in which it helps to condense DNA in the nuclei of developing sperm. The Atlas protein sequence evolves rapidly across *Drosophila* species. To investigate the functional consequences of this rapid evolution, we tested whether *atlas* orthologs of varying levels of divergence were able to restore fertility to *D. melanogaster* males lacking any endogenous copies of the gene. We found that as the degree of sequence divergence increased, the degree of fertility rescue diminished, despite all orthologs being robustly transcribed. In no case did we observe a significant deviation from the expected sex ratio of progeny produced. To investigate the cytological basis of the diminished fertility, we used confocal microscopy to observe nuclear condensation in developing spermatids and the subcellular distribution of the Atlas orthologs. Orthologs that restored fertility localized as expected to condensing spermatid nuclei, and these nuclei showed either no or very subtle changes in shape. Orthologs that failed to restore fertility had greater defects in both spermatid nuclear shape and Atlas localization. We also identified a protein that is required for *D. melanogaster* Atlas to be properly imported into spermatid nuclei. These experiments demonstrate significant functional consequences to Atlas's rapid evolution and suggest that the protein could potentially be involved in lineage-specific coevolution with other factors that affect sperm chromatin condensation.

338F Newly evolved genes required for *D. melanogaster* spermatogenesis are post-transcriptionally regulated through their 3' UTRs

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When newly evolved genes arise in genomes, they face the challenge of evolving regulatory mechanisms that enable optimal expression. This challenge is particularly acute for genes that function in spermatogenesis, a process in which transcription and translation are frequently decoupled. We investigated the regulatory mechanisms used by two orphan, putatively *de novo* evolved genes, *saturn* and *goddard*, which are required for *D. melanogaster* male fertility and function in distinct, post-meiotic processes during spermatogenesis. When making rescue constructs for these genes, we found that each required its 3' untranslated region (UTR) to restore fertility in a null genetic background. In the absence of their 3' UTRs, the proteins encoded by these genes are produced too early in spermatogenesis, leaving insufficient levels of protein in the later-stage cells where their activity is essential. Deletion analysis of the *goddard* 3' UTR revealed a specific region required for proper post-transcriptional regulation. We are currently testing whether a candidate site within this region that is predicted to be recognized by a testis-expressed RNA-binding protein is required for the gene's proper expression. Deletion analysis of the *saturn* 3' UTR revealed that no individually tested region of the UTR is required for male fertility. Rather, each deleted region causes subtle effects on the distribution of Saturn protein throughout the testes, suggesting that multiple portions of the UTR collectively contribute to the protein's wild-type expression pattern. These analyses suggest that both *goddard* and *saturn* experience negative, post-transcriptional regulation through their 3' UTRs and demonstrate how newly evolved genes must sometimes evolve regulatory mechanisms outside of their promoter regions.

339F Teaching a Centromere to Drive

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In most eukaryotes, the essential process of chromosome segregation is orchestrated by repetitive DNA sequences known as centromeres. Centromeric histone H3 (CenH3) histone variants bind specifically to centromeric DNA and recruit the molecular machinery necessary for faithful cell division. CenH3 loss or overexpression can lead to centromere dysfunction, which is associated with genome instability. Despite their essential function, both centromeric DNA and centromeric proteins (including CenH3) evolve rapidly. The 'centromere-drive model' posits that this unexpected rapid evolution results from competition during oogenesis between different meiotic chromosomes for inclusion in the egg instead of polar bodies. A vital principle of this model is that excess recruitment of CenH3 proteins to repetitive centromeric DNA confers a meiotic advantage. Past studies with heterozygous mouse strains containing centromeres with different lengths of repetitive DNA strongly support the model that higher CenH3 recruitment may lead to female meiotic success. However, these chromosomes also differ in many other sequence aspects, which may influence meiotic transmission. To overcome these difficulties, we propose to engineer and test a new approach to recruit CenH3 to repetitive DNA in the model fruit fly species *Drosophila melanogaster* (*D. mel*). We will utilize a novel tool for generating targeted centromeres in *D. mel* using a fusion of catalytically dead Cas9 (dCas9) and the *D. mel* CenH3, also known as Cid. With this tool, we plan to induce a chromosome with an expanded centromere by using gRNAs to target the dCas9-Cid fusion to the pericentromeric satellite repeat of a single chromosome, mimicking the rapid centromere expansions that can occur due to unequal crossing over in meiosis. These studies will evaluate the fitness consequences of excess CenH3 recruitment in cell division and unambiguously test the centromere-drive model.

340F Investigating the spatiotemporal regulation of Dumpy in novelty development

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The evolution of morphological novelties is a major focus of evolutionary developmental biology. While much work in this field has focused on the redeployment of ancestral networks to new developmental contexts through gene regulatory co-options, much less is known about how cellular effectors gain expression in novel structures. Here, we trace how a key cellular effector (Dumpy) became integrated into the network for a morphological novelty, the posterior lobe, present in the genitalia of *Drosophila melanogaster*. Previous work has shown a gained apical extracellular matrix (aECM) accumulation in lobed species around the posterior lobe during its development that correlates with its evolutionary origin. The cellular effector Dumpy, one of the main components that make up the aECM, is expressed in the posterior lobe, and disruption of *dumpy* causes a near ablation of the posterior lobe phenotype. To unravel enhancer regulation of Dumpy in genital development, we identified multiple enhancers active throughout genital formation. Currently, we have analyzed the spatiotemporal expression of each enhancer during the formation of the genitalia to understand how the complex three-dimensional assembly of aECM is constructed from imaginal disc to pupal stages. Additionally, we analyzed the reporter expression pattern of an orthologous *dumpy* enhancer from a species lacking the posterior lobe that led us to conclude that this enhancer's expression has been probably conserved. Overall, we have shed light on how gene regulatory regions of cellular effectors can be redundant and pleiotropic contributing to morphological novelties like the posterior lobe.

341F X chromosome meiotic drive and polymorphic Y chromosome-linked resistance in *Drosophila affinis*

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Meiotic drivers are selfish genetic elements that bias gametogenesis to enhance their own transmission, violating Mendelian segregation. When located on sex chromosomes, such drivers can skew progeny sex ratios, creating female-biased populations and reducing overall fitness. Genomes can evolve resistance on autosomes or the Y chromosome to counteract drive. In *Drosophila affinis*, two distinct X-linked meiotic drivers, identifiable by unique inversions, segregate at ~10% frequency in wild populations. Polymorphic Y chromosomes (small Y, medium Y, large Y, segmented Y) varying in size and shape also occur in the wild, and XO males lacking a Y chromosome are fertile. The two drivers differ in their resistance profiles in XO males, and polymorphic Y chromosome variants may trade off resistance between drivers. We hypothesize that Y chromosome diversity correlates with variation in resistance to these drivers. To test this, we established ~100 Y chromosome replacement lines from wild-collected males and assessed resistance of each Y chromosome against both driving X backgrounds. Y chromosome morphology was examined using metaphase chromosomes from larval ganglion squashes. Additionally, whole-genome sequencing of selected Y lines allowed us to assess divergence among Y chromosomes and its correlation with resistance. Unlike most characterized meiotic drive systems, both driving X chromosomes and their resistance alleles co-segregate in the wild in *D. affinis*, making this species an ideal system to study the dynamics of drive and resistance in natural populations.

342F Adaptation of a DNA virus to *Drosophila Species*

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Drosophila innubila Nudivirus (DiNV) is a double-stranded DNA virus that exhibits high virulence in members of the quinaria group. While much is known about DNA virus adaptation in invertebrates, questions remain regarding their host range, virulence, and genetic evolution. Here, we explore DiNV adaptation to a three *Drosophila* cell line (*Drosophila innubila*, *Drosophila virilis* and *Drosophila melanogaster*). We further tested host specific adaptation using reciprocal transplant experiments in cell culture. Additionally, viral DNA was extracted from both an ancestral DiNV passage from a *Drosophila innubila* cell line and evolved passages from all cell lines. Using short-read sequencing we compared genetic differences between evolved and ancestral DiNV strains. Our results indicate that the virus passaged in cell lines (evolved strain) demonstrates enhanced growth compared to the ancestral strain. Additionally, we show that the evolved virus exhibits host-specific adaptation to its corresponding host cell compared to the ancestral virus, with sequencing identifying potentially causative SNPs. The adaptation of DiNV to these cell lines highlights the significance of viral evolution, demonstrating that DNA viruses in invertebrates can develop host-specific adaptations that affect virulence, this provides insights into DNA virus-host coevolution.

343F Contribution of locally adapted variation to adaptive potential in experimental cages of *Drosophila melanogaster*

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Genetic diversity is central to the ability of a population to adapt to its environment, but neutral genetic diversity does not always have a simple relationship with fitness. Local adaptation of populations to distinct environments provides a potential reservoir of fitness relevant variation. Experimental cages comprised of *Drosophila melanogaster* from Zambia, highland Ethiopia, and France were founded with equal numbers of inbred lines from one, two, or all three of the source populations. In separate experiments, these 18 cages were selected over 10 generations against two distinct selection pressures: survival on a toxic food source dosed with a detergent and survival in a wounding assay. Survival and population size metrics were measured over the 10 generations of selection. For both experiments, the cages founded with lines from a single geographic source had smaller population sizes after 10 generations of selection than cages founded with two or three geographic sources.

344F Recombinant drive as a mechanism for recombination rate control

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Meiosis ensures equal allele segregation of alleles during gamete formation; however, certain genetic elements can skew transmission patterns, potentially impacting fitness. One hypothesized mechanism, recombinant drive, leverages asymmetric female meiosis to favor transmission of recombinant genotypes by preferentially including them into the pronucleus, causing increased production of recombinants and, therefore, recombination rate. In this study, we explore the possibility of recombinant drive as a mechanism to increase recombination rate when Drosophila melanogaster are under stress.

To probe recombinant drive effects, we adopted an experimental protocol in which nutrient deprivation in virgin females halts oocyte production and induces developmental arrest of existing oocytes at metaphase-I, a stage when crossovers have already been resolved. After days of aging, oocyte arrest is released by mating and a nutrient-rich diet. Comparing arrested (aged) and unarrested (fresh) oocytes in the resulting progenies, aged oocytes showed ~16% increase in recombination frequency (RF). This is attributable to three mechanisms: (1) elevated crossover frequency during prophase-I, (2) preferential transmission of recombinants via recombinant drive, (3) reduction in the viability of non-recombinant progeny. Since oocytes arrest after crossover formation, increased RF is unlikely to result from increased crossover rates, leaving the latter two as more plausible mechanisms.

To investigate differential viability between recombinants and non-recombinants, we compared hatchability of embryos resulting from arrested oocytes with and without balancers. Unexpectedly, arrested oocytes with balancers showed higher hatch rates, revealing that achiasmate chromosomes are less susceptible to segregation errors induced by metaphase-I arrest. This rules out decreased viability of nonrecombinant and argues that recombinant drive is the mechanistic basis for the increase in RF. Interestingly, we find that stress-induced recombinant drive effects were abolished in Cid (centromere-specific histone) protein mutant, implicating centromere-associated mechanisms at meiosis II in the preferential transmission of recombinant chromatids during asymmetric meiosis.

Our findings suggest that under stress, organisms use recombinant drive to increase recombinant offspring and adaptive allelic diversity by leveraging centromeric segregation mechanisms for transmission bias.

345F Recombinant mapping of an essential locus for male fertility on the early stage neo-Y chromosome of *Drosophila albomicans*

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The X and Y sex chromosomes are thought to originate from a pair of homologous autosomes. Sex-linkage, often due to the acquisition of a sex determination factor, leads to the gradual divergence of the two in both morphology and gene content as a result of cessation of recombination. On the non-recombining sex chromosome, i.e. the Y, recessive deleterious alleles and male beneficial alleles are expected to irreversibly accumulate. While *Drosophila* all share the same X chromosome, or Muller A, in *D. albomicans*, both the X and Y have recently fused to the autosome Muller DC from two independent Robertsonian fusion events, leading to the formation of the neo-X and neo-Y chromosomes. Here, we seek to understand how sex chromosomes begin to diverge by capturing how the neo-Y chromosome in *D. albomicans* is evolving, with the specific goal of identifying potential male beneficial and recessive deleterious alleles that are accumulating on the neo-Y. Through PCR genotyping and karyotype imaging from DAPI-stained mitotic squashes, we show that F1 trivalent males, generated from hybridization between *D.albomicans* and its sister species *D. nasuta* that lack the neo-sex chromosomes, produce aneuploid offspring at up to 62% frequency. Based on this finding, we developed a complex cross scheme using trivalent male hybrids to generate F2 XXY aneuploid daughters that have both the neo-X and neo-Y chromosomes. By PCR genotyping at three loci along Muller DC in their F3 offsprings, we find that recombination between neo-X and neo-Y occurs in these aneuploid females, producing sons with recombinant neo-Ys that exchanged with the neo-X. Interestingly, we identified a significant association between male fitness and recombinant genotypes based on a sample size of 246 F3 males, where recombinant males are more than twice as likely to be sterile compared to non-recombinant F3 males. These results indicate the presence of an essential locus for male fertility on the distal end of the neo-Y. Genotyping by whole-genome sequencing is currently underway to identify the specific crossover sites in the neo-Y recombinants, with the goal of identifying the essential male-specific gene(s) located on the neo-Y.

346F Adaptive evolution of a maternally deposited nuclear lamina protein preserves early embryonic genome integrity

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The zygotic genome is transcriptionally silent at fertilization and remains silent until the maternal-to-zygotic transition. Consequently, the earliest stages of embryo development depend on maternal factors deposited into the egg. Although maternally provisioned proteins support conserved biological processes in the embryo, including DNA replication and mitosis, many evolve rapidly under positive selection. The causes and consequences of this paradoxical adaptive evolution are not understood. To resolve this paradox, I integrate evolutionary genetics and gene editing with cell biological approaches to probe the maternally provisioned protein, Fs(1)Ya. Fs(1)Ya interacts with both histone H2B and Lamin and is essential for the first mitotic division in *Drosophila melanogaster* embryos. I discovered that Fs(1)Ya has evolved adaptively between two closely related sister species, *D. melanogaster* and *D. simulans*. This history of positive selection suggests that one or more Fs(1)Ya functions require recurrent evolutionary innovation. To determine these functions, I used CRISPR-Cas9 to replace the endogenous *fs(1)Ya* allele in *D. melanogaster* with the diverged version from *D. simulans*. I found that *D. melanogaster* embryos provisioned with *D. simulans* Fs(1)Ya do not hatch, indicating that Fs(1)Ya^{sim} is incompatible with the *D. melanogaster* genome. Confocal imaging revealed that Fs(1)Ya^{sim}-provisioning interferes with the critical steps preceding the first embryonic mitosis. Specifically, the “paternal pronucleus” exhibits a prolonged S-phase relative to the maternal pronucleus. Post-replication, the paternal pronucleus is aberrantly small. During the first mitotic division, paternal chromosomes become caught as a chromatin bridge, leading to early embryonic arrest. These observations suggest that the paternal *D. melanogaster* genome, which undergoes extensive chromatin remodeling upon fertilization, is incompatible with Fs(1)Ya^{sim}. I hypothesize that inefficient paternal H2B deposition into repeat-rich genomic regions, which also evolve rapidly between *D. melanogaster* and *D. simulans,*compromises DNA replication, leading to the observed chromosomal defects. To test this, I am imaging H2B deposition in Fs(1)Ya^{sim}-provisioned and Fs(1)Ya^{mel}-provisioned (control) embryos. My evolution-guided functional analysis of a fast-evolving, essential maternal protein reveals that the earliest moments of development require recurrent evolutionary innovation.

347F Genetic mechanisms underlying repeated evolution of intraspecific cuticular hydrocarbon variation across distantly related species

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Understanding repeated evolution across distantly related species provides a powerful opportunity to identify whether shared genetic mechanisms underlie similar evolutionary outcomes. While there are many studies identifying the genetic mechanisms underlying the repeated evolution of morphological or physiological traits, not many studies focus on determining the mechanisms underlying the repeated evolution of similar traits that leads to reproductive isolation across distantly related species. Cuticular hydrocarbons (CHCs) are a dual trait in *Drosophila*, influencing both desiccation resistance and mate recognition, and thus link environmental adaptation with reproductive isolation. In *D. serrata*, adaptation to different environments led to intraspecific variation in CHCs and reproductive isolation between different populations. This intraspecific variation in CHCs is due to polymorphisms in the fatty acyl-CoA reductase gene *CG17560*. In the distantly related *D. lummei*, we identified a similar pattern of CHC divergence in populations collected from locations from Russia and Japan with different ecological conditions. Preliminary studies showed differences in desiccation resistance and mating success between these two populations. RNA-seq of adult cuticles where CHC-producing oenocytes are located showed several genes differentially expressed between these two populations. We will determine whether the genetic mechanisms underlying this CHC divergence in *D. lummei* is similar to *D. serrata*. Our study contributes to understanding if repeated cases of reproductive isolation across distantly related species may have a common genetic mechanism.

348F Mapping a strong X-linked suppressor of selfish *Segregation Distorter* in *D. melanogaster*

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Segregation Distorter (*SD*) is a selfish male meiotic drive system in *Drosophila melanogaster* that biases its transmission by eliminating sensitive sperm, yet persists at low frequencies in natural populations due to evolved host suppressors. Here, we focus on a strong X-linked suppressor, *Su(SD)X*, from the Drosophila Genetic Reference Panel strain RAL-256. Previous studies in multiple different strains mapped X-linked suppressors to overlapping regions on the X chromosome around 15.5 - 16.0 Mb. We suspect that a strong suppressor corresponding to *Su(SD)X* from RAL-256 resides in this interval. To test this hypothesis, we generated a *Su(SD)X* chromosome with fluorescent markers flanking this interval on the X chromosome (*Su(SD)X*$^{GFP\text{-}DsRed}$) and then generated recombinants between *Su(SD)X*$^{GFP\text{-}DsRed}$ and a non-suppressing X in a sensitive genetic background. We scored transmission distortion (k-value) in 167 recombinant males, revealing a bimodal distribution consistent with one major suppressor locus. PCR genotyping of selected driving ($k \geq 0.90$) and suppressing ($k \leq 0.60$) recombinants narrowed *Su(SD)X* to a 93.7 kb window. This region contains 15 genes, 2 long non-coding RNAs, and 1 antisense RNA, with multiple loci emerging as strong candidates based on functional relevance to *SD*’s mechanism. We are currently doing fine-scale mapping via Illumina multiplex sequencing and functional validation to confirm the molecular identity and mechanism of suppression. This work reveals how host genomes counter selfish elements, providing insights into meiotic drive dynamics and genome evolution.

349F The Evolution of Functional Divergence: Quantifying Expression and Sequence Dynamics Across Tissues, Sex, and Species of Drosophila

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Divergence in both gene expression and protein-coding sequence drive the functional diversity of species. However, the relative roles of tissue specificity and sex bias as well as molecular features such as codon usage, GC content, and network centrality in shaping the evolutionary rates of genes remain unresolved. Here, we apply a comparative analysis to understand the relationship between tissue- and sex-specific gene expression with patterns of molecular evolution across eight species of Drosophila. Expression profiles cluster primarily by tissue identity rather than species prompting an investigation of the evolutionary forces underlying this divergence. Significant predictors of protein divergence from gene-level models using the Drosophila phylogeny include codon adaptation and chromosomal context with X-linked genes evolving significantly faster than autosomal loci. In contrast, a comparison between two closely related species, *D. melanogaster* and *D. simulans*, reveal that tissue specificity and sex bias accelerate molecular evolution, whereas genes with greater protein-protein interactions and higher GC are constrained. Across the eight species, a shift in gene expression variance was often biased towards female expression. Together, these analyses demonstrate how both lineage-wide molecular features and tissue- or sex-related function shapes diversity. By coupling expression and sequence evolution, our results show how tissues evolve through regulatory flexibility versus purifying constraint. This work also illustrates how sexual dimorphism and tissue specialization structure the functional landscape of the genome and offer a predictive basis for understanding how developmental and reproductive functions drive genomic diversity and the evolutionary potential of genes and tissues.

350S Evolutionary analyses of newly discovered domestications of *PIF* transposable element proteins in *Drosophila*

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Transposable elements (TEs) can contribute to the emergence of novel host genes through molecular domestication. Increasing evidence supports the recurrent domestication of *P instability factor* (*PIF*)/*Harbinger* superfamily of DNA TE proteins in eukaryotic genomes. *PIF* DNA TEs are characterized by having two ORFs: one encoding a catalytic transposase with a DDE domain, while the other encodes a protein with a Myb/SANT-like/MADF DNA-binding domain that facilitates nuclear localization of the transposase. In *Drosophila*, there are seven independent domestications of *PIF* TE transposases, known as *Drosophila PIF-like Gene 1-7* (*DPLG1-7*). Here, we describe two additional domestications of *PIF* transposase-derived genes in *Drosophila*, *DPLG8* and *DPLG9*. Phylogenetic and synteny analyses revealed that *DPLG8* is found only in *D. melanogaster*, *D. simulans*, *D. sechellia*, and *D. mauritiana*, with conserved synteny on the X chromosome. *DPLG8* is the youngest *DPLG* discovered to date, and shows male-specific expression. Analyses of substitution rates, polymorphism, and divergence in coding regions between *D. melanogaster* and *D. simulans* revealed that *DPLG8* is evolving under both purifying and positive selection. Both Tajima's *D* and Fay and Wu's *H* are negative for *DPLG8*, and we observed signatures of a recent selective sweep in *D. melanogaster*. The absence of the DDE catalytic triad in DPLG8 suggests loss of catalytic activity following domestication. In contrast, *DPLG9* is an older, independently domesticated gene present only in *D. hydei*, *D. montana*, *D. virilis*, and *D. novamexicana*, with conserved synteny except for independent inversions in *D. hydei* and *D. virilis*. Evolutionary analyses indicate that *DPLG9* is under strong purifying selection. Our findings demonstrate that *PIF* TE-derived genes have been repeatedly and incessantly co-opted by host genomes, and that the functions for which these genes were domesticated are under strong evolutionary pressures shaping the genome evolution in *Drosophila*.

351S Investigating the co-evolutionary dynamics of Drosophila recens and Wolbachia

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Wolbachia is a maternally inherited bacteria that manipulates host reproduction to ensure its own transmission, often to the detriment of host fitness. Wolbachia-host interactions are highly dynamic and can co-evolve rapidly, and over time may evolve toward a less virulent interaction. The North American mushroom-feeding fly Drosophila recens harbors a Wolbachia infection that induces strong cytoplasmic incompatibility (CI), where offspring of uninfected females and infected males die. We recently discovered that D. recens harbors two Wolbachia strains, and here we investigate their co-evolution with the host. First, we found that one strain is recently derived from the other, and that they both occur in natural populations. Second, we assayed the phenotype of each Wolbachia strain in D. recens hosts to determine if the derived strain is replacing the original strain due to lower virulence. We conducted assays of strength of cytoplasmic incompatibility, female fecundity, and Wolbachia titer. Third, we introgressed each Wolbachia strain into the closely related host D. subquinaria, which naturally hybridizes with D. recens, and compared the Wolbachia phenotype of each strain in the novel versus native host. This system provides a rare opportunity to observe host-endosymbiont co-evolution in real time and to characterize the dynamics of a potential strain replacement in natural populations.

352S The Hawaiian Drosophila Genomes Project: Uncovering the Genomic Basis of an Extraordinary Adaptive Radiation

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The Hawaiian drosophilid radiation, comprising ~700 described species belonging to two major groups, the Hawaiian *Drosophila* and the genus *Scaptomyza*, are an ideal system for studying the mechanisms that generate biodiversity. Their extreme isolation has driven remarkable bursts of diversification into varied ecological niches: many of them are found on a single island and are highly ecologically specialized on a single native plant family. Although many mainland drosophilid species (like *D. melanogaster*) are popular model organisms, and while the Hawaiian *Drosophila* Project of the 1960s was a major effort to catalog and understand the biology of Hawaiian drosophilids, scientific interest in this system has waned and the application of genomics to this group in recent decades has been limited. However, methodological advances that we have developed now make it possible to long-read sequence individual small insects (down to 25 ng gDNA) and make this an opportune moment to revisit this classic adaptive radiation with modern tools. Near-chromosome level genome assemblies can be obtained from single flies, provided they are freshly collected. We are now leading a collaborative effort to sample across the major Hawaiian Islands to obtain genome assemblies and population genomic datasets for a maximum representation of species, including fully annotated chromosome-scaffolded genomes for representatives of each major lineage. This scale of data will provide unparalleled resolution to study the within- and between-species processes that generated this extremely diverse group. Specifically, we will: resolve long-standing questions about the phylogenetic relationships within this group with genome-scale data, including a potentially complex history of speciation with gene flow; identify the genomic variation that underlies the Hawaiian radiation; and measure how natural selection has shaped their genomes. This project will occur in parallel with a broader effort to sequence non-Hawaiian drosophilids, which allows us to assess whether patterns and processes in Hawai'i are exceptional in the context of the family Drosophilidae, but also to build this dataset as part of an open genomic resource for our community. Finally, this may be one of the last opportunities to study the genomic mechanisms that generated one of the most extraordinary cases of diversification in nature, as the Hawaiian Drosophilidae are highly threatened by human impacts.

353S The Interaction of Cyclin G in the Insulin/TOR Pathway

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The Insulin/TOR pathway is evolutionary conserved and critical for growth and metabolism. An important positive regulator of this pathway is Cyclin G (CycG), interacting with the PP2A subunit B', specifically the widerborst (wrd) and well-rounded (wdb) gene found in the B' subunit. Mutations or loss of the CycG results in PP2A binding and dephosphorylating the Akt1, resulting in the inhibition of the TOR signaling pathway. This inhibition impacts metabolism, cell survival and gene transcription negatively, which effects the growth of an organism as a whole. The objective of this research is to understand how CycG is conserved among various Drosophila species, as well as how CycG binds to the well-rounded and widerborst genes on a molecular level. Protein sequences of CycG gene among ⅔ of Drosophila species were given, collected and validated from the GEP Genome Browser. This study examines the conservation and evolutionary processes using phylogenetics and multiple sequence aligments. With the use of AlphaFold Server and RStudio, further analyses will be performed on the specific domains in each and the binding interactions. Preliminary results suggest a dN/dS less than 1 in domains of CycG, showing conservation; the domain from wdb's protein sequence binding to the domains of the CycG and expect a similar AlphaFold profile among various of Drosophila species. The Insulin/TOR pathway has an important cyclin that interacts and helps regulate the pathway– CycG. Mutations to the conserved part of the cyclin can result in negative impacts on growth and regulation. This study assesses the structure and function of CycG: how it stays conserved as well as binding interactions between the wdb gene in the Insulin/TOR pathway across different Drosophila species.

354S Effects of developmental and adult temperature on lifespan in temperate and tropical *Drosophila*

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Thermal conditions during development and adulthood jointly shape adult lifespan, yet their interactive effects across species from different climates remain unclear. We used recently collected, outbred populations of temperate *Drosophila melanogaster* (Slovakia) and tropical *D. ananassae* (India) to examine how developmental and adult temperatures structure lifespan reaction norms in a comparative framework. Flies developed at ten (*D. melanogaster*: 13–31 °C) or nine (*D. ananassae*: 15–31 °C) developmental temperatures and, upon eclosion, were transferred to one of three adult temperatures (19, 25, 29 °C). Adult lifespan was measured in mixed-sex groups across all developmental × adult temperature combinations. This fine-scaled, fully factorial design allows us to examine lifespan reaction norms, test whether climatic origin predicts differences in optimal developmental temperatures, and quantify the consequences of thermal mismatch between developmental and adult environments within and across species. Initial analyses indicate that development at both low and high thermal extremes tends to reduce adult lifespan in both species. Together, these data provide a high-resolution comparative test of how developmental history modulates adult thermal performance and may shape species-specific vulnerability to changing climates.

355S A cytoplasmic factor underlies hybrid female sterility in *Drosophila yakuba* and *D. santomea*

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Species are fundamentally defined as populations that are reproductively isolated from one another. A key early step in the speciation process is the development of sterile male hybrids, which is typically followed over time by the development of sterile female hybrids. While much is known on the genetic basis of hybrid male sterility, a lot remains to be learned about hybrid female sterility. Our research utilizes a model system of two very closely related *Drosophila* species that produce mostly sterile F_1 hybrid females with *D. yakuba* mothers whereas their reciprocal counterparts (hybrid females with *D. santomea* mothers) remain fertile. To better understand this phenomenon, we examined gene expression differences between parental strains, and between them and their hybrid female offspring. These studies showed that transposable elements lack proper silencing in the ovaries of sterile F_1 females but have parental levels of transcription in fertile F_1 hybrids, indicating the influence of cytoplasmic factors. The cause of this phenomenon is not yet clear and cannot be said to be the source of the sterility in these females, but it is the first step towards identifying candidate mechanisms underlying female reproductive dysfunction in hybrids. Additional experiments also suggest a significant mitochondrial contribution to this cytoplasmic incompatibility.

356S Dietary stress reveals genotype by environment interactions in recombination rate plasticity

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Evolutionary genetics seeks to understand how evolutionary processes shape genetic variation. Meiotic recombination generates novel genetic variation. Despite its fundamental role in ensuring proper chromosomal segregation, meiotic recombination is highly variable. Recombination rate varies significantly depending on intrinsic and extrinsic factors, referred to as recombination rate plasticity, which includes environmental stress such as diet, temperature, and age. In addition, as organisms experience stress, their fitness declines. Fitness-associated recombination (FAR) hypothesizes that organisms increase recombination rate as an adaptive response to stress, predicting a negative relationship between RR and fitness.

In this study, we use *Drosophila melanogaster* from the Drosophila Genetic Reference Panel (DPRP) to test whether caloric density and genotype influence recombination rate. Previous work in our lab has shown that diet alters recombination, with a difference between low calorie (0.5x) and high calorie (2x), and some differences in genetic background based on two DGRP stocks. Here, we extended our study to include up to five DGRP stocks and additional intermediate dietary treatments. We measured recombination, fecundity, survivorship, nondisjunction, and body mass. Recombination and nondisjunction were quantified using seven X-linked visible markers, while egg-to-adult emergence assays were used to assess fecundity, fertility, and survivorship. Our results showed that dietary manipulation at the extremes (0.5× and 2×) did not significantly reduce survival. Also, our diets did not significantly alter nondisjunction rates. However, diet and genetic background had significant effects on recombination rate, suggesting genotype-by-environment (G×E) interactions, with two clear trends identified. Furthermore, increased recombination was not associated with reduced survival, regardless of strain, providing little support for the (FAR) hypothesis.

Our results highlight important factors such as condition and genetic background to consider in any study on genetic variation. Because recombination plasticity was not associated with reduced fitness, these changes do not appear to be adaptive, indicating that further work is needed to understand how evolutionary processes explain recombination rate plasticity.

357S Large-scale screening for meiotic drive in *D. melanogaster* populations

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Meiotic drive loci subvert Mendel's first law to selfishly increase their transmission. The selective costs imposed by meiotic drive are implicated in contributing to karyotype evolution, adaptive divergence of chromosomal and reproductive genes, and speciation. Meiotic drivers have been found sporadically over the last 100 years in animals, plants and fungi, with most discovered fortuitously due to their causing extreme deviations from Mendelian segregation. Yet little is known about the spectrum of meiotic drive in natural populations, and surprisingly even the model species *D. melanogaster* has not been screened comprehensively for meiotic drive. Here we report developing a screening method that tests for both female and male meiotic drive on all *D. melanogaster* chromosomes. We placed fluorescent markers at the telomeres and pericentromeres of all chromosomes (except the Y) that can be imaged in adults on a scanner and automatically scored to ~98% accuracy using the Fiji image processing package. This system allows us to readily genotype thousands of progeny per cross, providing power to detect deviations from Mendelian segregation as low as ~53:47. We have screened individual chromosomes extracted from 52 isofemale lines of a population collected in Zambia by crossing them to our fluorescent marker strains and scoring segregation in F1 progeny. We discovered a new isolate of *Segregation Distorter* (*SD*), which is consistent with previous reports that *SD* segregates at low frequency in most populations and validates our screening method. We have also identified several chromosomes from the Zambia population that consistently display moderate deviations from Mendelian segregation and that we are currently characterizing.

358S Leveraging Deep Learning to Study Drosophila Enhancer Evolution

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Gene regulation is fundamental to shaping morphological diversity, driven by cis-regulatory regions containing transcription factors motifs to orchestrate precise gene expression. Transcription factor motifs are often critical for function, yet they frequently undergo gains or losses during evolution.Thus, how enhancer sequences change while maintaining their function is not clear. Recent research has shown that chromatin accessibility depends on motif cooperativity that follows a more flexible motif syntax and enables low-affinity motifs to have an impact, providing an avenue by which motifs can arise de novo. To test if motif turnover occurs very frequently at the level of chromatin accessibility, we used Drosophila trichomes as a model system since it has previously been shown to show evolutionary changes at the level of cis-regulatory regions. To comprehensively map the chromatin accessibility landscape across several Drosophila species, we performed ATAC-seq on D. melanogaster, D. erecta, D. ananassae, and D. mojavensis embryos at the appropriate stage. This revealed that the amount of chromatin accessibility in regulatory regions is highly conserved across species, despite considerable sequence divergence. To identify functional motifs and interpret the cis-regulatory sequence rules in each species, we trained ChromBPNet models to predict bias-free base-resolution accessibility profiles from DNA sequence. We find that models trained on one species perform well in predicting the ATAC-seq data from another species, while the small differences reflect the evolutionary distances between them. This suggests that in addition to the accessibility amount, the motifs and syntax rules are also largely conserved across species. In contrast, model interpretation revealed that individual motif instances frequently turn over and change in affinity, supporting the hypothesis that the cis-regulatory rules of chromatin accessibility enable changes such as frequent motif turnover, change in affinity etc.

359S Natural Variation in Meiotic Drive and Susceptibility in Drosophila melanogaster

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We previously discovered that a D. melanogaster protamine gene, Mst77F, is required to suppress the potentially Y-chromosome-mediated meiotic drive against the X chromosome. Since meiotic drive is generally deleterious to the transmission of autosomal alleles due to lower male fertility, theory predicts that multiple suppressors of drive will arise within populations. Mst77F may be not the sole suppressor. We hypothesized that natural variants in distinct genetic loci might interact with and affect meiotic drive unleashed by the only half-dosage of Mst77F. To identify these natural variants, we generated hemizygous Mst77F flies, a sensitized background, carrying either the X or the Y chromosome from seven strains across different populations. By crossing these males to wild-type females, we then measured variation in fertility and drive strength, or in susceptibility to drive, of individual Mst77F hemizygous males from each genetic background. We found significant variation in both drive strength and drive susceptibility across different strains. We will combine this phenotypic variation with the sequencing information of different X and Y chromosomes to identify genetic loci associated with drive or susceptibility. Our study contributes to a better understanding of the pervasive effects of meiotic drive and the functions of protamines in suppressing sex-ratio distortion in natural populations.

360S Spiralling Invasions: Invasive species control through genetic Allee effect

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Invasive species are a significant factor contributing to the loss of native biodiversity and economic losses. Once introduced, these invasive species form smaller subpopulations at their boundaries, which further the invasion process. Eliminating these small subpopulations is the key to containment, but conventional means have proven ineffective. However, these smaller populations tend to have lower genetic diversity due to bottlenecks during migration. They may thus be vulnerable to extinction if variants present in these subpopulations are deleterious (i.e., if there is a genetic load). In this study, we explore the possibility of pushing these subpopulations into extinction by injecting the core population with deleterious recessive alleles, thus introducing a genetic Allee effect. To quantify the Allee effect, we used *Drosophila melanogaster* deficiency lines and large insect cages to mimic invading populations carrying a genetic load under various initial population conditions. Our experimental data suggest that the genetic load has a significant impact on the growth rate in small populations across multiple generations, with the effect proportional to the number of deleterious alleles and their initial frequency. As the next step, we aim to perform an allele frequency analysis to assess the purging rate of alleles, which will help us understand the persistence and effectiveness of this control method over a long-term period.

361S Violation of Mendelian segregation: Identifying selfish genes causal for sex chromosome drive in *Drosophila albomicans*

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Gametogenesis is a conserved biological process that produces gametes (sperm and egg cells) for reproduction. During gametogenesis, chromosomes split and segregate equally, ensuring that gametes retain only a single copy of each chromosome. However, selfish genetic elements such as meiotic drive genes are known to frequently 'cheat' equal segregation principles to propagate their own transmission to the gametes. When meiotic drivers occur on sex chromosomes in males, they cause unequal transmission of X and Y chromosomes, resulting in a skewed sex ratio among offspring—a phenomenon known as sex chromosome drive. Sex chromosome drive often reduces host's reproductive fitness, and an imbalanced sex ratio can be detrimental to the species. As a result, strategies to counteract drive evolves to restore equal segregation of X and Y chromosomes, but the identities of meiotic drive genes in most cases, and the mechanisms of host suppression, remain unknown. Only a few meiotic drivers have been identified at the gene level so far, and none have had their molecular mechanisms fully elucidated. Here, we study sex chromosome drive in hybrids between *D. albomicans* and *D. nasuta*—two relatively young *Drosophila* species in the *immigrans* group. Our work builds on a prior genetic study that identified four quantitative trait loci (QTL) associated with sex chromosome drive in *D. albomicans*. Since these QTLs are predicted to contain genes responsible for sex chromosome drive, we performed third-generation sequencing, and testis transcriptomics from the *D. albomicans* driving strain and characterized 1008 annotations, of which, 526 genes are expressed in the testis within the major effect QTL (QTL1). As sex chromosome drive occurs primarily in hybrids, we hypothesize that the loss of suppression in hybrids may result in the misexpression of meiotic drive genes. To identify putative meiotic drive gene(s) within QTL1, we performed comparative transcriptomics from testes in *D. albomicans* and *D. nasuta* and identified 6 genes that are upregulated in F1 *D. albomicans* (♀) X *D. nasuta* (♂) hybrids, of which, *MYH01-18694* is a multi-copy gene on the *D. albomicans* neo-X chromosome that we are currently investigating its potential role in sex chromosome drive. Additionally, we also seek to validate our putative meiotic driver using three *D. albomicans* strains that lack sex chromosome drive. Overall, our study aims to uncover novel sex chromosome drive genes, which will pave the way for mechanistic investigations into sex chromosome drive and its effects on genome and sex chromosome evolution.

362S Genetic variation modulates body weight and metabolic responses to high-fat and high-sugar diets in *Drosophila melanogaster*

Sumaira Shabbir, Yulin Bai, Yuyan Chen, Mutheshree Rajesh, Sabir Ali, Abigail Trudnak, Xuan Zhuang Biological Sciences, University of Arkansas, Fayetteville

Genetic variation significantly influences how our bodies process food, which is crucial for comprehending intricate metabolic conditions. Obesity and related health problems represent a growing global health challenge, driven by interactions between genetic background, environmental factors, and behavior, especially diet. While all animals require carbohydrates, proteins, and fats, there can be considerable differences in how well various species, populations, and even individuals handle different diets and metabolize nutrients.

Drosophila melanogaster is a powerful model system for investigating diet–genotype interactions due to its well-characterized genetics and conserved metabolic pathways. Prior research has shown that high-sugar (HSD) and high-fat diets (HFD) induce obesity-like phenotypes in flies, including increased triglyceride storage, insulin resistance, and altered carbohydrate metabolism, closely paralleling metabolic dysfunction observed in humans. Despite this, we still have a limited understanding of how natural genetic variations affect body weight and important metabolic indicators when flies are exposed to different diets.

To address this gap, we examine variation in macronutrient tolerance using the Drosophila Genetic Reference Panel (DGRP), a collection of approximately 200 inbred strains derived from a single natural population. We reared these genetically distinct strains on control, HSD and HFD, then measured their body weight and metabolic phenotypes like glucose, glycogen, triglyceride, and protein levels. We will then use genome-wide association study to identify genetic loci associated with diet-induced phenotypic variation and to characterize gene-environment (GxE) interactions underlying metabolic susceptibility.

We anticipate substantial phenotypic variation among DGRP strains in body weight and metabolic profiles in response to different dietary stresses, and that these traits will have high heritability. The expected outcomes include the identification of diet-specific genetic effects and candidate genes involved in nutrient-dependent metabolic dysregulation. Together, these findings will advance our understanding of how genetic diversity shapes metabolic health, provide insight into conserved mechanisms underlying human metabolic diseases such as obesity and diabetes, and highlight the importance of GxE interactions in metabolic regulation.

363S Genetic analyses and functional validation reveal diet-dependent GxE effects on *Drosophila* development time

Yulin Bai, Sumaira Shabbir, Yuyan Chen, Sabir Ali, Abigail Trudnak, Mutheshree Rajesh, Xuan Zhuang University of Arkansas

Diet-dependent developmental plasticity is widespread in natural populations, yet the genetic factors underlying genotype-by-environment (GxE) effects remain poorly understood. Here, we examined development time, a strongly diet-responsive trait, to identify genetic determinants of variation between high- and low-sugar diets. Using recently developed Drosophila recombinant outbred populations, we performed genome-wide association analyses (GWAS) within each diet and a meta-analysis (meta-GWAS) across diets in multiple populations, identifying 14 candidate genes whose variants were reproducibly associated with diet-dependent differences in development time. These loci were enriched for conserved metabolic regulators, including genes with human orthologs linked to metabolic phenotypes, highlighting the evolutionary conservation of metabolic pathways between *Drosophila* and humans. To validate the function of the prioritized candidate genes, we used genetically modified strains obtained from the Bloomington Drosophila Stock Center to test their effects experimentally. Of the 14 genes tested, 11 significantly shifted development time in at least one diet condition, providing experimental evidence that these loci contribute to diet-dependent developmental variation. Notably, four genes (*Pak*, *Tap*, *Eip75B*, and *Cerk*) exhibited significant GxE effects, with genotype-dependent changes in development time across diets, in agreement with predictions from the genetic mapping results. Collectively, these findings refine the set of candidate genes for diet-dependent development time and demonstrate that integrating diet-stratified association mapping with targeted functional validation is an effective strategy for studying GxE mechanisms.

364T Selective adhesion facilitates gut symbiont-host specificity through a conserved molecular mechanism

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Animals form symbiotic relationships with resident gut microbiota that are both stable and host-specific. These specific associations play critical roles in the fitness of both the host and microbiota, yet the mechanisms underlying specificity remain unclear. Lactobacilli are prevalent symbionts in mammals and insects, with distinct strains colonizing different hosts. We previously isolated a strain of *Lactiplantibacillus plantarum* (LpWF) from wild-caught *Drosophila melanogaster* that specifically colonizes a symbiotic niche within the foregut, and demonstrated that a pair of serine-rich repeat adhesin (SRRP) genes within the LpWF genome are major determinants of foregut colonization. To elucidate the molecular mechanisms of foregut colonization by LpWF, we biochemically manipulated the SRRP binding regions and found that they specifically recognize distinct subregions of the symbiotic niche. Glycan microarray screening found that the SRRPs specifically bind fucosylated glycan structures. Through glycomic mass spectrometry, we confirmed that the symbiotic niche is dominated by a unique glycan profile that contains structurally similar glycans to those bound in the array. Ectopic expression and cell surface display of the binding regions in mutant *L. plantarum* strains and *Lactococcus lactis* demonstrated sufficiency of the SRRPs to promote spatially-specific localization of bacteria to the foregut, suggesting that adhesion plays a key role in specifying colonization of the symbiotic niche. Cryo-EM, truncation, and single residue substitution analyses of the binding regions identified a glycan binding pocket within a structurally conserved ß-helix domain. Structural phylogenetic analysis of SRRP ß-helix domains from diverse host-associated lactobacilli revealed that the binding pockets show highly distinct sequence characteristics and predictive binding properties. We currently aim to identify the Drosophila-expressed ligands of the SRRPs via tissue-specific knockdown of glycosylation genes and trace the evolutionary histories of ligand recognition by SRRP proteins to further investigate mechanisms by which animals establish and regulate specific host-microbe associations. Overall, our study reveals a molecular mechanism of host tissue recognition through glycan binding specificity by *L. plantarum*.

365T Drosophila complement-like Mcr acts as a wound-induced inflammatory chemoattractant

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Sterile tissue injury is accompanied by an acute inflammatory response whereby innate immune cells rapidly migrate to the site of injury guided by pro-inflammatory chemotactic damage signals released at the wound. Understanding this immune response is key to improving human health, and recent advances in imaging technology have allowed researchers using different model organisms to observe this inflammatory response *in vivo*. Over recent decades, offering a unique combination of live time-lapse microscopy and genetics, the fruit fly *Drosophila* has emerged as a powerful model system to study inflammatory cell migration within a living animal. However, we still know relatively little regarding the identity of the earliest signals that drive this immune cell recruitment and the mechanisms by which they act within the complex, *in vivo* setting of a multicellular organism. Here, we couple the powerful genetics and live imaging of *Drosophila* with mathematical modeling to identify the fly complement ortholog-macroglobulin complement-related (Mcr)-as an early, wound-induced chemotactic signal responsible for the inflammatory recruitment of immune cells to injury sites *in vivo*. We show that epithelial-specific knockdown of Mcr suppresses the recruitment of macrophages to wounds and combine predictive mathematical modeling with in vivo genetics to understand macrophage migration dynamics following manipulation of this chemoattractant. We propose a model whereby Mcr operates alongside hydrogen peroxide to ensure a rapid and efficient immune response to damage, uncovering a novel function for this protein that parallels the chemotactic role of the complement component C5a in mammals.

366T From protein to pathogen: Toxin-receptor co-evolution across biological scales

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Evolutionary arms races are ubiquitous in nature and lead to the origin of novel traits through co-evolution. Protein toxins often lie at the heart of the Red Queen-like dynamics between hosts and pathogens. Toxic cargo genes are deployed by both hosts and pathogens, evolve rapidly, and are even co-opted by their targets through horizontal gene transfer (HGT) for their own benefit. While many toxic proteins relevant to human disease have been studied in detail, there remain important gaps in our knowledge of how these evolutionary dynamics play out across different levels of biological organization, as we lack tractable models to test these dynamics at the organismal level in natural hosts. Addressing this gap is crucial to understand fundamental processes that shape host-pathogen biology. My new laboratory at UCSF will use genetic approaches to elucidate molecular mechanisms of co-evolution between toxic proteins and their cellular targets at the host-pathogen interface across biological scales (proteins, cells, organisms). To this end, we are developing a naturally occurring and genetically tractable *Bacillus thuringiensis* (Bt) toxin–*Drosophila* system to address this goal with potential applications towards agriculture and human health. We are particularly interested in using existing genetic tools and developing new genetic techniques in *Drosophila* to address the following biological questions: How does toxin specificity evolve? How do toxins potentiate pathogenic lifestyles in otherwise commensal bacteria? How do host immune systems respond and evolve to these pathogens, and what functional constraints are there on the evolution of immune proteins?

367T Time of Day Variation in the Gut Microbiome and Feeding Behaviors of *Drosophila melanogaster*

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Interactions between the gut microbiome and the host circadian rhythms give rise to a complex set of oscillations in behavior and physiology. Using 16S sequencing, we detected time of day variation in the composition of the fly gut microbiome. This variation was abolished under constant light, which inactivates the fly circadian clock. In contrast, the time of day variation in the host microbiome was maintained in *period* mutant animals with genetically ablated circadian rhythms. To investigate the connection between microbiome and feeding behavior, the CAFÉ assay was used to monitor feeding behavior. As expected, non-sterile wild-type flies showed rhythmic feeding behavior over time in constant dark conditions (DD). Non-random choice of feeding time was also apparent in non-sterile *period* and *timeless* mutant populations in DD. Experiments to measure feeding time preference in sterile flies are currently underway.

368T Genetic Dissection Reveals Region of 2R as Basis of Broad Immune Susceptibility Previously Attributed to Thor2 Flies

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Thor encodes eIF4E-BP, which inhibits cap-dependent mRNA translation under oxidative stress, starvation, and bacterial infection in *Drosophila*. Previous studies had indicated that *Thor* mutant flies are highly sensitive to Gram-positive and Gram-negative bacterial infections. However, while performing genetic background isogenization, we discovered that the broad infection-sensitivity phenotype could be separated from the *Thor* mutation through a single recombination event, indicating that it has a distinct genetic basis. We mapped infection sensitivity to the 2nd chromosome, which also carries the *Thor* gene. We then generated 54 recombinant 2nd chromosome lines between the original *Thor* mutant strain and wild type Canton-S. For each recombinant line, we quantified susceptibility to infection by the Gram-negative bacteria *P. rettgeri* and *S. marcescens* infection and performed low-coverage genome sequencing. We identified a 300 kb region of 2R that fully explains infection sensitivity and is independent of the Thor mutation on 2L. To identify the possible gene(s) responsible for this increased mortality phenotype, we evaluated sensitivity to infection in transheterozygotes of the original *Thor* line and a revertant $Thor^{rev}$ strain over ~60 deficiency and gene disruptions spanning the region of interest. While no single candidate from these screens completely explained the immune phenotype, we consistently observe that inheritance of the implicated 2R region shows a dominant phenotype when hemizygous or heterozygous, and is strongest when homozygous. We infer that sensitivity to infection may be due to combined effects of more than one mutation in the identified chromosomal region, possibly acting in a dose-dependent manner.

369T 4E-BP is Required for Translation of Bomanins in Early Infection with *E. faecalis*

Kate L Browning, Brian P Lazzaro Entomology, Cornell University

Response to infection requires rapid protein translation by the host. *Thor*, which encodes eIF4E-BP, has been shown to inhibit cap-dependent mRNA translation under oxidative stress, starvation, and bacterial infection in *Drosophila*. We find that *Thor* mutants are less resistant to systemic *E. faecalis* infection, with higher bacterial loads at 16 hours and increased mortality between 24–96 hours post-infection. Prior work suggested that Thor promotes antimicrobial peptide (AMP) translation in the larval gut. Therefore, we hypothesized that adult Thor may similarly promote AMP translation following systemic infection. Bomanin proteins are critical for surviving *E. faecalis* infection, and we found reduced translation of *Bomanin* mRNA transcripts in *Thor* mutant flies at 3 hours post-infection. However, AMP transcripts were equally bound by ribosomes in *Thor* mutant and wildtype genotypes at 6 hours post-infection, indicating that the translational impairment was transient. Our data also revealed constitutive upregulation of multiple inflammatory and oxidative stress-related genes in *Thor* mutants, indicating that absence of *Thor* disrupts organismal homeostasis even in the absence of infection. In prior work, we showed that flies carrying high bacterial loads are more likely to die from infection, whereas flies with low loads are more likely to survive. We therefore contrasted the transcriptional and translational profiles of wild type and *Thor* mutant flies with high versus low bacterial loads at 16 hours post-infection. We found that high-load flies show decreased translation of cytosolic ribosomal proteins relative to low-load flies, a difference that was modest in Canton-S but striking in *Thor* mutant flies. Altogether, these data support a model where combined homeostatic and infection stresses may compound to result in a more severe translational and metabolic disruption, including failure to effectively prioritize AMP mRNAs.

371T Genetic variation in larval sessile crystal cell number

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Three major types of hemocytes carry out cellular immunity functions in *Drosophila melanogaster*: plasmatocytes (macrophage functions), crystal cells (granulocyte functions) and lamellocytes (encapsulation of parasitoid wasp eggs). Crystal cells make up 5% of the hemocytes in larvae and perform many vital roles including oxygen transport, melanization and clotting. We carried out a Genome-Wide Association Study (GWAS) on sessile-crystal-cell-number from 78 isolines (a minimum of 10 wandering third instar larvae (w3L) per isoline) from the Drosophila Genetics Reference Panel (DGRP) in order to identify new genes involved in crystal cell development. Crystal cells were melanized during a 10 minute, 70° C heat treatment and then counted. We found 131 ($p<1 \times 10^{-4}$) polymorphisms associated with differences in sessile crystal cell number. These polymorphisms map to 67 genes, with several polymorphisms mapped to the same gene. Using the DRSC scRNA-seq Database, we found that 67% of these genes are expressed in hemocytes or lymph glands, the hematopoietic organ of the larvae. Additionally, 13 of the polymorphisms were also found in a GWAS of oxidative stress resistance. The putative target genes include domino (dom), which is known to be required for hemocyte generation. We knocked-down ten of the top hits using the ubiquitous driver da-Gal4 and UAS-RNAi. Knock-down of six of the eight genes reduced larval crystal cell number. Knock-down of dom and prickle (pk) caused lethality prior to w3L. The da-Gal4>UAS-pkRNAi larvae were delayed, small and thin and failed to pupariate. Interestingly, they developed melanotic tumors. Phalloidin staining showed that these tumors were made of lamellocytes, indicating that an immune response had been triggered. Pk knockdown with hemocyte specific drivers (lozenge, hemolectin and hemese) or a neuronal driver (ELAV) did not cause lethality or tumors. We are currently testing additional tissue-specific drivers to determine where pk is required to avoid triggering lamellocyte and tumor formation.

372F Fly viral atlas: Cellular tropism of *Drosophila* RNA viruses and their impact on the host

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Drosophila RNA viruses are diverse and often persist in wild and lab populations, yet their cell tropism is poorly understood. In the Fly Cell Atlas (represents comprehensive *Drosophila* single-cell transcriptome) data, we detected four natural RNA viruses (persistent infections): Nora virus, *Drosophila* A virus (DAV), *Drosophila* C virus (DCV), and Newfield virus. Nora and DAV were abundant and widespread across tissues and cell types, while DCV and Newfield virus RNA transcripts were only found in oenocyte and fat body. We found that *Drosophila* responses with canonical viral immunity related genes (e.g. *Vago*, *Vir-1*) against these persistent viral infections. Additionally, we observed that during persistent viral infections transposable element (TE) transcripts get upregulated in the somatic cells. Transposable elements (TEs) are traditionally associated with the germline, but recent studies and our data show they are also expressed in somatic cells as well. Using Fly Cell Atlas, we found that distinct somatic cell types express specific TE subtypes, indicating regulated and cell-type-specific TE activity often overlooked in transcriptomic studies. Here, we present Fly Viral Atlas, a single-cell atlas of RNA virus and transposable element (TE) expression in *Drosophila*, providing new insights into viral tropism and TE dynamics across tissues.

373F Tumor induced *Acetobacter* expansion leading to kidney stone and ascites development

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Ascites is a pathological condition where fluid accumulates in the patient's abdominal cavity. Although the occurrence of ascites is infrequent in cancer patients, it often poses complications like poor prognosis of the disease and significant hurdles in treatment. Our study focuses on investigating the mechanisms underlying the development of ascites in cancer patients, a condition that remains poorly understood. To investigate this, we utilized the *Drosophila* genetic toolkit to develop a tumor model that mimics the cancer-associated ascites. We generated a larval salivary gland transition zone (TZ) tumor model by overexpressing an activated form of the Notch intracellular domain (NICD). The endogenous JNK and JAK/STAT signaling within TZ creates a tumor-prone environment that, upon constitutive activation of NICD, drives neoplastic tumorigenesis. The resulting primary *TZ>NICD* tumors can be allografted into the adult fly abdomen, where they continue to proliferate and the hosts develop ascites. These tumors can be retransplanted and passaged into new hosts; thus, we refer to them as high-passage tumors. The *TZ>NICD* high-passage tumor-bearing flies with ascites exhibited alterations in their gut microbial homeostasis, characterized by the highest expansion of an *Acetobacter* species. Our previous studies have demonstrated that tumor hosts activate the NF-kB innate immune deficiency (IMD) pathway in response to gut bacterial translocation into the hemolymph. Given the role of the *Drosophila* renal Malpighian tubules (MTs) in maintaining fluid and ion balance, we investigated whether gut dysbiosis activates NF-kB/IMD specifically in the MTs, hence disrupting fluid homeostasis. *Diptericin-lacZ* reporter and quantitative RT-PCR confirmed consistent IMD upregulation in the MTs of tumor hosts. Remarkably, the removal of *Acetobacter* species alone was sufficient to suppress IMD activation in the hosts' MTs and prevent ascites formation. Previous studies have shown that IMD upregulation in the MTs fuels purine metabolism, thereby leading to uric acid (UA) accumulation in the MTs. Similarly, we found that *Acetobacter* removal not only reduced IMD upregulation but also alleviated UA buildup and kidney stone formation in the hosts. Moreover, bacterial mono-association studies have demonstrated that *Acetobacter* expansion directly triggers IMD activation and systemic UA accumulation in tumor hosts. MTs-specific knockdown of the IMD signaling receptor PGRP-LC, or the transcription factor Relish, significantly rescued abdominal fluid retention, further demonstrating the contribution of IMD-mediated kidney stone formation to ascites development in tumor hosts. Our findings have identified a tumor-microbiome-immunity axis that causes ascites in *Drosophila*, suggesting that microbial regulation may be a potential target for mitigating cancer-associated nephrotic disorders.

374F The *drop-dead* mutant fly as a model for gut–brain–immune interactions in neurodegeneration

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Neurodegenerative diseases affect millions of people worldwide, imposing high economic costs, straining healthcare systems, and causing profound emotional burdens for patients and caregivers. Emerging as a critical aspect of these diseases is the gut-brain axis: a hypothesis that suggests the brain and gut communicate bidirectionally to influence brain health. Here, we expand upon this hypothesis and investigate a gut–brain–immune connection using a *Drosophila melanogaster* neurodegenerative model based on a mutation in the *drop-dead* (*drd*) gene. *drd* mutant flies show rapid, severe neurodegeneration, significant disruption of gut structure and function, and an activated immune system, making them an ideal system to study the connections among all three arms of this axis. Previous work showed that *drd* flies have an increased humoral immune response in the form of antimicrobial peptide production in multiple tissues, including the gut and the brain, but it was unclear whether a cellular response occurred simultaneously. To this end, we tagged hemocytes, the fly's mobile, phagocytic defense cells, with a fluorescent marker and imaged control and *drd* adult brains. We found a profound increase in hemocyte recruitment to the surface of *drd* brains compared to wildtype, indicating that our neurodegenerative model mounts a cellular immune response in addition to a humoral one. Subsequently, we asked whether removal of the mobile immune response would rescue lifespan. We genetically ablated hemocytes in *drd* and control flies by inducing *reaper* expression with the hemocyte-specific driver *HmlD-GAL4*. Preliminary data shows similar lifespan in *drd* mutants without hemocytes compared to *drd* mutants with hemocytes. However, we have yet to investigate brain structure, brain function, and the impact of gut-specific immune pathway knockdowns on *drd* mutants without hemocytes. This work provides a foundation for linking the gut, immune system, and brain in neurodegenerative disease. A deeper understanding of this axis may yield new mechanistic insights and open avenues for developing treatments for these disorders. *Supported by Marquette University and a GAANN fellowship from the U.S. Department of Education.*

375F Comparison of axenic and conventional bacterial supplementation in w^{1118} flies

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There has been extensive research demonstrating how the microbiome affects motor function in *Drosophila melanogaster*. While bacterial mono-association of axenic flies is the gold standard, bacterial supplementation is more translatable to probiotic supplementation in humans. We undertook an experiment to compare the difference between bacterial mono-association and supplementation in w^{1118} flies and used a climbing assay to measure motor function. This assay utilizes a TriKinetics Multibeam Monitor to record the location of 16 individual flies every second over a 20-minute period. Using the standard embryo bleaching protocol, axenic (germ-free) w^{1118} embryos were made and then mono-associated with *Acetobacter pomorum*, two strains of *Acetobacter tropicalis*, *Lactobacillus plantarum* or *Lactobacillus brevis*. Another group of conventional microbiome flies were had the same bacteria supplemented on their non-sterile diet. A significant difference in total climbing events and total movements were recorded in the flies mono-associated with the *Acetobacter* genus compared to the axenic flies. Interestingly, mono-association with the two *Lactobacillus* species had no effect on the flies. This suggests that *Acetobacter* species specifically increased the flies' motivation and ability to climb. Interestingly, there was no effect on locomotion in flies that were supplemented with the respective bacteria. This study reveals that supplementation of bacteria in the diet does not show significant impact in climbing ability, even when bacterial mono-association of axenic flies does. Furthermore, *Acetobacter* species increased both movement and motivation to climb while the *Lactobacillus* species had no effects.

376F Host Genotype Impacts Metabolic Response to Chronic Bacterial Infection

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Certain bacteria naturally establish sublethal chronic infections in survivors of an initial acute phase of infection. Chronic infections exert a continuous metabolic burden on *Drosophila melanogaster* hosts, in part by necessitating a sustained immune response. Previous work has shown that *Drosophila* genotypes vary in their ability to withstand infection, but the role of genotype in determining the metabolic response to chronic infection has not been investigated. In this study we asked whether genotypes vary in metabolic phenotypes resulting from chronic infection and whether metabolic depletion scales with immune response across genotype. To investigate this, we used a panel of genetically diverse, wild-derived *Drosophila* lines and their outbred F1 progeny as hosts, and two bacterial species, *Providencia rettgeri* and *Serratia marcescens*, as chronically infecting pathogens. We measured infection survival and chronic bacterial loads to determine infection severity and found that while chronic bacterial loads were similar across genotypes for each pathogen, genotypes varied in their ability to survive acute infection. We asked whether mortality phenotypes might be associated with chronic metabolic outcomes, so we used starvation to compare metabolic stores in low- and high-mortality genotype pairs for each pathogen. We found that some genotypes starve faster when infected while others do not. We suspected that depletion of essential metabolites was responsible for decreased starvation resistance, so we measured triglyceride (TAG) and glycogen levels in genotype pairs. We found no glycogen depletion across genotypes, but that infection-induced TAG depletion corresponds with lower starvation resistance. We then asked whether metabolic depletion was associated with an increased immune response, so we compared antimicrobial peptide (AMP) expression in each genotype pair. We found that genotypes varied in both uninfected AMP expression levels and expression induction resulting from infection. However, there was no direct association between AMP expression and metabolic depletion, suggesting that genotypes differ in infection-induced mechanisms besides AMP production which also contribute to metabolic depletion. Overall, we report that genetic variation strongly influences chronic metabolic outcome, and that genotype-by-pathogen interactions result in unique host metabolic and immune responses to chronic infection.

377F In-host evolution of gut bacteria significantly reduces fly lifespan

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The effect of the gut microbiome on aging has been tested through aged microbiome transfers to young hosts, resulting in a reduction in lifespan. Aged microbiomes usually have an overgrowth of immunoinflammatory Proteobacteria, however, the mechanisms increasing the abundances of these bacteria are unclear. A hypothetical driver for these observed patterns are bacterial genetic mutations that appear during host-aging. To test this, we fed a defined community of commensal bacteria isolated from a wild caught fly to young axenic flies and aged them. The aged microbiomes from these flies were transferred to new young axenic flies and their lifespan measured. All species of the defined microbiome persisted throughout host-lifespan, and the resulting aged commensal microbiome conferred shorter lifespans to the naive flies, reducing median lifespan by almost 10 percent. Whole genome sequencing of bacterial species uncovered recurring genetic mutations in a gene encoding a heptosyltransferase enzyme in *Acetobacter cerevisiae*, a Proteobacterium. This mutation impacted bacterial surface proteins, making the bacterial cell morphology more filamentous and allowing overproliferation in the aged microbiome. Additional longevity experiments with heptosyltransferase knock-out mutants validated that the mutation was a causative factor in reduced host longevity and recapitulated the overgrowth observed in the aged microbiome. Revertant mutants with normal cell morphology resulted in an elongation of fly lifespan, increasing median lifespan by almost 30 percent, equivalent to germ-free flies. The results of this study highlight the impact of microbiome surface protein mutations on overall host health and the role of in-host mutations of bacteria in driving aging.

378F Genetic and evolutionary basis of Drosophila resistance to parasitoid wasp infection

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Parasitoid wasps are a common natural enemy of many insect species, including *Drosophila melanogaster*. We have performed a long-term experimental evolution study where we allowed replicated populations of *Drosophila* to evolve for ~132 generations in the presence to two different strains of the parasitoid wasp *Leptopilina boulardi*, one of its most prevalent parasites. The selected populations evolved greatly increased resistance through a more efficient melanotic encapsulation response. While populations that adapted to a virulent and highly immune-suppressive parasite strain became resistant to a less virulent parasite, the reverse was not the case. To understand the immunological basis of resistance, we used a fluorescent reporter assay to measure activation of the JAK-STAT pathway. In the control populations the virulent parasite suppressed JAK-STAT activation, but this response was restored when the populations adapted to this parasite. Therefore, escaping immune suppression may be critical to the evolution of resistance. However, these populations spontaneously melanized their own tissues, which is characteristic of an over-active JAK-STAT response, suggesting autoimmunity may be the price paid for high levels of resistance. These findings provide insight into the genetic and physiological factors that underlies the evolution of specific immunity in insects.

379F Microscopic Mercenaries: How Teratocytes manipulate Host Immune Defenses

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Little is known about the mechanistic basis of how long-lived metazoan parasites (i.e. worm-like parasites such as schistosomes, blood flukes) manipulate host immunity in order to thrive within their hostile host environment, largely due to poor laboratory models for these infections. We leveraged the genetic tools in *Drosophila melanogaster* to understand how worm-like larval stages of parasitoid wasps affect host immunity. *Leptopilina heterotoma* is an obligate parasitoid wasp that develops within *D. melanogaster* larvae. We rediscovered an uncharacterized cell type called “trophic cells” produced by *L. heterotoma* in infected *D. melanogaster* hosts, which were initially discovered in 1969 (Nappi & Streams). These cells are hypothesized to be similar to teratocyte cells in *Aphidius ervi*, a parasitoid wasp that targets pea aphids. Teratocytes are malignant cells that are released by the parasitoid embryonic serosa (an epithelial layer of cells surrounding the embryo) into the host hemocoel, where they manipulate host development and immunity to facilitate their early development. *A. ervi* teratocytes secrete factors such as fatty acid binding proteins (FABP) and enolases into the hemolymph that are critical to the parasitization of their hosts. FABPs are commonly involved in lipid metabolism, and enolases activate plasminogen into plasmin to assist in the tissue invasion process by interfering with clotting in worm-like parasitoids of humans, suggesting that teratocytes serve both as virulence factors and provide nutritional benefit to the developing parasite.

We functionally characterized these ‘teratocyte-like’ cells from *L. heterotoma* throughout parasitoid development. Across a time course from 48, 72, and 96 hours post-infection, we assessed changes in cellular morphology and abundance throughout development of the parasitoid. Using flow cytometry to isolate these cells for RNAseq, we determined genes expressed by these cells that could potentially contribute to successful parasitism of *L. heterotoma*. Our findings elucidate the function of these ‘teratocyte-like’ cells in the parasitization process and how they contribute to successful parasitism and host-immune suppression.

380F Conserved Functions of JNK Signaling in blood cell Differentiation and Inflammatory Responses

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The hematopoietic organ of the Drosophila larva, the lymph gland, serves as a simplified yet powerful model for studying mammalian hematopoietic niches. It comprises the three fundamental components characteristic of hematopoietic niches across species: blood cell progenitors, differentiated blood cells, and a specialized niche that regulates progenitor maintenance and differentiation through signaling interactions. Two types of mature blood cells are found in the Drosophila larva: macrophage-like plasmatocytes and platelet-like crystal cells. Upon immune challenge, a third type of blood cell, the lamellocyte, differentiates to encapsulate foreign bodies too large to be phagocytosed by plasmatocytes. This response is reminiscent of the formation of multinucleated giant cells (MNGCs) in mammals, which similarly form to surround large foreign bodies or arise during granulomatous diseases such as tuberculosis. Recent studies have identified a population of intermediate progenitors residing between blood cell progenitors and differentiated blood cells in the lymph gland. This population of intermediate progenitors has the potential to give rise to all three mature blood cell types, yet the mechanisms controlling their differentiation remain poorly understood. Single-cell transcriptomic profiling revealed enrichment of c-Jun N-terminal kinase (JNK) pathway targets in this population, but the specific role of JNK signaling has not been characterized. Here, we uncover a previously unrecognized role for JNK signaling in regulating intermediate progenitor fate determination. We demonstrate that proper blood cell maturation requires finely tuned levels of JNK activity within these cells. Insufficient JNK signaling traps progenitors in the intermediate state, preventing their differentiation into plasmatocytes and crystal cells, while excessive activation disrupts normal development and biases cells toward lamellocyte formation. Furthermore, we show that JNK-driven lamellocyte production occurs through both cell-autonomous and non-cell-autonomous mechanisms, with Mmp1 identified as a key downstream effector required for lamellocyte release from the lymph gland into circulation. Since the JNK pathway in mammals has been implicated in macrophage activation and MNGC formation, and its overactivation has been associated with chronic inflammatory and granulomatous diseases, this conservation underscores the translational relevance of our findings. These results pave the way for future studies aimed at identifying JNK modulators that could help alleviate or treat inflammatory conditions such as tuberculosis.

381S Characterization of the Lipid Composition of the Lamellocyte Membrane

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Lamellocytes are a specialized Drosophila immune cell type that encapsulate and melanize invaders too large to be engulfed, such as parasitoid wasp eggs. These cells are induced following immune challenge and, consistent with their encapsulation role, are enormous, up to 80 µm in diameter, yet extremely thin. To better understand their unusual morphology and plasma membrane organization, we are investigating lamellocyte membrane composition using both mass spectrometry and Raman spectroscopy. Raman imaging provides powerful spatial resolution for visualizing lipid distributions at subcellular scales, but its application to native cell membranes has been limited by high background signals from cytoplasmic components. Lamellocytes, with their flat morphology and minimal cytoplasm, offer a unique opportunity to overcome this limitation and directly probe membrane lipid composition in intact cells. We will present findings from the combined use of lipidomics and Drosophila genetics to reveal the membrane architecture of these unusually tractable immune cells.

382S *Interactions Between Host Genetics, Diet, and Pathogen Infection in Drosophila*

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Insect immune responses to bacterial pathogens vary widely among host genotypes, but the mechanisms by which nutritional conditions modulate these genetic differences in disease susceptibility are not well characterized. *Chromobacterium subtsugae* is a Gram-negative bacterium that produces insecticidal metabolites along with the pigment violacein. It is also used commercially as a bioinsecticide against agricultural pests, though the mechanism by which it kills is not known. To investigate possible host factors that contribute to *C. subtsugae* susceptibility, we investigated survival and pathogen load across 40 *Drosophila* lineages that are part of the Core 40 set of the *Drosophila* Genetic Reference Panel (DGRP) following oral infection. We compared responses to infection between *Drosophila* on the Broderick standard diet versus *Drosophila* on a Yeast Peptone Dextrose (YPD) diet across a 10-day period. We observed moderate mortality of *Drosophila* on the Broderick standard diet across most lineages (10-18 deaths per 20 flies). However, YPD diet dramatically accelerated mortality in specific lineages, including lines that exhibited near-complete mortality (≥20/20 flies) by 72-96 hours post-infection (hpi). We also identified lineages showed minimal susceptibility enhancement. To distinguish pathogen-mediated from diet-induced mortality, we conducted three independent replicates comparing *C. subtsugae* or mock infection susceptibility across fast-dying (Dredd, DGRP_358, DGRP_707, DGRP_399, DGRP_732) and resistant (DGRP_705, DGRP_379, DGRP_208 lineages. Our results suggest that the accelerated mortality was likely diet-induced rather than pathogen-dependent. Interestingly, *C. substugae* infection alters fly climbing ability. Given that *C. substugae* produces dopamine precursors, we will also discuss our exploration of the neuronal impacts of a *C. substugae* infection and changes to *Drosophila* behavior. Altogether, this novel pathogen model provides a useful tool to study how host-microbe interactions can lead to the loss of host homeostasis.

383S Roles of CaM-dependent kinase kinase 2 in enteroendocrine cells in the axis of the gut-immune response

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Calcium/calmodulin-dependent protein kinase kinase 2 (CaMKK2) is a multifunctional serine/threonine kinase that integrates calcium signaling with metabolic and transcriptional regulation. mTOR signaling alters CaMKK2 alternative splicing shifting the ratio between the Longform (LF) and shortform (SF) isoforms which share catalytic domains but differ in their C-terminal regions. Isoform-specific differences in subcellular localization, protein–protein interactions and downstream signaling have been identified in vitro, but their physiological relevance remains unclear. Utilizing the model organism *Drosophila melanogaster*, we employed the GAL4/UAS system to express the human CaMKK2 LF and SF isoforms in a tissue-specific manner across a panel of GAL4 driver lines. Expression directed in enteroendocrine cells (EECs) induced melanotic nodule formation indicative of systemic immune activation. SF expression yielded a higher frequency of nodules than LF, whereas expression in other tissues did not elicit immune phenotypes. To assess upstream signaling regulation, we activated protein kinase A (PKA) using forskolin and observed LF-driven nodules were markedly reduced, whereas SF-driven nodules persisted. These results demonstrate CaMKK2-driven immune activation is both isoform and tissue specific. Beyond immunity, Longform expressions reduced larval weight compared to Shortform, indicating a distinct growth phenotype. We therefore hypothesize that CaMKK2 isoforms differentially regulate peptide-mediated signaling from EECs, driving distinct systemic, immune and physiological responses. To test this, we employed two systems originally developed to study peptidergic transmission in mice, a pH sensitive reporter for large dense-core vesicle release and LDCV-localized peptidase targeting peptide hormones. Together, this study established a novel *Drosophila* model for investigating isoform-specific functions of human CaMKK2 and reveals their roles in peptide mediated immune signaling from the gut to immune cells.

384S The Role of Prophenoloxidase Pathway in Drosophila Defense Against a DNA Virus

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Hosts from diverse species combat viral infections using different immune mechanisms, and *Drosophila* serves as an important model for studying antiviral immune responses. *Drosophila innubila* Nudivirus (DiNV) is a double-stranded DNA virus that primarily infects its native host, *Drosophila innubila*, yet it remains unclear how the host utilizes its immune system to fight viral infection. In this study, we explored the role of the Prophenoloxidase (PPO) pathway in defense against DiNV in *D. innubila*. Initial RNA sequencing results reveal that PPO1 and PPO2 are down regulated in the presence of DiNV infection across three earlier timepoints. To further test its role in later timepoints, we injected *D. innubila* flies with either mock, uninfected, or infected treatments across six timepoints and performed qPCR to quantify viral genome increase and prophenoloxidase gene expression over time. Overall, our results highlight the role of melanization in insect host defense against DNA viruses, with PPO1 and PPO2 being major contributors to this response. These findings provide new insight into antiviral host defense mechanisms against DNA viruses in *Drosophila.*

385S Single-cell transcriptomics reveals early *Wolbachia* infection dynamics in *Drosophila melanogaster*

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Intracellular bacterial symbionts must navigate complex host cellular environments to establish persistent infections while avoiding immune clearance. The alphaproteobacterial endosymbiont *Wolbachia pipientis*, prevalent in arthropods and nematodes, manipulates host biology through diverse mechanisms including reproductive manipulation, developmental reinforcement, and viral blocking. The *w*Mel strain, natively associated with *Drosophila melanogaster*, has proven particularly effective for biocontrol applications, blocking viral transmission and suppressing reproduction when transinfected into mosquito populations. Despite these successful applications, the molecular mechanisms underlying initial infection establishment remain poorly understood. *Wolbachia* strains demonstrate host cell-type preferences and induce tissue-specific phenotypes, yet bulk RNA-sequencing studies have yielded inconsistent signals of differential expression, likely due to averaging across cells with heterogeneous infection titers, states, and developmental stages. Understanding early somatic infection at single-cell resolution is therefore critical both for elucidating fundamental infection biology and for assessing biosafety risks, as artificially transinfected insects released into ecosystems could enable *Wolbachia* to establish novel infections in non-target species. Here, we employed single-cell RNA sequencing to investigate how *w*Mel colonization influences the host transcriptome during early infection in *D. melanogaster* JW18 neuroblast-derived somatic cell lines. Using both 10X Genomics Chromium and the lower-cost PIPseq platforms, we demonstrate that nascent *w*Mel infections induce distinct transcriptional changes that create novel cellular states diverging from uninfected controls. Through unsupervised clustering, pseudotime analysis, and marker gene identification, we reveal how *Wolbachia* reprograms host somatic cells to evade immune responses, establish infection, and facilitate nutrient acquisition. These findings uncover the hidden role *Wolbachia* plays in eukaryotic cellular biology and provide mechanistic insights from the native host system that will enable future efforts to develop advanced control measures leveraging cell-type specific phenotypes of *Wolbachia* strains.

386S Dietary phytochemicals modulate the physiology and host-microbiome interaction in *Drosophila*

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Diet is a key modulator of gut microbiota, thereby influencing host physiology. We examined host–microbiome modulation in phytochemical-enriched diets, including capsaicin, curcumin, resveratrol, and thymoquinone. We integrated gut RNA-seq transcriptomic analysis with microbiome and host phenotypic data in *Drosophila melanogaster*. Thymoquinone increased body weight and triglyceride levels in females, while also promoting *Enterococcus* enrichment. Resveratrol elevated protein content in males, whereas curcumin reduced glucose levels. In addition, curcumin and capsaicin disrupted circadian behavior, while resveratrol promoted more consolidated sleep. However, only capsaicin and resveratrol modulated *Acetobacter*, *Pseudomonas*, and *Lactiplantibacillus* in a sex-dependent manner. Integrative analysis revealed that, although *Levilactobacillus* exhibited low relative abundance, it contributes to the modulation of host gene expression under enriched diets. Notably, *DCTN5-p25* and *mRpL28* showed significant correlations with *Lactiplantibacillus* abundance, particularly under capsaicin-supplemented diets and in females fed thymoquinone-supplemented diets, respectively. Overall, our findings highlight that host–microbiome–diet interactions in *Drosophila* are both sex-specific and compound-dependent.

387S The Role of the Toll Inflammatory Signaling Pathway on Crystal Cell Differentiation in Drosophila

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Hematopoiesis (blood cell development) is a critical process that generates immune cells important for defense against pathogens. The Toll and Imd signaling pathways play important roles in the inflammatory response to Gram-positive and Gram-negative bacteria, respectively. In *Drosophila* these pathways regulate the secretion of antimicrobial peptides that protect against infections, and they also have developmental roles. However, their impact on hematopoiesis has not been fully explored. This project›s purpose is to understand the impact of these pathways on crystal cell differentiation. We performed heat-induced melanization assays to visualize crystal cells (CCs) in third instar *Drosophila* larvae, comparing wild-type larvae to larvae expressing RNAi to knock down genes in the Toll pathway. We imaged melanized CCs using microscopy and quantified the number of CCs found in sessile pockets along the body wall using ImageJ. We also used CC-specific fluorescent markers to count CCs in live larvae. We dissected out the lymph glands and imaged them using confocal microscopy to quantify the ratio of CCs in this organ. A knockdown of the Toll pathway component *cactus* in CCs, showed a statistically significant decrease of CCs in the circulating hemocytes and sessile pool hemocytes suggesting that the Toll pathway may be sufficient for CC development. Knockdown of *MyD88* with the same driver showed a significant decrease in CCs in sessile pockets via live imaging and melanization, leading us to question why inhibition of the pathway shows a similar phenotype to an activation of the pathway. I will conduct further RNAi knockdowns of other components of the Toll Pathway and image larvae in different time points to understand when in larval development the phenotype develops. Understanding hematopoietic signals can provide insights into blood cell diseases caused by defects in differentiation.

388S The immune signaling pathways Toll and Imd play a role in Drosophila hematopoiesis

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The Toll and Imd pathways regulate immune responses and development in Drosophila melanogaster. This study investigates how these pathways influence crystal cell (CC) differentiation, a key cell type involved in wound healing, hypoxic response, and innate immunity. We compared wild-type larvae with RNAi knockdowns targeting tube, Myd88, cactus, Dif (Toll pathway), and Relish (Imd pathway). Crystal cell differentiation was assessed by quantifying crystal cell melanization in third-instar larvae. Knockdown of these genes significantly reduced CC differentiation, indicating that both pathways are required for crystal cell formation. RNAi efficiency was validated by qPCR. To determine cell autonomy, we performed live imaging using the crystal cell driver lozenge (lz) and found that cactus and Relish knockdowns exhibited cell-autonomous effects. Consistent with these findings, bleeding assays revealed decreased circulating crystal cells in cactus knockdowns. Together, these results suggest that the Toll and Imd pathways are required for crystal cell differentiation in a cell-autonomous manner. Understanding how these immune pathways influence hematopoiesis in Drosophila may provide insight into conserved mechanisms of blood cell differentiation in humans.

389S Linking Host Control of the Microbiota and Host Adaptive Evolution in Experimentally Evolved Populations of *Drosophila melanogaster*

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Experimental evolution has revealed that host life-history traits can evolve rapidly and predictably under laboratory selection, yet the role of the microbiota in this adaptive process has historically been ignored. In this project, we combine microbiota data from long-standing experimentally evolved *Drosophila melanogaster* populations with longitudinal data from populations undergoing active selection for accelerated development to ask how host adaptive evolution and host control of the microbiota are linked. Across several different selection regimes, we demonstrate that long-standing selection on populations of *Drosophila melanogaster* is associated with genotype-specific shifts in microbiota composition, including differences in the presence and relative abundance of *Wolbachia* and acetic acid bacteria. These findings suggest that host adaptive evolution occurs alongside and potentially through changes in host genetic control over the microbiota. Building on this analysis further, we also track developmental rate and microbiota composition across generations during ongoing selection for faster development in order to determine whether changes to the life history or the host's ability to manipulate its microbiota evolve first. By combining microbiota data from long-standing selection lines with longitudinal data collected during ongoing selection, this work provides a look into how the microbiota responds to and participates in host adaptive evolution.

390T Building Bridges in Genomics Education: Regional Nodes as Catalysts for Distributed Faculty Collaboration

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The Genomics Education Partnership (GEP; thegep.org) is a nationwide Community of Practice with 260 faculty from 209 institutions which aims to bring authentic research experiences in genomics to undergraduate students. The GEP provides students with experiential learning and research opportunities in genomics and bioinformatics via Course-based Undergraduate Research Experiences (CUREs). Since 2019, more than 14,000 students have participated in courses that utilized GEP curriculum and in research projects that focused on the structural annotations of genes in specific pathways or genomic regions. GEP institutions include Community Colleges, Primarily Undergraduate Institutions, Minority-Serving Institutions, and Research Universities. In 2023–2024, ~46% of the GEP students were Pell Grant eligible, and ~26% were first-generation college students.

In 2019, the GEP implemented a distributed leadership model which included the organization of its members into 13 geographically-based groups called Regional Nodes. The goals of the Regional Nodes include developing local communities to expand our membership, lowering barriers to participation, and providing opportunities for undergraduates to participate in regional scientific communities. The number of faculty (ranges from 7 to 36) and geographical area within Nodes were highly variable. The Regional Node Director and Co-Director positions were created within the GEP leadership structure to facilitate event organization and gather data to determine if Node activities were meeting our stated grant goals. Node leaders coordinated wide-ranging types of events including in-person or online workshops focused on professional development and curriculum implementation, scientific talks, new member recruitment and training, journal clubs, and opportunities for students to present posters or talks. We are currently assessing the success of our Regional Node initiative based on survey data from faculty and students. In particular, we are interested in the factors motivating engagement in Node events, the barriers to participation, and the perceived benefits of attendance at events. We will report on how each of these factors contribute to the success of the Regional Nodes and the GEP's long term goals.

The GEP is supported by NIH IPERT-R25GM160660 to LKR and has started a 501(c)(3) nonprofit wing. Data presented here was generated in part with support from NSF IUSE-1915544 and NIH IPERT-R25GM130517 to LKR.

391T Building scientific identity through a CURE modeling diabetes and nutrition in an introductory biology course

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Course-based undergraduate research experiences (CUREs) offer scalable opportunities for students to practice science authentically. At Minority Serving Institutions like Northeastern, intentionally creating access and belonging is essential for supporting STEM students. In our required introductory majors course, *Essential Skills for Biologists*, we partnered with the eCLOSE Institute to implement a CURE focused on diabetes and nutrition, which are topics that connect directly to many students' lived experiences and community health disparities.

Students began by examining patterns of diabetes incidence through social, economic, and structural lenses, grounding their work in culturally relevant contexts. They then designed and carried out experiments using two *Drosophila* diabetes models to test how specific nutrients influence fly development. The course emphasized iteration, teamwork, and reflection as students refined techniques, analyzed data, and interpreted their findings within mechanisms of diabetes. Importantly, students contributed their original data to a national research network, reinforcing the collaborative and cumulative nature of science.

Assessment focused on gains in research skills, scientific identity, and sense of belonging. At the end of the semester, students reported high confidence in data collection and quantitative analysis, as well as a stronger sense of themselves as scientists. These outcomes highlight how CUREs that integrate culturally relevant themes and intentional scaffolding can support diverse students in building both the skills and the identities essential for success in biology.

392F From genotype to phenotype: generating novel mutations in *doublesex* using CRISPR/Cas9 in an undergraduate laboratory course

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I will present on a multi-week lab project I have developed for an intermediate-level laboratory course in which undergraduate students use CRISPR/Cas9 to generate novel mutations in the *doublesex* (*dsx*) gene in *D. melanogaster* and study their impact on phenotype and predicted protein sequence. The lab uses existing transgenic lines that are available from stock centers to induce the expression in the germline of Cas9 and two guide RNAs (gRNAs) that target the coding region of *dsx*. Students collect virgin females from a UAS-*dsx* gRNA line and cross them to a line with UAS-Cas9 and nanos-GAL4 transgenes. They then collect male progeny that have inherited all three transgenes and thus have CRISPR/Cas9 activated in their germlines. The resulting double-stranded breaks in dsx can be repaired either through homology directed repair or by the error-prone non-homologous ending joining, which can result in indels. Students cross these males with the potential to pass down frameshift mutations in *dsx* to females with a deficiency that deletes *dsx* and neighboring genes. Loss of *dsx* expression in resulting progeny results in a robust, intersex phenotype that students can readily learn to recognize; they image the intersex mutants and then use PCR and sequencing to determine the new *dsx* sequence and predict the impact on the resulting protein sequence. The basic outline of the project is straightforward and very feasible for an intermediate-level laboratory course like genetics or developmental biology. This project reinforces many core concepts that students are learning in class but also provides an opportunity for impactful conversations on topics including the evolution of sex and sex determination pathways, disorders of sexual development in humans, and the use of CRISPR-gene editing in research and therapeutics. There are also opportunities for add-on activities including designing PCR primers and discussing primary literature articles on *dsx*, depending on the goals of the instructor. I have used this lab in conjunction with a comparative genomics project through the Genomics Education Project (GEP) in which students annotate genes in the sex development pathway across the Drosophila genus.

393F Expanding undergraduate research experiences through the Fly-CURE: A collaborative Drosophila genetics network

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The Fly-CURE is a course-based undergraduate research experience (CURE) centered on the genetic mapping of cell growth mutants identified in an EMS screen in Drosophila melanogaster. There are currently over 30 faculty implementing the Fly-CURE in genetics classrooms across a variety of institution types (community colleges, MSIs, public and private universities). To date, over 3,000 undergraduate students have participated in the project, resulting in 17 peer-reviewed publications, with over 800 student co-authors. Student participants in the Fly-CURE report gains in their sense of science identity, self-efficacy in research methods, and intent to pursue future research experience, regardless of their institution type, demographics, or identity. The Fly-CURE curriculum is in the process of expanding via an NSF-funded Research Coordinated Network (RCN) to develop courses in bioinformatics, behavioral genetics, molecular biology, and developmental biology. Each RCN module is designed such that it can be implemented as a stand-alone CURE, mixed and matched to create unique interdisciplinary CUREs, or scaffolded with the genetics Fly-CURE. We are currently recruiting faculty to participate in our RCN, specifically, the molecular biology CURE. Faculty participants will be provided stipends for curriculum training and implementation, a social network of faculty, a community of like-minded scientists, continued scholarship opportunities, and support for tenure and promotion. Supported by NSF Awards DUE #2021146 and RCN-UBE #2316218.

394F Fly-CURE Behavioral Genetics: Challenges and Successes in Implementation Across Institutions

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Fly-CURE is a multi-institutional consortium in which faculty implement a genetics laboratory Course-based Undergraduate Research Experience in their own classrooms. Students perform real-world, inquiry-based research in which they work together to map an unknown mutation that results in a defect in the development of the *Drosophila* eye. We have recently established a Fly-CURE Research Coordinated Network to develop new CUREs for Bioinformatics, Molecular Biology, Developmental Biology, and Behavioral Genetics courses. As the eye is composed of ~7200 neurons, mutations that affect eye development may also result in neuronal dysfunction that may lead to behavioral changes. Therefore, in the Fly-CURE Behavioral Genetics expansion, students use a variety of assays to characterize different behavioral phenotypes, including locomotor behaviors (walking, soot, climbing, and flight), sensory behaviors (larval food preference, adult odor choice, and soot), and response to stress (ethanol sensitivity, traumatic brain injury, cold/heat shock).

In 2024/2025 Fly-CURE Behavioral Genetics was piloted across two institutions (Oregon State University (OSU) and nearby Lewis & Clark College) and across three instructors (2 at OSU and 1 Lewis & Clark) teaching upper division biology courses. After this successful implementation, we recruited our first cohort of nation-wide Behavioral Genetics participants who participated in a virtual 2-day workshop during the summer and implemented in Fall/Winter of 2025. We held weekly check-ins to troubleshoot issues that arose, to revise protocols and develop new course materials, and to discuss future implementation strategies. Here, we discuss the challenges and successes we faced in expanding from nearby to cross country institutions. We also compare the students' results from different institutions and from sections within the same institution and discuss what factors may lead to differences in results. We also share future strategies for addressing these differences in order to have robust, replicable protocols that can be easily and widely implemented across institutions.

395T Muscle Hnf4 rewires methionine transsulfuration to fuel intestinal tumor growth

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Tumors induce systemic metabolic rewiring by modulating transcription factor (TF) activities in distal tissues to support their growth. While transcriptomic data are commonly used to correlate TF expression with metabolic enzyme expression, such correlations do not directly indicate TF or metabolic activities. To address this gap, we developed an integrative workflow that combines Network Component Analysis (NCA), high-throughput metabolomics, and ^{13}C-isotope tracing to identify TFs whose inferred activities and associated metabolic pathway activities are altered under pathological conditions.

Applying this approach to a *Drosophila* gut-tumor model driven by *yorkie* activation (Yki flies), we identified Hepatocyte nuclear factor 4 (*Hnf4*) as one of the TFs with the highest inferred activity among 149 candidates in thoracic tissues, specifically at the mid-stage of tumor progression. Consistent with this, Hnf4 nuclear localization was increased in Yki fly thoraces, indicating elevated activity. Hnf4 overexpression in wild-type muscle recapitulated muscle degeneration induced by Yki tumor at middle stage and triggered systemic metabolic remodeling, decreasing tricarboxylic acid (TCA) intermediates and amino acid pools by 41–66%, while increasing metabolites associated with methionine metabolism, such as S-adenosyl-L-methionine (SAM) and cystathionine, by 157% and 405%, respectively. These metabolites and associated genes were likewise elevated in Yki flies.

Mechanistically, Hnf4 overexpression in thoracic muscle induced expression of *Glycine N-methyltransferase* (*Gnmt*), a key regulator of SAM and cysteine homeostasis, and its expression was similarly upregulated in Yki thoracic muscle. Using $^{13}C_5$-methionine tracing, we confirmed increased methionine transsulfuration pathway activities in both Yki and Hnf4-overexpressing flies' thoraces. Notably, thoracic knockdown of *Hnf4* in Yki flies reduced *Gnmt* expression, suppressed tumor growth, and extended lifespan, whereas knockdown of putative cysteine transporters in the tumor also suppressed tumor growth and extended lifespan.

Together, these results establish muscle Hnf4 as a metabolic regulator that promotes tumor growth through the *Hnf4–Gnmt* axis and transsulfuration-mediated cysteine production. Our findings highlight the utility of our integrative approach to uncover transcriptional drivers of systemic metabolic remodeling in cancer.

396T A drug repurposing screen in *Drosophila* finds antibiotics and antihypertensive drugs as potential therapeutics for the rare disease WARS2 deficiency

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Mitochondrial aminoacyl-tRNA synthetase proteins (mt-aaRSs) are critical for charging tRNAs in the mitochondria. Deficiencies in mt-aaRSs result in rare diseases called mtARS disorders. These disproportionately affect high-energy tissues like the brain and muscle, and mtARS disorder patients often have seizure or movement disorders. mtARS disorders have few treatment options and unclear causative mechanisms. Due to low patient population, it is challenging to conduct clinical trials for new treatments, yet the need for therapeutics is high. The tryptophanyl tRNA synthetase, WARS2, is essential in *Drosophila* and humans - resulting in WARS2 deficiency. To model this disorder in *Drosophila*, we knocked down *WARS2* in the eye - resulting in a small, developmentally impaired eye. To find potential therapeutics using this model, we performed a drug repurposing screen of 1,520 drugs that are 98% FDA or EMA-approved. We identified 72 drugs (Z score ≥ 1.5, ~5% hit rate) that partially rescued our model. Our top hit categories included antibiotics, antihypertensives, and COX inhibitors (e.g. NSAIDs). In particular, antibiotics are implicated in harming mitochondria, and we hypothesize that certain antibiotics might help turnover mitochondria under stress from *WARS2* knockdown. Further testing revealed that our top antibiotic drug and top antihypertensive drug worked at multiple doses, suggesting a robust improvement of our model. In sum, we find new data connecting these pathways to mitochondrial health, and these drugs represent potential new therapies for WARS2 deficiency.

397T GABAergic neurons are a key cell type in a *Drosophila melanogaster* model of PARK14/*PLA2G6*-Associated Neurodegeneration

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Loss of function mutations in the human gene *PLA2G6* give rise to a spectrum of inherited neurodegenerative diseases collectively called *PLA2G6*-Associated Neurodegeneration (PLAN), which includes PARK14 autosomal recessive early onset parkinsonism. Using RNAi to knock down the *Drosophila PLA2G6* homolog *iPLA2-VIA*, we asked whether subsets of neurons, identified by their neurotransmitter usage, were more susceptible than others to loss of this gene. To model movement disorders resulting from PLAN, we used negative geotaxis climbing assays. The studies were conducted by undergraduate students in an upper-level Genetics course lab. Our results showed that loss of *iPLA2-VIA* in all neurons, or in GABAergic neurons alone, strongly perturbed locomotor ability in aged flies. Knockdown of *iPLA2-VIA* in both dopaminergic and serotonergic neurons affected locomotor ability weakly, while knockdown in other neuronal subsets did not disrupt locomotion. These data suggest that GABAergic neurons are a major site of iPLA2-VIA activity. We currently are testing whether the same knockdowns reduce lifespan, as another read-out of neurodegeneration. Furthermore, we found that reintroducing the wild-type *iPLA2-VIA* PB allele into GABAergic neurons alone rescued both climbing performance and lifespan of a knockout mutant, confirming these cells as a primary locus of activity. Climbing performance was improved only slightly upon reintroduction of wild-type *iPLA2-VIA* into the dopaminergic neurons. Thus, although much research on this gene has focused on the dopaminergic neurons, whose degeneration leads to clinical parkinsonism, our results highlight the importance of GABAergic neurons to PLAN, consistent with clinical findings in human PLAN patients. Furthermore, although inherited parkinsonian disorders like PARK14 have been suggested to share an underlying etiology with the much more common sporadic Parkinson's disease (PD), our data, considered along with human pathology studies indicating that most GABAergic neurons are spared in sporadic PD, may indicate that sporadic PD and PARK14 have distinct etiologies despite overlapping clinical presentations.

398T Monitoring age-dependent locomotor decline of *PARK14/iPLA2-VIA* mutant *Drosophila melanogaster* using a Mathematica-based video-tracking protocol

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Parkinson's disease (PD) affects over 11 million people worldwide. Most cases are sporadic, arising from a combination of poorly understood stochastic, environmental, and genetic factors. Nevertheless, several monogenic parkinsonian disorders have been documented, some of which are highly similar to sporadic PD. For these inherited parkinsonian disorders, over 20 causative "PARK" genes have been identified, and variants of these genes have been proposed to contribute to sporadic PD as well. Our lab and others have shown that loss of function mutations in the *Drosophila melanogaster* ortholog of human *PARK14* (aka *PLA2G6*), *iPLA2-VIA*, lead to age-dependent locomotor decline and attenuated lifespan. Recent work from our lab also has shown that depletion of this gene in dopaminergic, serotonergic, or GABAergic neurons leads to locomotor decline, as assessed by negative geotaxis climbing assays. To better characterize the movement defects that result from loss of *iPLA2-VIA* globally and in discrete subsets of neurons, we developed a video-tracking protocol. After transfer and acclimation to a circular arena, flies were individually video-recorded during 3 minutes of free movement. We wrote Mathematica code to extract the location coordinates of each fly throughout the length of the video, and we used the coordinates to calculate various metrics including speed, velocity, acceleration, path length, curvature, rest time, location bunching, and turning angles. Our preliminary data analysis indicates that *iPLA2-VIA* mutants lose agility and ability in numerous locomotor parameters between the ages of 15 and 25 days post-eclosion.

399T Use of large-scale *Drosophila* genetic reagents to facilitate human disease research

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The widespread adoption of whole-genome and whole-exome sequencing has revolutionized human genetic research, making the identification of genetic variation among individuals easier, faster, and more cost-effective than ever before. However, translating these discoveries into an understanding of human health remains challenging due to limited knowledge of the molecular functions of many genes and the difficulty of predicting how observed variants affect gene function. *Drosophila melanogaster* offers a powerful solution to this gap, thanks to its extensive genetic toolkit that enables functional annotation of genes and assessment of variant effects.

A key strength of this approach lies in the synergy between thousands of alleles for Drosophila genes, such as MiMIC and CRIMIC T2AGAL4/KozakGAL4 alleles generated by the Gene Disruption Project, and thousands of UAS-human cDNAs produced by the Drosophila Human cDNA Project. Together, these resources allow researchers to determine fly gene function, assess evolutionary conservation, and test the functional impact of human disease-associated variants. To date, we have generated approximately 3,800 GAL4 alleles and 5,500 UAS-human cDNA transgenic stocks. Using these collections, available through Bloomington and Kyoto Stock Centers, we can currently test the functionality of 2,055 human genes in flies.

In collaboration with clinicians, we have screened numerous rare disease-associated variants, contributing to the discovery of over 60 novel human disease genes. In many cases, combining expression pattern analysis with phenotypic data has revealed previously unknown gene functions in specific cell populations (e.g., *Tau*, *Gba1a*, *IntS11*, *Cdkl*). Furthermore, by uncovering biological processes underlying rare diseases, we have identified drugs in flies that show efficacy in patient-derived cells (e.g., *Acox1*, *iPLA2-VIA*, *pits*). Our studies have also uncovered pathways implicated in common genetic disorders such as Parkinson's disease and Alzheimer's disease.

We are expanding our collections and developing strategies to enhance their versatility, including promoter and tag exchange for alternative binary systems (e.g., LexA, QUAS) and conditional rescue of gene function using CRIMIC alleles. These efforts will further establish Drosophila as a premier model for functional genomics and human disease research.

400T The effects of *myoglianin* knockdown on a *Drosophila melanogaster* model of myotonic dystrophy type 1

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Myotonic Dystrophy Type One (DM1) is a multisystemic disorder caused by the repeat expansion of a CTG sequence in the *DMPK* gene that is characterized primarily by low muscle tone. Another common symptom of DM1 is insulin resistance due to aberrant splicing of the insulin receptor in skeletal muscle (*INSR*), which can often develop into Type Two Diabetes. While the cause and features of DM1 are known, there is no known cure and very few effective treatments. If muscle growth is facilitated, some of the symptoms of DM1 may be alleviated. Myostatin, a muscle-secreted cytokine, both presents as a biomarker for and regulates muscle growth. In *Drosophila melanogaster*, the homolog of myostatin is myoglianin, coded by the *myo* gene. Our lab uses a *Drosophila* DM1 model that expresses expanded CUG repeat RNA using the Gal4/UAS system and modulates the expression of *myo* by RNAi knockdown. Preliminary data from this study suggests that expression of expanded CUG repeats significantly decreases climbing ability, measured by climbing velocity (cm/sec). Successful knockdown of *myo* has been confirmed via RT-qPCR and genes encoding components of the INSR transduction pathway show reduced RNA expression. The flies from this experiment will be assessed for whether *myo* knockdown can alleviate muscle function phenotypes in DM1 flies and how signaling of the insulin pathway is affected by the knockdown of myo in our DM1 model.

401T Pvr Modulation in a *Drosophila* model for myotonic dystrophy type 1

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Myotonic dystrophy type 1 (DM1) is an inherited multisystemic disorder that causes severe muscle wasting. DM1 results from CTG-repeat expansions in the untranslated region of the *dystrophia myotonica protein kinase* (*DMPK*) gene. While the cause of DM1 is known, the underlying mechanism behind DM1 muscle wasting is not fully understood. Previous research using a mouse model of DM1 with severe muscle wasting showed deregulated PDGFRβ signaling. PDGFRβ is a receptor that regulates cell growth, survival, and differentiation. Our lab is evaluating the role of the *Drosophila melanogaster* PDGFRβ ortholog, pvr, in muscle maintenance and the progression of DM1 phenotypes. We are using the Gal4/UAS system in *Drosophila* to overexpress pfv3, the Pvr ligand, as well as over-expression of activated pvr protein in the presence and absence of expanded CUG-repeat RNA (CUGexp). These models will allow assessment of the role of pvr signaling in the progression of DM1 phenotypes. Pvf3 and Pvr will be over-expressed in both the control (UAS-CUG20) and DM1 fly model (UAS-CUG250) using *Mhc*-Gal4 to drive skeletal muscle-specific expression of both CUGexp repeats and the modifier. We will measure climbing velocity (mm/second) and flight capability (landing height in cm) to assess muscle function in these flies. Representative samples will be prepared for histological analysis to measure muscle atrophy. RT-qPCR of RNA isolated from thoraces will allow evaluation of expression levels of *pvr* and pvf3 to test the efficacy of over-expression, as well as Pvr signaling downstream targets *hid*, *dpp*, *thor*, and *myc*. Matings to generate appropriate genotypes are currently underway, along with preliminary testing of gene expression levels of Pvr targets in CUG repeat-expressing flies and in flies over-expressing the activated receptor or ligand. We expect this research will shed light on the role of pvr signaling for proper muscle functionin DM1 flies.

402T *Drosophila* modeling to uncover causal genes and mechanisms within human sleep GWAS loci

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Sleep is a complex trait influenced by numerous factors, including genetics. Recent genome-wide association studies (GWAS) in humans has provided strong evidence for the genetic basis of healthy sleep. However, while a handful of genetic loci have been investigated to understand the causal genes driving genetic associations, many more remain unstudied. Here, we use *Drosophila* to identify the causal genes and mechanisms that mediate sleep-associated genetic signals in humans. Using insomnia-associated loci with known effector genes, we demonstrate that *Drosophila* modeling can delineate pathways that distinctly regulate sleep quantity, quality, or timing. We further identify causal genes and mechanisms at two previously unresolved loci. GWAS for multiple sleep traits revealed a region of chromosome 12 containing common variants associated with chronotype, ease of waking, and napping. *Drosophila* modeling implicates a role for *ALG10*/*ALG10B* and *N*-glycosylation in regulating sleep quantity and quality. Glycoproteomic analyses revealed *N*-glycosylated sleep-related targets, including a single target CG2915 (newly named CPA43F) which fully phenocopies *Alg10* neuronal knockdown. At another locus linked to insomnia and altered sleep health scores, we provide evidence for *MFSD6* as the causal gene driving these sleep phenotypes. Neuronal knockdown of its *Drosophila* ortholog, *jet fue*l (*jef*), shortens circadian period and alters temperature-dependent remodeling of sleep behavior. Together these findings highlight the power of *Drosophila* models to resolve causal genes from human GWAS driving sleep phenotypes and uncover conserved pathways regulating sleep.

403T Effects of developmental nicotine exposure on egg-laying behavior

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Around 8 million people are killed every year due to the use of tobacco (WHO: Tobacco, 2025). Nicotine exposure in humans has been shown to cause low birth weight, increased mortality, and higher incidence of drug use later in life. Similar negative effects from nicotine exposure have been observed in the model organism *Drosophila melanogaster* (Velazquez-Ulloa, 2017). Nicotine has also been shown to negatively impact reproduction in humans by reducing fertility, causing premature births, and risking infant mortality (Martin et al., 2002). In this study, we seek to examine the reproductive effects of nicotine by characterizing the egg-laying behavior of *D. melanogaster* fruit flies of the *wB* strain. First, we characterized whether flies exposed to nicotine during development preferred nicotine-laced 5% sucrose agar or control 5% sucrose agar for laying their eggs compared to control flies. We also determined the effects of developmental nicotine exposure on the amount of eggs laid by female flies in a 24 hour period using a modified version of Wieschaus and Volhard's egg laying procedure (2016). We counted the amount of eggs and larvae to assess the reproductive effect of nicotine as well as any developmental delay. Both control and nicotine-exposed *wB* flies showed significant preference for a nicotine substrate in the two different assays we conducted. In addition, we found that nicotine-exposed *wB* flies showed a significant decrease in the amount of eggs laid and the number of larvae compared to control. To begin investigating embryo viability after the female fly is exposed to nicotine, we are characterizing the embryonic muscle contractions in eggs laid by nicotine-exposed females compared to eggs laid by flies rared on control food. To test this, we have imaged dechorionated stage 17 embryos on a microscope over the span of one minute to characterize the different movements. Our preliminary results suggest there are differences in the quality of the movements. Next, we plan to analyze the ovaries of developmentally nicotine exposed female flies to see if egg development is affected. We will dissect and stain ovaries of both control and nicotine-exposed flies, then image and compare any physical differences. In the future we would like to see if nicotine has proportional effects on reproduction in mutant *D. melanogaster* strains that are either resistant or sensitive to nicotine exposure we have previously characterized. This will allow us to identify genetic factors that contribute to these effects of nicotine.

404T ModelMatcher: an online resource to facilitate cross-disciplinary collaborations between scientists, clinicians, patients and beyond

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Advancement of clinical genomic technologies such as whole-exome and whole-genome sequencing is leading to identification of novel genetic causes of human diseases. In order to understand the functional consequences of disease-associated genetic variants and to reveal the underlying pathogenic mechanisms, it is critical to facilitate interdisciplinary collaborations between clinicians and basic scientists who share interests in the same/orthologous genes. While a number of national and regional efforts, such as the Undiagnosed Diseases Network (UDN) in the USA and Rare Diseases Models and Mechanisms Network (RDMM) in Canada, have been established to facilitate such collaborations, efforts to stimulate international collaborations on a large scale have been lagging behind.

To fill this gap, we have been developing ModelMatcher (https://www.modelmatcher.net), a bioinformatic tool that allows scientists to identify clinical collaborators on a global scale. By using this web browser based tool, which is connected to the MARRVEL search tool (http://marrvel.org/), RDMM International scientist registries (https://rdmminternational.org/) and MatchMaker Exchange clinical databases (https://www.matchmakerexchange.org/), Drosophila researchers can quickly gather information about the human and other model organism orthologs of their genes of interest and further identify potential clinical and scientific collaborators around the world as well as patient organizations who may be interested in supporting your research program. While there is enormous value in pursuing fundamental scientific questions that does not have obvious or immediate clinical value, active involvement in a clinical project may help further increase the significance of specific research projects, increasing the impact of the basic scientific work performed on the fly gene of interest.

405T Refining Tribbles biosensors to detect Slbo (C/EBP) substrate interactions in vivo

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Acute myeloid leukemia (AML) is a blood cancer that affects the bone marrow and progresses quickly, spreading to other parts of the body. AML tumors are marked by increased levels of the Tribbles (Trib) adaptor proteins Trib1 and Trib2, proteins that direct the proteasomal degradation of the differentiation factor C/EBP, which is crucial for proper blood cell differentiation. Tribbles family members are scaffolding proteins and link substrates like C/EBP to E3 ligases for ubiquitin addition and proteasomal turnover. The notion that Trib1 and Trib 2 act as oncogenes to direct C/EBP degradation to prevent blood cell formation and promote tumor overgrowth retains an attractive simplicity, however in some contexts Tribs act as tumor suppressors, so their precise mechanism of action remains unclear. Understanding how Trib-C/EBP interactions promote AML could lead to new drug development strategies.

Tribbles and C/EBP interactions are conserved in Drosophila and we have established two biosensors to detect Tribbles-C/EBP interactions in vivo: (1) a Trbl GFP knock-in to measure Trbl expression and (2) a novel C/EBP degron-fluorescent protein fusion to detect Tribbles-directed substrate turnover in vivo. Coupled with our specific antibody to measure Tribbles protein levels, we present on-going work to examine the influence of C/EBP on Trbl expression, Trbl protein turnover and Trbl activity. Our research will identify novel genes that act in a conserved Trbl signaling pathway and our results will have significance for diagnoses and treatments in humans

406T The Role of *RAB5A* Variants in Bipolar Disorders

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Bipolar disorder has one of the most noteworthy links to inheritance of common neuropsychiatric disorders, with twin studies indicating it may have a heritability as high as 87%. Regardless, specific variants that predispose probands to bipolar disorder have been difficult to identify. We have identified *RAB5A* as a gene with multiple variants associated with bipolar disorder and known interactions with other candidate bipolar disorder genes, such as a *SHANK3*. To evaluate if *RAB5A* bipolar disorder variants alter protein function, we generated multiple *RAB5A* variant lines (*RAB5*^{D136N}, *RAB5*^{Q44R}, *RAB5A*^{Q79L} and *RAB5*^{Q79E}) and screened for changes in adult viability, survival, body morphology, behavior, electrophysiology and sub-cellular structure. We identified a significant decrease in viability for animals ubiquitously overexpressing the *RAB5*^{D136N} and *RAB5*^{Q79E} variants, and expression with Nubbin-Gal4 produced morphological differences in the wings of *RAB5*^{D136N} and *RAB5*^{Q79E} flies. We further examined larval body wall muscle and identified significant alterations in the number and size of late endosomes. RAB5^{D136N} and RAB5^{Q79E} expressing flies displayed a unique perinuclear clustering of late endosomes, and RAB5^{D136N}-expressing flies demonstrated enlarged puncta in comparison to controls. This redistribution of late endosomal and autophagic markers is indicative of a defect in endosomal maturation and cargo turnover, and likely denotes an impaired transition between functional late endosomes and lysosomes. This alteration was also identified in human medulloblastoma (Daoy) cells transfected with Flag-tagged *RAB5A* variants. In aggregate, this demonstrates a significant loss of early endosomes with the expression of *RAB5A*^{D136N} in human cells, while *RAB5A*^{Q79L} cells had no significant change in the size or intensity of EEA1 positive early endosomes, but trended towards a reduction in early endosomal number. As *RAB5A* is a critical regulator of endosomal maturation, we wanted to examine its effect on synaptic function using the *Drosophila* larval neuromuscular junction. We observed a significant increase in the frequency of the spontaneous miniature excitatory junction potentials (mEJP) in our constitutively active Rab5^{Q79L}, Rab5^{Q44R} and Rab5^{D136N} animals compared to the reference. In total, our results confirm that these missense variants impact protein function, and that the *RAB5*^{D136N} variant causes multiple significant phenotypes within a *Drosophila* model.

407T Modeling X-Linked Adrenoleukodystrophy with a comprehensive study of *Abcd1 (CG2316)* in *Drosophila*

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X-linked adrenoleukodystrophy (X-ALD) is a progressive neurodegenerative disorder caused by loss-of-function (LOF) mutations in the *ATP-binding cassette subfamily D member 1 (ABCD1)* gene. *ABCD1* encodes an ATP-binding cassette (ABC) transporter that localizes to peroxisomes and facilitates very long-chain fatty acids (VLCFAs) transport into them. Peroxisomes are the tiny vesicles where the VLCFAs are broken down by the enzymes into acetyl CoA through beta-oxidation. LOF of *ABCD1* thus causes accumulation of VLCFAs in the neurons. *ABCD1* being on X chromosome, the disorder exhibits striking clinical heterogeneity: some male patients develop an early-onset neuroinflammatory demyelinating disease, whereas others—including adult males and most heterozygous females—present with a chronic, progressive myelopathy. Adrenocortical failure occurs in nearly all affected males and may be the first diagnostic manifestation. Thus X-ALD is considered a single peroxisomal component defect and does not play a direct role in peroxisome assembly.

In *Drosophila,* knockdown of its ortholog-*Abcd1* (CG2316), has provided some insight into lipid accumulation in retina but complete null phenotype has remained unstudied. Here, we present a comprehensive genetic analysis of *Abcd1*.

Ubiquitous *RNAi*-mediated knockdown caused salivary gland defects, peroxisomal number defect, VLCFA accumulation and lethality. However, a CRISPR-generated four-base-pair deletion (*Abcd1*$^{\Delta 4}$) produced a homozygous-viable null. These larvae also exhibit reduced salivary gland cell size and decreased peroxisomal numbers and VLCFA accumulation. Along with that the viable adult flies display locomotion defects and shortened lifespan. Attempts to rescue this null phenotype with human *ABCD1* cDNA were impossible as its overexpression appears to inhibit or overwhelm peroxisomal biogenesis to levels similar to null mutations in fly *Pex2*, *Pex16* and *Pex3.* Also, the overexpression of human cDNA displayed wing crumpling phenotype in the adult which is characteristic of the peroxisomal gene *Pex2* LOF; whereas overexpression of a known X-ALD–associated LOF variant did not. Interestingly, utilization of a genomic duplication of *Drosophila Abcd1* displayed rescue of all the *Abcd1*$^{\Delta 4}$ null phenotypes. Currently, we are working towards testing the effect of different X-ALD related drugs on these flies.

408T Mob4's phospho-binding motif is essential for viability and neuronal function

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Mob4 expression has been found to be significantly downregulated in Alzheimer's disease patients. Mob4 is a core component of a large, multi-protein complex called the Striatin-Interacting Phosphatase and Kinase (STRIPAK) complex. While Mob4 is considered a core complex component, little is known about how it molecularly functions within the complex. Mob family proteins are known to act as adaptor proteins. Using Mob4 depletion and replacement experiments and genome engineered mutants, we demonstrate that a C-terminal phospho-serine/threonine binding motif within Mob4 is required for neuronal function, viability, and lifespan. Within neurons, Mob4 function impacts axonal transport, climbing ability, and wing expansion. All the observed neuronal phenotypes from Mob4 depletion are rescuable by wildtype Mob4 but not by the phospho-binding defective Mob4. We also probed the function of the N-terminus of Mob4 and found that it is dispensable for viability and neuronal function but is important for Mob4 protein stability. Our current efforts are aimed at understanding the phospho-specific interacting partners of Mob4 to further understand its molecular function in neurons and other cell types.

409T *Drosophila* modeling uncovers a previously unrecognized steroid hormone defect in a patient with *BUD13*-related progeroid laminopathy

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Progeroid laminopathies are rare genetic disorders characterized by accelerated aging and severe nuclear envelope (NE) abnormalities. These syndromes are often linked to variants in NE components or their interactors. Recently, a novel progeroid disease caused by loss-of-function variants in *BUD13* has been reported (Kornak *et al*., *Genet Med*, 2022). These patients present progeroid features with lipodystrophy and NE defects.

BUD13 is a highly conserved gene from yeast to humans and plays a key role in mRNA splicing. To investigate the molecular mechanisms of this disease, we established a *Drosophila* model of BUD13-associated progeroid laminopathy. Using CRISPR/Cas9, we generated a knockout/in allele and visualized fly *Bud13* expression patterns by GAL4/UAS. From human cDNA overexpression experiments, we concluded that the patients' variants behave as loss-of-function alleles, consistent with a previous report based on patient-derived fibroblast cells.

RNAi-mediated knockdown of fly *Bud13* successfully recapitulated key patient phenotypes, including lipodystrophy and NE abnormalities. To investigate the impact of *Bud13* knockdown on splicing and gene expression, we performed bulk RNA-seq analysis. Transcriptional profiling revealed increased intron retention in mitochondrial genes and upregulation of detoxification-related genes. TEM analysis and immunostaining confirmed the mitochondrial defects and elevated ROS levels.

Interestingly, based on the high expression of *Bud13* in the ring gland in fly larvae, we revealed a novel role of this gene in ecdysone signaling. In collaboration with clinicians, we identified a previously unreported steroid hormone abnormalities in an individual with *BUD13*-associated progeroid laminopathy. This case highlights the power of *Drosophila* as a genetic disease model not only to validate known phenotypes but also to uncover previously unreported symptoms. Such insights inform clinical understanding and have the potential to improve the quality of life for affected individuals.

410T Tumor-secreted vesicular Hedgehog disrupts kidney filtration to drive cancer cachexia

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Cancer cachexia, a devastating syndrome characterized by severe energy wasting and high mortality in patients with malignant tumors, is increasingly understood as a multi-organ dysfunction. Previous studies leveraging various animal models have illuminated that tumor-secreted proteins can remotely impair metabolic organs like muscle, fat, pancreas, and kidney tubules, contributing to cachectic features. However, interventions targeting these organs have struggled to extensively reverse systemic energy wasting or mortality, indicating that other critical, unidentified pathogenic organs or tissues are involved in a more systemic capacity. In this study, we discovered that restoring perturbed kidney filtration, a vital process for both vertebrates and invertebrates to eliminate circulating waste and toxins and alleviate systemic stresses, significantly improves both energy wasting and mortality in response to malignant tumors. Using the *Drosophila* yki^{3SA}-gut tumor model, we demonstrated that malignant tumors unconventionally release Hedgehog (Hh) via extracellular vesicles (EVs) to primarily target pericardial nephrocytes, the functional analogs of kidney glomerular podocytes, leading to impaired renal filtration and hemolymph protein aggregation. This profound dysregulation subsequently caused systemic energy wasting, including muscle dysfunction, lipid loss, circulating carbohydrate elevation, as well as ovary atrophy, and ultimate host mortality. Our integrated multi-omics analysis and *in vivo* screening further elucidated the molecular mechanisms, by which tumor-secreted vesicular Hh activates Ptc-Smo-Ci signaling in nephrocytes to disrupt slit diaphragm integrity, endocytic homeostasis and the endolysosomal degradation pathway via induction of a novel target gene, *Cathepsin protease L1* (*CtsL1*). Crucially, we also confirmed the conserved Hh-mediated glomerular dysfunctions in tumor-bearing mice and humans. Collectively, our findings definitively establish the tumor-kidney axis as a key pathogenic core in cancer cachexia and provide novel insights into the mechanisms of tumor-impaired kidney filtration.

411T Unexpected Molecular Mechanism of Orc6-Based Meier-Gorlin Syndrome: Insights from a Humanized Drosophila Model

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Meier-Gorlin syndrome (MGS) is a rare autosomal recessive disorder characterized by microtia, primordial dwarfism, and skeletal abnormalities. Patients with MGS often carry mutations in genes encoding the subunits of the Origin Recognition Complex (ORC), components of the pre-replicative complex and replication machinery. ORC6, an essential ORC subunit, plays a critical role in both DNA replication and cytokinesis. In eukaryotes, Orc6 has a homology with transcription factor TFIIB. We have solved the structure of human ORC6 and shown that metazoan Orc6 is composed of three independent domains: N-terminal, middle and C-terminal. We identified a structured motif in the Orc6 C-terminal domain which defines the dynamics of inter-subunit interactions within metazoan ORC. The MGS mutation in the C-terminal motif of Orc6 impedes the interaction of Orc6 with the core ORC. Another MGS mutation is localized in the N-terminal domain of the protein and directly affects DNA binding. Furthermore, approximately 30% of reported ORC6-related MGS cases exhibit compound heterozygosity for the ORC6 variants c.2T>C (p.Met1Thr) and c.449+5G>A. The c.2T>C mutation disrupts the start ATG codon by changing it to ACG, potentially initiating translation at an alternative downstream in frame Methionine (Met20), while c.449+5G>A results in in-frame exon skipping. Both mutations are predicted to produce significantly truncated ORC6 proteins with impaired functionality. Using humanized ORC6 based Drosophila model, we demonstrated that these truncated proteins fail to rescue orc6 deletion. Instead, our findings reveal that the strong Kozak sequence, naturally present in human ORC6 mRNA, promotes translation from a non-canonical ACG codon. Rescued flies demonstrated a phenotype that we observed earlier for other MGS mutants in Drosophila. These results provide compelling evidence that MGS patients with c.2T>C/c.449+5G>A mutation rely on full size ORC6 protein initiated from a non-canonical ACG start codon. In summary, using the humanized *Drosophila* model of the Meier-Gorlin syndrome we discovered the molecular mechanisms underlying MGS phenotypes. Our studies revealed the importance of evolutionary conserved and variable domains of ORC6 protein and allowed studies of human protein functions and the analysis of the critical amino acids in live animal heterologous system.

412T Regulation of anne gene expression in the larval midgut

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Although polyamine biosynthesis is well characterized, the mechanisms governing their transport remain poorly understood. Because polyamines are found in all forms of life and their imbalance is implicated in diseases such as cancer and neurological disorders, identifying the molecular components that regulate polyamine uptake, and homeostasis is of significant biological and clinical importance. Our work focuses on *anne*, a *Drosophila melanogaster* gene encoding a P5B-type ATPase involved in polyamine transport across membranes. This project aims to determine how changes in endogenous polyamine levels influence the expression of *anne* in the larval midgut which is the major site of polyamine uptake into the body. Anne produces multiple protein isoforms and our work examines the contribution of each isoform to polyamine uptake. Our findings will provide new insight into conserved mechanisms of polyamine regulation and inform future work on human disorders associated with altered polyamine metabolism.

413T Altered regulation of IKK genes in a *Drosophila* model of the human neurodegenerative disease SCA3

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This research focuses on the human neurodegenerative brain disease spinocerebellar ataxia type 3 (SCA3), which is characterized by a trinucleotide (CAG) repeat expansion in the ataxin-3 gene. This encodes for a polyglutamine expansion in the translated ataxin-3 protein, causing neurodegeneration and the problematic phenotype, such as motor impairments. Chronic inflammation has been implicated in a worsening of the disease progression. Specifically, the transcription factor Nf-kB (relish) has been demonstrated to be influential. The IKK complex, a dimer that phosphorylates this transcription factor, is the focus of this study. The two subunits are Kenny and Ird5, which are homologous to IKK2 and NEMO respectively in humans. We use *Drosophila melanogaster* that are either diseased, expressing a CAG trinucleotide 84 repeats in length (84), or undiseased, with a repeat length of 27 CAG (27) in the human ataxin-3 gene. The genes are expressed in the eyes using the GMR-GAL 4 driver. These lines were crossed to *Drosophila* with altered Kenny or Ird5 expression, as well as an unchanged control. We hypothesized that altering regulation of genes that control Relish translocation may alter immune response dependent degeneration.

414T Altered TNF signaling in a *Drosophila* model for SCA3

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Spinocerebellar Ataxia 3 (SCA3), otherwise known as Machado-Joseph Disease (MJD), is a polyglutamine disease similar to Huntington's in which misfolding of the Ataxin-3 protein due to excess polyglutamine repeats causes protein aggregation and neurodegeneration. Our investigation explores a potential relationship between the tumor necrosis factor (TNF) stress-response pathway and degeneration caused by SCA3. Dying cells may release Eiger which may up-regulate JNK and promote neurodegeneration. We asked if neurons dying due to disease protein expression may be influencing neurodegeneration in neighboring cells due to TNF signaling. TNF ligand Eiger can bind one of two TNF receptors, Grindelwald and Wengen, in order to activate this neurodegenerative pathway. To test this we reduced expression of these genes individually and observed their effect on neurodegeneration in our Drosophila model for SCA. Data was obtained through quantitative and qualitative assessment of fly retinas. These results will provide information about the usefulness of targeting TNF signaling to reduce degeneration in human neurodegenerative diseases.

415T *Drosophila* Glioma associated Inflammation leads to Hemocyte-Infiltration through Breakdown of the Blood–Brain Barrier

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Glioblastoma (GBM), a grade IV astrocytoma, is the most aggressive type of brain tumor in humans. Despite aggressive treatment involving surgery and chemo-radiation, GBM inevitably recurs. GBM patients show mutations in genes encoding the phosphatase and tensin homolog (*PTEN)* and tumor protein p53, and amplification of Epidermal growth factor receptor 8 (*EGFR)*. In GBM patients, chronic low-grade inflammation is associated with poor prognosis. The brain is predominantly regulated by the innate immune system (e.g., myeloid cells) rather than adaptive immunity (e.g., T cells). Recent work suggests GBM associated myeloid cells promote chronic inflammation and aggressive GBM tumor behavior. However, the role of inflammation in myeloid cells versus tumor cells, and its impact on development and progression in GBM is poorly understood. In *Drosophila* glioma models, we study glial-neuronal interactions, and the role of innate immune system in glioma progression. Taking advantage of the power of *Drosophila* genetics, we study two glioma models where both EGFR/MAPK and PI3K pathways are constitutively activated, first by overexpression of *UAS-EGFR*$^{\lambda top}$ and *UAS-PI3K92E*^{CAAX} (Model 1), and second, by overexpression of *UAS-Pten*^{RNAi}, and *UAS-Ras*^{V12} (Model 2).These *Drosophila* glioma models cause development of aggressive neoplasms under the glia-specific repo-GAL4 driver. We validated our models using the confocal/IHC data of neuroblasts, neuron-glia differentiation in GBM models. Our qPCR and IHC data of antimicrobial peptides (AMPs), activated JNK cascade and hemocyte specific markers revealed activated immune pathways and immune infiltrations in fly glioma models. Here, we present our results on the role of *Drosophila* hemocytes (homologous to human myeloid cells) in promoting GBM through (a) their interactions with glial cells and (b) degradation of the blood brain barrier and infiltration in glioma brain and tumor cells. Overall, the study provides insight into how immune-mediated modifications effect tumor microenvironment and progression in GBM.

Key words:Glioma/GBM, hemocytes, AMPs, JNK.

416T Comparing protein nitration levels in vulnerable and non-vulnerable parkin-null *Drosophila* neurons

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Loss-of-function mutations in the *PRKN* gene cause a rare form of Parkinson's disease (PD). Parkin-null *Drosophila* have dopaminergic neurodegeneration, the hallmark pathology of familial and idiopathic forms of PD. We reported elevated mitochondrial H_2O_2 and glutaredoxin activity (indicating antioxidant demand) in parkin-null fly protocerebral posterior lateral region 1 (PPL1) dopaminergic neurons. The rest of the human/fly brain is relatively resistant to degeneration, and we recently observed evidence suggesting that PPL1 may lack the antioxidant compensation required to protect cells from parkin loss; however, we have not yet used uniform protocols to compare specific oxidative stress markers between PPL1 and the central brain. Nitric oxide (NO) promotes inflammation and synaptic maintenance in a context-dependent manner, making the implications of altered NO signaling observed in PD difficult to discern. Using anti-nitro tyrosine amino acid residue labeling, we reported decreased central brain nitration in young parkin-null flies. To shed light on whether nitration decreases are reflected in PPL1, we measured protein nitration in PPL1 and central brains of young and aged parkin-null *Drosophila*. Preliminary results indicate that central brain and PPL1 nitration are decreased in young parkin-null flies. At the central brain level, parkin-null female nitration is reduced compared to males, but PPL1 nitration levels are unaffected by sex. Aged parkin-null flies have decreased PPL1 nitration, while central brain nitration is similar to control. Aged male parkin-null fly central brain nitration is decreased relative to females. To address whether nitration reflects inflammatory or synaptogenic NO signaling, we used nitric oxide synthase (NOS)-driven GFP to observe decreased NOS levels in the central brain of aged but not young parkin-null flies. The single *Drosophila* NOS isoform (dNOS) has the highest sequence homology to mammalian neuronal NOS (nNOS), which is essential for synaptic maintenance. Our preliminary data suggest that PPL1 dNOS may function more like nNOS, since parkin-null flies have decreased PPL1 nitration. Central brain nitration patterns loosely reflect dNOS expression, suggesting a more mammalian inducible NOS (iNOS)-like inflammatory function; however, whether dNOS is exhibiting a more nNOS- or iNOS- like activity is unclear. Mammalian nNOS is regulated by glutathionylation, and in addition to our observations of disrupted parkin-null PPL1 mitochondrial glutathione redox equilibrium, we have also reported increased glutathione levels in parkin-null central brains. These results inform future work exploring the interplay between parkin, glutathione, and NO signaling pathways.

417T Discovery of the Alp/Enigma gene family in *Drosophila* identifies an essential role for the PDZ domain protein Uchmaz in muscle assembly

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Z-discs define sarcomere boundaries and anchor actin filaments in muscle cells, yet many of their structural components remain uncharacterized. The objective of this study is to identify and characterize new components of the Z-disc in Drosophila muscle. In this paper, we identify three new members of the Alp/Enigma family in *Drosophila*, including *Uchmaz* (*CG42319*), a PDZ domain-containing protein, that we characterize in detail. Here, we used CRISPR/Cas9 to generate multiple Uchmaz knockout alleles, which are viable but result in a flightless phenotype. Mutants exhibit disrupted sarcomere organization in the indirect flight muscles (IFMs), including fragmented and misaligned Z-discs and reduced accumulation of α-actinin and other Z-disc markers. We find that Uchmaz localizes to the Z-disc and is dependent upon α-actinin for its localization to the Z-disc, via a direct physical interaction. These results identify Uchmaz as a component of IFM myofibril architecture required for muscle function. Since a human ortholog of *Uchmaz*, *PDLIM2*, is associated with severe muscle dysfunction in muscular dystrophies and myofibrillar myopathy, our studies provide insight into conserved mechanisms of Z-disc assembly and its relevance to human muscle disease.

418T Differential recruitment of signaling pathways downstream of Ras regulate pleiotropic effects of neurofibromin

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Neurofibromatosis type 1 (NF-1) is a monogenetic inherited disorder resulting from loss-of-function mutations in the *Neurofibromin 1* gene. NF-1 occurs at an incidence rate of approximately 1 in 3000 and clinical manifestations are highly variable: common symptoms include tumors in the nervous system, behavioral abnormalities, and metabolic alterations. *Neurofibromin 1* encodes a large protein, neurofibromin (Nf1), that acts as a tumor suppressor via its regulatory effects on Ras activity. This modulatory function affects many signaling pathways downstream of Ras, including MAPK (Raf/MEK/ERK) and P13K/Akt/mTOR, both of which contribute to metabolic homeostasis. Critically, while there are limited pharmacological options for symptom management, there is no current treatment for this disorder.

The fruit fly, *Drosophila melanogaster*, presents a powerful model to identify targets for therapeutics. In flies, Nf1 is ~60% homologous to the human protein and, importantly, retains its central GAP-related domain (GRD) that is required for Ras regulation. Our lab has established that loss of Nf1 in flies leads to both metabolic and behavioral phenotypes through neuronal mechanisms. Subsequent genetic analysis determined that behavioral alterations are mediated by MEK signaling, not through Akt. Conversely, metabolic alterations observed in Nf1-deficient flies were found to be mediated by coordinated actions of MEK/ERK and Akt/mTOR/S6K/4E-BP signaling. Changes in mitochondrial morphology in muscle, but not neurons, was also observed. Taken together, this data presents multiple signaling cascade targets that differentially affect behavioral and metabolic phenotypes in NF-1.

419T Comparisons of motor and neural phenotypes in TBI and PD Drosophila models

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Traumatic Brain Injury (TBI) can occur as a consequence of sustained head trauma. Delayed onset symptoms can increase the risk of permanent cognitive changes and the onset of neurodegenerative diseases such as Parkinson's Disease (PD) or Parkinson's like behavioral symptoms. While several studies have explored the general mechanisms behind TBI and PD development, a model assessing the link between these conditions has not yet been established. This study explores a *Drosophila* model of TBI in direct comparison to a PD model, assessing behavioral and neurological measures. The fly alpha-synuclein model for PD was created by crossing Ddc-Gal strain to a UAS-synuclein heterozygous stain producing Ddc/synuclein flies that produce the synuclein protein in dopaminergic cells and phenocopy PD. The other products of the cross were used as controls. Four groups were established: PD, PD+TBI, TBI alone, and control flies. TBI was achieved using a fly head specific injury model (Katzenberger et al., 2013; Alphen et al., 2022) using unanesthetized five-day old flies. Climbing assays were used to assess changes in motor ability with age for all groups (Manjila and Hasan, 2018). Immunohistochemistry was performed using a tyrosine hydroxylase antibody (company) to monitor preservation of dopaminergic neurons throughout life span. The control and TBI alone showed no decrease in climbing ability over the course of the experiment. PD and PD+TBI showed decreased climbing ability initially as well as significant declines in climbing ability with age. TBI enhanced the climbing deficits in PD flies. The immunohistochemistry data shows a pattern of dopaminergic cell loss that parallels the climbing deficits although this data is preliminary. Future experiments will look at neurodegeneration more generally in this model system. In conclusion, TBI exacerbates PD predisposition and symptomology but doesn't appear to generate PD like climbing defects alone.

420T Cortex glial subtypes are regionally specialized and differentially regulate seizure susceptibility

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Glial cells are ubiquitous in the nervous system and are now recognized as heterogeneous across brain regions. In *Drosophila*, subtypes within the same glial class have been identified, but their functional relevance is unclear. Here, we employed a genetic approach to evaluate if cortex glia located in different parts of the brain differentially regulate seizures. We developed transgenic tools to label and manipulate cortex-glial subpopulations across developmental stages, achieving subtype-specific expression in the optic lobe (OL), central brain (CB), and ventral nerve cord (VNC) using Gal4, split-Gal4, Gal80, LexA, and Killer Zipper systems. Using these drivers, we performed region-specific rescues in two seizure models where cortex glia was shown to play important role: ceramide phosphoethanolamine synthase (*cpes*) mutants with light-inducible seizures and $Na^+/Ca^{2+},K^+$ exchanger (NCKX; zydeco, *zyd*) mutants with temperature-inducible seizures. We found that the requirement for cortex glial function is region specific and non-redundant. Specifically, OL- and CB-restricted cortex-glial expression of UAS-CPES, but not VNC-restricted expression, significantly suppressed light-inducible seizures in *cpes* mutants. Conversely, VNC-restricted, but not OL or CB, cortex-glial expression of UAS-NCKX suppressed temperature-sensitive seizures in *zyd* mutants. Further in a third model, VNC-specific cortex-glial expression and activation of dTrpA1 alone was sufficient to induce temperature-sensitive seizures in wild-type flies. These results demonstrate that regionally specialized cortex glial subtypes have distinct, local roles in regulating seizure susceptibility, providing evidence for the functional importance for glial subtype heterogeneity.

421T Identification and Functional Analysis of Novel Neurofibromin-Interacting Proteins

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Neurofibromatosis type 1 (NF1) is a neurodevelopmental disorder arising from loss-of-function mutations in the *NF1* gene, with symptoms including tumors of the nervous system and cognitive and behavioral issues. *NF1* encodes the Ras-GTPase activating protein (Ras-GAP) neurofibromin, which negatively regulates Ras signaling by catalyzing the hydrolysis of active Ras-GTP into inactive Ras-GDP. This activity is conferred by its GAP-related domain (GRD) and is essential for proper development and function of the nervous system. Pathogenic mutations within the neurofibromin GRD have been shown to affect Ras-GAP catalytic activity, though it is also possible that they impact neurofibromin function in other ways – for instance, by disrupting its interaction with other proteins, beyond Ras.

High conservation between human neurofibromin and the *Drosophila* ortholog (dNf1) facilitates modelling of patient-derived missense mutations in the fly. As expected, neuronal expression of *UAS-dNf1* transgenes bearing GRD mutations failed to rescue established *dNf1*$^{-/-}$ phenotypes including growth, sleep, and circadian rhythmicity deficits. To investigate how GRD mutations impact the interaction of dNf1 with other proteins, three mutant *UAS-dNf1* transgenic lines were used for proteomics via affinity purification-mass spectrometry. Curiously, in the presence of a GRD mutation, many interactions were strengthened or otherwise altogether novel. We speculate that dNf1 with impaired GAP activity serves as a 'protein trap,' allowing typically transient interactions between dNf1 and its associated proteins to be captured.

Since *dNf1*$^{-/-}$ flies are small due to a neuroendocrine deficit (reflecting the typically shorter stature of individuals with NF1), functional analysis of putative dNf1-interacting proteins was carried out via pan-neuronal RNA interference to reveal those involved in the regulation of fly growth. Several hits whose knockdown in otherwise wildtype flies led to reduced or increased pupal size were identified. We hypothesize that such proteins may work together with dNf1, either in concert or antagonistically, to regulate growth and other dNf1-dependent processes including sleep and circadian rhythm. Genetic interaction experiments are ongoing to test this possibility, using novel mutant lines generated via CRISPR. We are also examining whether the interactions identified in our proteomics analyses are conserved between human neurofibromin and their corresponding orthologs.

422T The differential effects and mechanisms of the MEK-ERK pathway in neurodegeneration and axon integrity in fly and mouse models

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The MEK-ERK axis of MAPK signaling is a highly conserved signaling pathway that regulates diverse cellular processes, and its dysregulation is implicated in cancer and neurological disorders. TDP-43 is a critical DNA/RNA-binding protein associated with neurodegenerative diseases such as ALS and FTD. In a transgenic RNAi screen, we uncovered that knockdown (KD) of *dMEK* suppressed TDP-43 toxicity without altering its protein or phosphorylation levels. We found that the downstream gene *dERK* was abnormally upregulated in TDP-43 flies. Neuronal overexpression (OE) of *dERK* or TDP-43 induces profound upregulation of antimicrobial peptides (AMPs), indicating a robust innate immune overactivation. Critically, this immune response and the associated neurotoxicity could be suppressed by downregulating the MEK-ERK pathway in neurons. Furthermore, neuronal KD of AMPs improved motor function, while forced activation of immunity diminished the mitigating effect of *dMEK* KD. The FDA-approved MEK inhibitor (MEKi) trametinib markedly suppressed this immune overactivation, alleviated motor deficits, and prolonged the lifespan of TDP-43 flies. In contrast, trametinib did not extend lifespan in of AD or SCA3 flies, indicating a specific role for MEK-ERK in TDP-43 pathogenesis.

Axon degeneration is a key pathological feature in neurological disorders. While we and others have proposed MEKi as a potential therapy for ALS and other brain diseases, it is known to cause peripheral nerve lesions in cancer therapy. In our most recent study, we resolved this paradox by finding that MEK inhibition enhances, whereas its activation protects against, injury- and chemotherapy-induced axon degeneration in mouse DRG neurons. Mechanistically, the Raf-MEK-ERK cascade promotes the critical axon survival factor Nmnat2 via ERK phosphorylation-dependent transcription. Conversely, trametinib decreases *Nmnat2* expression, thereby inducing axon degeneration. Unlike the peripheral DRG neurons, the CNS cortical and spinal motor neurons maintain *Nmnat2* transcription via CREB, independently of MEK-ERK, and are consequently resistant to MEKi. These findings demonstrate a neuron subtype-specific role and mechanism of MEK-ERK in regulating axon integrity.

In summary, therapeutic targeting of the Raf-MEK-ERK pathway for neurodegenerative diseases requires BBB-permeable inhibitors, and the risk of CNS nerve lesions—as observed with trametinib in the PNS—must be carefully evaluated. Our work reveals the candidacy of MEKi for treating ALS and uncovers a context-dependent axon survival paradigm, explaining the specific vulnerability of PNS neurons to MEKi. Notwithstanding these complexities, our findings support the potential use of MEKis for ALS and other CNS diseases, while the influence on *Nmnat2* expression must be carefully assessed in the targeted neuronal populations.

423T Early social isolation sensitizes *Drosophila* for subsequent TDP-43 pathological spread, activation of endogenous retroviruses, and neurodegeneration

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are two fatal neurodegenerative disorders characterized by motor defects and cognitive dysfunction. A key pathophysiological hallmark of both disorders is loss of nuclear localization and abnormal cytoplasmic aggregation of TAR DNA-binding protein 43 (TDP-43). Such pathology is observed in the affected brain regions of ~95% of ALS and ~40% cases of FTD patients. The functional roles of TDP-43 include transcriptional regulation, mRNA splicing, mRNA transport and repression of retrotransposons (RTEs) and endogenous retroviruses (ERVs). Previous studies in *Drosophila*, in human postmortem cortical tissues and in cell culture, reveals that TDP-43 pathology activates expression of RTEs and ERVs. RTEs and ERVs are mobile genetic elements that replicate through an RNA intermediate to insert cDNA copies at de novo chromosome locations. We have previously reported that TDP-43 aggregation causes dysfunctional RTE/ERV overexpression and that ERV expression triggers TDP-43 pathology. This feedback amplification between TDP-43 pathology and ERV expression also contributes to toxic signaling from glia-to-neurons, leading to both cell intrinsic and non-autonomous cell death. But the upstream triggers that initiate or drive this phenomenology are not identified.

Although dominant mutations in TDP-43 or a number of other genes explain a small fraction of cases, the majority of ALS and FTD cases are sporadic, with no known genetic causes. Thus, most cases exhibit aggregation pathology of TDP-43 protein that contains wild type amino acid sequence. Although the environmental factors that influence onset and progression of disease are largely unexplored, epidemiological studies suggests that anxiety, depression and loneliness are associated with increased risks of neurodegenerative disease. We examined the impacts of several of ethologically relevant behavioral stressors in *Drosophila*. We report that early life social isolation is sufficient to exacerbate effects of subsequent induction of pathological levels of TDP-43 in glial cells. Social isolation stress drives more rapid activation of mdg4-ERVs, more aggressive propagation of TDP-43 protein pathology from surface glial cells to nearby neurons and shortened life span ensues. We demonstrate that flies require a holistically complete social interaction to prevent these effects of social isolation. Our findings have implications for the association between psychological stressors such as loneliness and risk of neurodegenerative diseases in humans.

424F The microcephaly-associated protein Abnormal Spindle promotes proper neurogenesis and neuronal cell fate via its interaction with Protein Phosphatase 2A.

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Mutations in *abnormal spindle (asp)* disrupt brain growth and development, yet the underlying mechanisms responsible have yet to be defined. This is primarily due to a lack of known protein interactors, which can provide insight into the cellular pathways essential for this process. Here we report the identification of >20 novel interactors of Asp's N-terminus, which we previously showed to be essential for brain growth and development. Using a genome-wide Yeast two-hybrid (Y2H) fragment library from the third instar larval brain, we obtained domain level resolution of these interactions. The highest confidence partners were the β-regulatory subunits of Protein Phosphatase 2A (PP2A), including widerborst (wdb) and well-rounded (wrd). Computational modeling and mutational analysis confirmed that this interaction is driven through a short linear motif (SLiM) in Asp's unstructured region. Mutations in Asp's SLiM motif led to microcephaly, suggesting that this interaction is critical for Asp's ability to promote proper brain growth. We also uncovered an essential role for the Asp-PP2A interaction in the development and function of the larval Inner Proliferation Center (IPC), particularly in the establishment and maintenance of the neuroblast population essential for the generation of T4/T5 neurons. Cellular defects included a loss of migratory IPC progenitor cell populations, disruption of neuroblast layer architecture, and reactivation of stem cell markers in T4/T5 neurons, leading to dedifferentiation and loss of neuronal identify in the adult lobula complex. Together, these results uncover a new role for Asp as an essential determinant of neuronal identity via its interaction with PP2A in progenitor cells, ensuring proper brain growth and development.

425F Modeling a human Pericentrin MOPD II variant in *Drosophila*

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Centrosome dysfunction is linked to developmental disorders affecting brain and body size, including microcephaly and primordial dwarfism. However, the cellular mechanisms underlying these rare conditions remain poorly understood. In this study, we investigate a rare variant in Pericentrin (PCNT), a centrosome-associated protein, discovered in a single family with Majewski/microcephalic osteodysplastic primordial dwarfism type II (MOPD II). Unlike the majority of pathogenic PCNT variants that cause severe protein truncation, the p.Lys3154del variant (ΔK3154) involves a single amino acid deletion in the protein's only conserved functional domain, providing a unique opportunity to explore PCNT function in MOPD II. To model the *PCNT*$^{\Delta K3154}$ allele, we examined the effects of *Drosophila* Pericentrin-like protein (PLP) carrying an orthologous deletion (*plp*$^{\Delta R}$). Our results show that *plp*$^{\Delta R}$ animals exhibit developmental delay and smaller tissues that recapitulate MOPD II phenotypes. Behavioral assays revealed defects in gravitaxis and mechanosensation, suggesting impaired sensory cilia function. We also find that *plp*$^{\Delta R}$ cells exhibit accelerated mitosis, increased apoptosis, and reduced pericentriolar material recruitment. *In silico* structural modeling, yeast two-hybrid, and co-immunoprecipitation experiments show that *plp*$^{\Delta R}$ disrupts PLP dimerization and the interaction with Asterless/Cep152, another centrosome protein. Overall, modeling the human MOPD II patient variant, *PCNT*$^{\Delta K3154}$ in *Drosophila* reveals how a single amino acid deletion affects biological processes from the molecular level all the way to the organismal level. Our work offers new insights into the defective cellular mechanisms underlying MOPD II in patients with the *PCNT*$^{\Delta K3154}$ variant, potentially linking the etiology of this disease to the loss of a single protein-protein interaction.

426F Age- and Sex- Dependent Mitochondrial and Metabolic Dysregulation in a *Drosophila* Model of PLA2G6-Associated Neurodegeneration

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PLA2G6-associated neurodegeneration (PLAN) is a rare and progressive disorder in humans resulting from mutations in the PLA2G6 gene, which encodes a calcium-independent phospholipase A2 enzyme critical for phospholipid remodeling and membrane homeostasis. The loss-of-function mutations in the *iPLA2-VIA*, the ortholog of PLA2G6 gene in *Drosophila*, exhibit age-dependent locomotor defects, reduced lifespan, and female-specific fertility defects. Given that iPLA2-VIA localizes to mitochondria and its loss leads to mitochondrial aggregation and apoptosis in female germ cells, we hypothesized that systemic mitochondrial function and metabolic homeostasis are disrupted in iPLA2-VIA mutants. Thus we evaluated mitochondrial structural and functional impairments in organ, sex, and age-dependent manner, and altered metabolic pathways in the mutant flies. We also confirmed that characteristics of neurodegeneration and aging are prevalent in these flies.

TEM of heads, thoraxes, abdomens, and ovaries revealed mitochondrial abnormalities like a reduction in number, disrupted cristae, membrane damage, and elongated morphology in the mutants. Accordingly, ATP production was reduced, and oxidative stress was enhanced due todownregulation of SOD2. Additionally, altered expression of PGC1α, Mfn2, and Drp1, indicated dysregulated biogenesis and fission–fusion dynamics of mitochondria. Metabolomic and lipidomic profiling of young (<1 week) and aged (3 weeks) iPLA2-VIA mutant and control flies revealed that the young mutant flies resembled with the old control flies. SMURF (?) assays confirmed an accelerated loss of intestinal barrier integrity in the mutants, which is a marker of an aggravated aging process. H&E staining of fly head sections was used to examine brain morphology, revealing prominent vacuolar lesions and tissue degeneration in mutant brains, consistent with hallmark features of neurodegeneration. This work provides mechanistic insight into how mitochondrial impairment drives disease progression in PLA2G6-associated neurodegeneration and establishes *Drosophila* as a powerful model for exploring therapeutic targets for PLAN.

427F Zika Virus NS4B Protein Activates the UFMylation pathway via UBA5 to Cause Microcephaly

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Zika virus (ZIKV) is a single-stranded RNA virus belonging to the Flaviviridae family along with West Nile and Dengue virus. With the *Aedes aegypti* mosquito as its vector, the virus crosses the placental-blood barrier to affect the growing foetus after the pregnant mother is bitten. ZIKV infections lead to phenotypes clinically termed as Congenital Zika Syndrome (CZS). CZS includes neurodevelopmental defective phenotypes such as seizures, muscle pains, cognitive defects and microcephaly, which are not curable. We previously established the usefulness of *Drosophila* to understand pathways that ZIKV affects during development. Protein-protein interaction (PPI) data suggest an interaction between ZIKV non-structural protein 4B (NS4B) and human Ubiquitin-like activating enzyme 5 (UBA5). UBA5 acts as the E1 protein (similar to ubiquitination) in the ufmylation pathway which allows for post-translational modification of proteins by adding the UFM1 molecule. Mutations in this gene can lead to early-onset infantile encephalopathy, specifically Development and Epileptic Encephalopathy 44 (DEE44). Using *Drosophila*, we validate the NS4B-UBA5 interaction. We show that expression of human UBA5 severely exacerbates NS4B-induced phenotypes in neuronal stem cells (NSCs). Interestingly, reduction of Uba5 using RNAi rescues NS4B phenotypes. Future work involves determining the mechanism behind this interaction and its significance during neurodevelopment. Findings from this study will contribute to drug development to combat both ZIKV infections and DEE, since there are no effective treatments against either.

428F Neprilysin-like 15 Modulates Sex-Specific Metabolic Homeostasis Through Reduced Insulin Signaling and Dietary Restriction-Like Adaptations in *Drosophila melanogaster*

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Metabolic flexibility is crucial for balancing energy utilization, stress resistance, and longevity. Here, we identify Neprilysin-like 15 (*Nepl15*), a catalytically inactive member of the neprilysin family, as a critical regulator of sex-specific metabolism in *Drosophila*. Loss of *Nepl15* rewires multiple physiological networks without affecting fertility or fecundity. Male mutants exhibit reduced lipid and glycogen storage, enhanced mitochondrial membrane, stable ATP levels, and condensed, cristae-rich mitochondrial morphology in thoracic flight muscle phenocopying dietary restriction. In contrast, female mutants display increased glycogen accumulation with modest lipid reduction, reflecting distinct metabolic reprogramming. Optical Coherence Microscopy of the adult hearts revealed a stronger heart performance in the mutants in old age. Functionally, *Nepl15* mutants maintain normal climbing ability, and survival under exercise, suggesting preserved energy efficiency.

Metabolomic and lipidomic analyses reveal broad shifts in carbohydrate, amino acid, and purine pathways. Female mutants showed significant decrease in oxidative stress, consistent with upregulated *Sod2* and *Sirt6* expression.

At the signaling level, male mutants display reduced insulin pathway activity (decreased pAkt, increased Foxo nuclear activity, and suppressed mTOR expression), indicating a systemic metabolic slowdown reminiscent of dietary restriction-induced longevity pathways.

Collectively, these findings position *Nepl15* as a molecular integrator of mitochondrial integrity, redox balance, and insulin signaling, governing sex-dependent metabolic resilience and lifespan modulation in *Drosophila*.

429F FlyCAR: A CAR-macrophage model in Drosophila melanogaster

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Chimeric Antigen Receptors (CARs) are repurposed membrane receptors that redirect cellular responses toward a novel ligand by replacing the extracellular domain of a receptor with single-chain antibody derivatives. Engineered CAR-T cells have achieved great success against blood cancers; however, their use against solid tumors is challenging, partly due to their limited infiltration into the tumor microenvironment. As an alternative, phagocytic CAR-Macrophages as a treatment against solid tumors have gained attention in recent years because macrophages can infiltrate solid tissues better than T cells. Here, we proposed to use Drosophila as a model organism to study such interactions. Using regular and imaging flow cytometry and time-lapse confocal imaging, we observed that Drosophila S2 cells transfected with FlyCAR displayed elevated phagocytosis frequency towards target cells expressing membrane-tethered GFP, and we did not observe significant off-target phagocytosis against S2 cells expressing intracellular GFP. This highlights the evolutionary conservation between Draper and Megf10 and opens up the possibility of studying CAR-Macrophages in Drosophila in vivo. We are currently conducting experiments to assess FlyCAR phagocytic efficiency using the Drosophila efferocytosis reporter pHlorina. New transgenic fly lines are also being constructed to test whether expressing FlyCAR in Drosophila hemocytes can rescue tumor-related host mortality in vivo.

430F Investigating sleep and circadian rhythm disruption and resulting metabolic impacts in flies modeling C9ORF72-FTD

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Circadian rhythms are a defining feature across virtually all life forms. Disturbances in rhythmicity correlate with disease risk and severity in many neurodegenerative diseases. Sleep reports and actigraphy studies of patients with Frontotemporal Degeneration (FTD) have revealed reduced morning activity and total sleep, increased nighttime activity, and sleep fragmentation. However, the molecular nature of this behavior is not fully understood, nor is the effect of this arrhythmicity on other physiological processes, such as metabolism, which is known to be regulated by the core circadian clock genes. Exploring the interconnection between CR and metabolism in FTD is intriguing, given the known disruptions in glucose metabolism during clinical and preclinical disease stages, as well as altered lipid catabolism during disease progression. Studies in Drosophila models of C9orf72-FTD have revealed the role of repeat-associated non-ATG (RAN) translation and dipeptide repeat proteins (DPRs) in the causation and progression of disease. This model would be particularly ideal for studying the connection between disrupted CR and metabolic dysregulation, given patient findings that C9orf72-FTD is associated with disruptive sleep events more so than other genes implicated in FTD, and metabolic dysregulation has been implicated in disease progression in animal models and human patients with C9orf72 repeat expansions. Our preliminary data suggest that CR disruptions and altered metabolism are also evident in the C9orf72-FTD Drosophila model. Investigating the molecular nature of these phenotypes will provide additional insight into how each of these processes is affected individually and synergistically. Understanding these connections may provide fruitful biomarkers of early diagnosis before other behavioral and physiological symptoms emerge, as well as targets to improve patient quality of life.

431F Functional suppression of amyotrophic lateral sclerosis/frontotemporal dementia (ALS/FTD) associated neurodegeneration by a reduction in stress granule-associated genes encoding RNA binding proteins

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Stress granules (SG) are dynamic membraneless, ribonucleoprotein structures that assemble as a protective response to stress in the cell. In the presence of chronic stress, SGs fail to dissolve, leading to persistent aggregation of proteins and stalled mRNA translation. While SG dysfunction is a hallmark of many neurodegenerative diseases, including amyotrophic lateral sclerosis/frontotemporal dementia (ALS/FTD), it is not clear whether SG dysfunction is causative or a response to cellular changes associated with neurodegeneration.

Many cases of ALS/FTD are associated with mutations in *TARDBP* (TDP-43) and the expansion of a hexanucleotide repeat in *C9orf72*. Using a motor neuron-driven *dTARDBP* patient allele (*TBPH[N493D]*) and two Drosophila models of *C9orf72-(G4C2)* expansion, we tested if a knock down of SG-associated genes using RNAi could alter neurodegeneration phenotypes displayed by these disease models, including synaptic defects at the NMJ, reduced motor function, and shortened lifespan. Four SG-associated genes (*Heterogeneous nuclear ribonucleoprotein at 27C (Hrb27C/dDAZAP), Tudor staphylococcal nuclease (Tudor-SN/dSND), maleless (mle/dDHX9),* and *ovarian tumor (otu/dOTUD4)*) were selected from a screen that identified ~150 novel human SG components (Markmiller et al., 2018) based on the ability of RNAi knockdown to reduce the rough eye phenotype indicative of neurodegeneration, associated with GMR>hTDP-43[M337V] and GMR>hFUS[R521C]. To better understand the functional consequences of altering the level of these SG genes in actually alleviating neurotoxicity within the motor circuit, we assessed the integrity of the NMJ, motor function, viability, and lifespan in different models of ALS/FTD. We found the ability to suppress neurotoxicity associated with *C9orf72-(G4C2)* ALS and *TBPH[N493D]* ALS varied between SG genes, as well as between disease phenotypes assayed. These results highlight the dynamic role of SG-associated RBPs in ALS pathology.

432F Effects of disease-associated, activating mutations in Rac on cell migration and cannibalism in Drosophila border cells

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Ras-related C3 botulinum toxin substrate 2 (Rac2) is a small signaling GTPase crucial for proper immune system development and function. It cycles between GTP-bound (active) and GDP-bound (inactive) states, regulated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). Human patients with dominant activating mutations in Rac2 (Rac2+/G12R, Rac2+/G15D, Rac2+/P34H, Rac2+/E62K, and Rac2+/N92T) exhibit a spectrum of immunodeficiencies from T- and B-cell lymphopenia to severe bone marrow hypoplasia. However, the mechanisms by which activated Rac2 causes immunodeficiencies remain unknown. Rac proteins are highly conserved in sequence and function from flies to humans, and key insights into possible mechanisms have recently come from studies of border cells in the Drosophila melanogaster egg chamber. Our lab found that border cells expressing constitutively active RacG12V engulf and kill the neighboring nurse cells, resulting in egg chamber destruction. Thus, hyperactive Rac is sufficient to cause one cell to eat and kill another living cell in vivo. Intriguingly, our lab further found that hyperactivating Rac2 mutations also cause mouse and human macrophages to engulf and kill T cells, offering a potential explanation for the disappearance of circulating T cells observed in Rac2+/E62K patients and mice. Building on this work, here we propose to use Drosophila as a model to overexpress a variety of dominant activating Rac2 mutations found in human patients. Expressing dominant activating mutations in vivo will be crucial for elucidating the cell-autonomous and cell non-autonomous effects of hyperactive Rac on motility, phagocytosis, and tissue destruction. We further propose to test the effects of Rac mutations on Drosophila hematopoiesis, which takes place in the lymph gland and has recently emerged as a powerful model for blood cell development and function. Leveraging the egg chamber and lymph gland models will improve our understanding of the effects of Rac mutations on cell behaviors in vivo and contribute to elucidating the pathogenesis of human hematopoietic disorders.

433F Understanding the role of *Drosophila* trachea (Vasculature) in brain tumorigenesis

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Background: The cognitive control centre of the body, the central nervous system (CNS), is generated from neural stem cells (NSC) during development to give rise to neurons and glial cells. Brain and CNS tumours such as medulloblastoma and high-grade glioblastoma are among the most malignant tumours in children and adults, respectively. Tumour progression can be influenced by environmental factors, which include oxygen availability. In *Drosophila*, oxygen transport is carried out by the trachea, which has also been shown to interact with other brain cell types, playing a part during neurogenesis. However, its role in brain tumorigenesis is poorly understood.

Aims and method: to investigate how oxygenation influences dedifferentiation induced tumours, we used a brain tumour model (knockdown of a transcription factor called Prospero which promotes differentiation) in the CNS of *Drosophila* and assessed (i) whether the oxygenation tubules named trachea is important for tumour growth; (ii) what type of cells in the CNS produce the ligand to promote trachea branching (iii) the cell type in the CNS that is sensitive to hypoxia

Results: Our result shows that i) Manipulating FGF receptor gene *breathless* (*btl*) required for trachea branching, reduces brain tumour size. ii) The FGF ligand, *branchless* (*bnl*) is expressed in the glia, and glial-specific knockdown of *bnl* reduced tumour size, as did hypoxia (2.5% oxygen). iii) The glial cells surrounding the tumour are the cell types that can respond to hypoxia, and Sima, the HIF1a homolog in *Drosophila* affects tumour growth

Conclusions: Our data show the importance of the trachea within the brain tumour microenvironment in tumour growth. We are now trying to understand how regulators downstream of hypoxia in the glial cells regulate tumour growth using targeted DamID. This investigation will shed light on how the brain microenvironment influences tumour growth and enables us to specifically target the niche during cancer treatment.

434F The TMEM161B ortholog, Emei, is an essential protein localised to the endoplasmic reticulum and is a member of a conserved protein superfamily

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TMEM161B is a multi-transmembrane spanning protein that we have shown is required for cardiac rhythm, cardiac morphogenesis, embryo growth and survival in both zebrafish and mouse. Mutations in this gene have also been identified in patients with congenital brain defects. Using trRosetta and tFold, we have generated 3-dimensional (3D) models of TMEM161B structure. We find transmembrane domain 4 (TM4) diagonally crosses TMs 6-8, creating a unique protein fold or “pocket” not previously been described in PDB by DALI analysis. The protein is predicted to embed in the membrane, with the pocket sitting immediately on top of the plasma membrane, suggestive of a docking site. We identified three other families that meet this criterion, indicating that TMEM161B is a member of a new, uncharacterised superfamily. We have analysed the subcellular localization of the Drosophila TMEM161B orthologue, Emei, via generation of a transgenic line using the tagged FlyFos TransgeneOme Library. This line incorporates a C-terminally GFP-tagged Emei within a fosmid transgene. Emei co-localises with endoplasmic reticulum markers. We have also used CRISPR-Cas9 to generate emei null alleles. These mutants arrest development at the early pupal stage and exhibit dysmorphic imaginal discs with elevated levels of apoptosis. The Emei-GFP fosmid can rescue the pupal lethality indicating that it is a useful tool for investigation of Emei function. Future studies will focus on investigation of the role of emei in imaginal disc development and shared functions amongst the superfamily members.

435F A novel role for the E2F transcription factor and the ER stress sensor IRE1 in cytoplasmic DNA accumulation

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The E2F family of transcription factors are key regulators of the cell cycle in all metazoans. While they are primarily known for their role in cell cycle progression, E2Fs also play broader roles in cellular physiology, including the maintenance of exocrine tissue homeostasis. However, the underlying mechanisms that render exocrine cells particularly sensitive to E2F deregulation remain poorly understood. The Drosophila larval salivary gland (SG), like its mammalian counterpart, is an exocrine tissue that produces large quantities of “glue proteins” in the endoplasmic reticulum (ER). Here, we show that E2F activity is important for the exocrine function of the Drosophila SG. The loss of *de2f1b*, an alternatively spliced isoform of Drosophila *E2F1*, leads to elevated DNA damage and accumulation of cytoplasmic DNA (cytoDNA) in the SGs. Surprisingly, we found that IRE1, a key sensor of the unfolded protein response, is required for ER homeostasis during development that is critical for preventing cytoDNA accumulation in the SG. Importantly, we found evidence demonstrating that IRE1 activity is attenuated in *de2f1b*-deficient SGs, contributing to ER dysfunction and cytoDNA accumulation. Together, these findings reveal an unanticipated link between ER homeostasis and cytoDNA processing and offer mechanistic insights into why exocrine tissues are particularly vulnerable to E2F deregulation.

436F Optimized Anti-Epileptic Screening for Pharmacoresistant Epilepsy Using Drosophila

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Epilepsy affects millions in the U.S. alone, often causing severe lifelong disability or death. While a range of anti-epileptic drugs (AEDs) can suppress certain seizures, about one-third of patients remain treatment-resistant or require frequent medication changes to control their condition (pharmacoresistant epilepsy). Screening for new anti-seizure medications is challenging and time-consuming, particularly with *in vivo* models of human epilepsy. Seizures often stem from excitatory/inhibitory imbalance, traditionally linked to neurons, but growing evidence shows glial dysfunction plays a key role. Our lab developed a glial specific *Drosophila melanogaster* model of pharmacoresistant Duplication 15q syndrome (Dup15q). Standard-of-care broad-spectrum AEDs often fail to control seizures in Dup15q patients over time, necessitating the identification of alternative therapeutic compounds. Previously, we identified multiple FDA-approved compounds with seizure suppressing effects, but the assay relied on the bang sensitivity assays (BSA) with an observer-based scoring, making it slow and subjective. To improve the quality and speed of screening new compounds, we have developed a streamlined, automated screening system by incorporating multi-fly video tracking (Iowa FLITracker) paired with machine learning (Hidden Markov Classifier) and tethered fly electrophysiology to quantify unbiased seizure-associated behavior. Currently, we are validating our screening tool by testing 10 drugs known to suppress repo>Dube3a induced seizures to confirm the validity of the new assay (**R61 phase**). Thus far, we have seen a significant reduction in seizure-like behavior with minoxidil, mirtazapine, and vortioxetine. In the R33 phase we will test ~3500 FDA-approved and natural product compounds to identify potential drugs for repurposing in individuals with Dup15q syndrome (**R33 phase**). As these compounds already have known safety profiles, positive hits may be fast-tracked for off-label use in humans without further testing. This project will not only identify new drugs that can be repurposed to treat pharmacoresistant epilepsy in Dup15q syndrome but will also establish powerful *in vivo* screening tools for anti-seizure drugs, applicable to existing or new Drosophila models of human epilepsy.

437F Elucidating multiple hereditary exostoses (MHE) pathogenic mechanisms using Drosophila model

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Multiple hereditary exostoses (MHE), also known as hereditary multiple osteochondromas (HMO), is a developmental bone disorder caused by mutations in the heparan sulfate (HS) polymerase genes *Ext1* or *Ext2*. On the cell surface, HS chains attach to core proteins to form HS-proteoglycans (HSPGs), which serve a key role in developing tissues by establishing normal morphogen gradients, including those formed by Bone Morphogenic Protein (BMP) ligands. Exostoses consist of a genetic mosaic of *Ext1* or *Ext2* heterozygous (+/-) and homozygous mutant (-/-) cells, implicating somatic loss-of-heterozygosity (LOH) mutations in *Ext1*- or *Ext2*-heterozygous individuals. However, since exostoses do not arise from the clonal expansion of mutant cells, and because exostoses follow a normal bone developmental program, it has been suggested that exostoses do not represent true neoplasms. Therefore, LOH and the classical “two-hit” model of tumor development fail to completely explain the molecular mechanisms underlying the ectopic bone growth observed in MHE. To model this disease, we created a conditional knockout model of *Ext1* ortholog *tout velu* (*ttv*) in *Drosophila* that generates *ttv*-genetic mosaics in developing wing tissue. This system provides a clear readout of ectopic BMP signaling in the form of ectopic wing veins. Our results show that somatic clones heterozygous for a mutation in *ttv*, representing a “one-hit” event, induce mild ectopic wing vein phenotypes. This result suggests that biallelic inactivation of *Ext1*/*ttv* is not required to produce disease phenotypes, and that *Ext1*/*ttv* genetic mosaicism in the developing tissue is a critical component of MHE pathogenesis. Because of HSPGs’ known role in shaping morphogen gradients, we propose that ectopic BMP signaling, caused by disruptions to the morphogen gradient at the interfaces between *ttv*-mosaic cells, is the primary driver of exostoses formation in MHE.

438F Loss of calcium-binding protein *Cbp53E* leads to delayed repolarization of photoreceptor cells in Drosophila

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Calcium functions as an important second messenger in a wide variety of intracellular processes. In photoreceptor cells, calcium is involved in activation, deactivation, and adaptation in response to light stimuli. Calcium-binding protein 53E (Cbp53E, also known as calbindin-32 or cbn), a protein with 6 EF-Hand domains thought to act as a calcium buffer, was previously identified to have elevated expression levels in the eye of drosophila. While a recent study showed that transgenic flies lacking Cbp53E have aberrant axonal arborization at the neuromuscular junction, nothing is known about the role of Cbp53E in the visual system. We performed electroretinogram (ERG) recordings on Cbp53E mutant flies to test whether eye function was affected. Here, we report that Cbp53E null mutants exhibit a prolonged repolarization (or slow termination) phenotype which can be rescued by expressing Cbp53E in photoreceptor cells. The human homologs Calbindin 2 and Calbindin 1 also rescue the drosophila ERG phenotype. This supports a role for Cbp53E in regulating intracellular calcium levels of photoreceptor cells and contributing to normal sensory neuron response dynamics *in vivo* in drosophila and suggests a similar function in human photoreceptor cells.

439F A role for cellular senescence in a *Drosophila* TDP-43 model of Amyotrophic Lateral Sclerosis and Frontotemporal Dementia

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Cellular senescence is a state of persistent cell cycle arrest, typically entered to prevent uncontrolled cell growth. Senescence plays important roles in many biological processes, including wound healing and inhibition of cancer progression. More recently, the accumulation of glia and neurons with senescent markers has been described in both aging and neurodegenerative disease (ND). Because of the known secretory phenotype of senescent cells that drives inflammation, their presence could contribute to the cell non-autonomous toxicity that is observed in such diseases. In many NDs, neurons and glia that experience dysfunctional states impact the physiology of nearby healthy cells. Although not yet attributed to senescence, such cell non-autonomous effects have been observed in models of Amyotrophic Lateral Sclerosis (ALS) and frontotemporal dementia (FTD), disorders that involve aggregation pathology of the TAR DNA binding protein (TDP-43).

To examine the possibility that cellular senescence underlies or contributes to such cell non-autonomous effects, we have used an established *Drosophila* TDP-43 model. In this model, focal overexpression (OE) of human TDP-43 in a subset of glia triggers both nuclear loss and cytoplasmic aggregation of TDP-43 in a cell autonomous manner. It also drives cell non-autonomous effects in nearby neurons, including aggregation of TDP-43, accumulation of DNA damage, and ultimately, cell death. We are testing the idea that senescence contributes to the cell non-autonomous toxicity between glia with TDP-43 pathology and nearby neurons. Using an established reporter of senescence, upregulated AP-1 activity, our data supports the notion that TDP-43 pathology triggers senescence, predominantly in glia. Our findings also support the idea that different glial cell types have different vulnerabilities to becoming senescent, and potentially, different capacities for contributing to cell non-autonomous effects. With single nucleus RNA sequencing (snRNA-seq), we hope to identify cell-type-specific transcriptomic changes associated with senescence. Cell-type-specific genetic manipulations will also be used to dissect mechanisms by which senescence contributes to neurodegeneration in this model system.

440F Whole-brain single-cell transcriptomic profiling in CHCHD10-related ALS-FTD *Drosophila* models

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Mitochondrial dysfunction is emerging as a central driver of neurodegeneration in amyotrophic lateral sclerosis and frontotemporal dementia (ALS-FTD). The brain constitutes a dynamic microenvironment where diverse cell types and signaling pathways continuously interact; thus, a systems-level perspective is essential to understand disease progression. To delineate mutant CHCHD10-induced ALS-FTD at single-cell resolution, we established a CRISPR/Cas9-mediated knock-in Drosophila model carrying the CHCHD10^{S81L} mutation, orthologous to the human ALS-FTD–associated variant, together with isogenic wild-type and knockout controls. Phenotypes of KI and KO.

Single-cell RNA sequencing of adult brains yielded over 15,000 high-quality cells classified into 76 distinct neuronal and glial clusters, revealing widespread and cell-type–specific transcriptomic perturbations across genotypes. Significantly, cell-type specific vulnerability, differential mitochondrial stress responses, differential senescence and aging signatures were detected between KO and KI models. These findings demonstrate that mitochondrial dysfunction caused by mutant CHCHD10 reshapes the cellular landscape of the brain and elicits diverse molecular responses in the brain. Further analyses will define cell-type–specific alterations and reveal how CHCHD10 loss or mutation reshapes intercellular networks within the neural microenvironment, providing a foundation for development of targeted therapies for ALS-FTD

441F Defining the Role of Serrate in Indirect Flight Muscle Regeneration in *Drosophila melanogaster*

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Mutations in the human JAG2 gene, which cause a novel form of muscular dystrophy, can lead to deficiencies in the Jagged 2 ligand responsible for facilitating the renewal and differentiation of muscle stem cells into muscle fibers. In *Drosophila melanogaster,* the Serrate gene is an ortholog of the human JAG2 gene and shares many functional similarities, including its role in the Notch Signaling Pathway. In our previous work, we successfully modeled human muscular dystrophy induced by the mutant JAG2 gene in *Drosophila* by inducing Serrate deficiency using RNA interference with the UAS/Gal4 system. This identified the connection between JAG2 deficiency and impairment of the Notch Signaling Pathway, highlighting the improvement of this pathway as a potential therapeutic strategy. However, how JAG2 controls the muscle stem cell fate, including muscle fiber regeneration, and how its deficiency is related to cellular stress responses induced during disease pathogenesis remain poorly understood. In the present study, we investigate how Serrate deficiency affects the fate of adult muscle stem cells and the regeneration of muscle fibers in *Drosophila melanogaster*. We hypothesize that a deficiency of the Serrate gene results in flies with a lower regenerative capacity than their wild-type counterparts. Using the serrate-deficient model flies that we established, we will present how serrate deficiency affects to indirect flight muscle regeneration and adult muscle stem cells after mechanical injury. This study will evaluate the regenerative capacity of adult indirect flight muscles in Drosophila in the serrate-deficient condition in vivo. The results of this project will be shared at the Annual Drosophila Conference and in subsequent lab meetings.

442F Investigating developmental lethality caused by pan-neuronal expression of human amyloid beta 42 in Drosophila melanogaster

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia worldwide. One of its pathological characteristics is the accumulation of extracellular amyloid plaques within the brain. These plaques are primarily composed of the peptide amyloid beta (Aβ), which are generated through sequential proteolytic cleavage of the amyloid precursor protein (APP) by β- and γ-secretases. Among the various forms of Aβ, the 42-amino acid species (Aβ-42) is believed to play the most significant role in AD pathology. Aβ-42 is more prone to aggregation than the shorter Aβ40 isoform, leading to enhanced oligomerization. Familial AD mutations often increase the relative production of Aβ-42 or alter the Aβ-42/Aβ-40 ratio, thereby promoting amyloid deposition and accelerating disease onset. Experimental evidence indicates that even subtle changes in this ratio can have profound effects on neuronal viability and synaptic function (Suzuki et al., 1994; Kuperstein et al., 2010).

We previously generated transgenic fly lines carrying Aβ-42 under GAL4-UAS control. We observed that, Drosophila melanogaster lines with two copies of UAS-Aβ-42 expressed pan-neuronally under the Appl-Gal4 driver, do not survive to adulthood; flies carrying only a single copy, however, do survive to adulthood. This indicates that elevated levels of Aβ-42 can induce developmental lethality. To investigate this phenomenon further, and to identify juveniles carrying two copies of Aβ-42, we generated flies carrying Aβ-42 with a Green Fluorescent Protein (GFP) marker and Aβ-42 with an mCherry marker, to determine the developmental stage at which homozygous expression of Aβ-42 becomes lethal. The validity of the GFP/mCherry construct is analyzed by comparison with a control cross lacking the GFP and mCherry transgenes. As D. melanogaster is a widely used model organism for AD, we hope that identifying the specific stage at which lethality occurs will facilitate the identification of the mechanisms by which Aβ-42 induces developmental lethality. This may provide insight into the mechanisms contributing to Aβ-42 toxicity.

443F Establishing an Eye Model for Studying Human Urate Homeostasis using White-eyed GMR-GAL4

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The human gene ABCG2 (Gene ID: 9429) has been implicated in maintaining urate homeostasis and in disorders such as gout. ABCG2 is a predicted ortholog of the Drosophila white gene (-w / 31271-). The goal of this project is to validate the orthology between these genes and consequently develop the possibility for studying ABCG2-associated diseases in Drosophila eyes. To test this possibility, we generated white-eyed GMR-GAL4 lines by targeting the mini-white marker with the CRISPR/Cas9 technology, similar to how DarkJedi GAL4 lines were established (Park et al. 2024). These lines were developed from home over the summer as an undergraduate research opportunity. To express human ABCG2 in fly eyes, UAS transgenic lines were made with pUASTattb cloning with the human gene ABCG2. Preliminary results showed that human ABCG2 expression in the eye, using the traditional GMR-GAL4 driver, made fly eyes darker than the corresponding controls. Specifically, it indicated a correlation through a stronger orange color in the eyes of ABCG2 compared to a control. We will present the effects of human ABCG2 gene expression in Drosophila eyes using the white-eyed GMR-GAL4 line and also discuss how this home-based project could create a potential human disease model to control urate homeostasis and related diseases.

444F DIP2A regulates neural DAG homeostasis: functional analysis of disease-associated variants in Drosophila

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Diacylglycerol (DAG) is a central lipid signaling intermediate that regulates diverse cellular processes including protein kinase C activation, membrane curvature, vesicle trafficking, and synaptic function. Dysregulated DAG levels have been implicated in multiple neurodevelopmental and neurodegenerative disorders, yet the molecular mechanisms linking DAG dysregulation to neural dysfunction remain poorly understood.

Disco-interacting protein 2 (DIP2) is an evolutionarily conserved protein from yeast to humans. Yeast studies suggest that its fatty acid/acyl-CoA ligase-like domains (FLDs) participate in fatty-acyl activation. We previously demonstrated in Drosophila that DIP2 is required for proper axonal bifurcation and guidance of mushroom body (MB) axon in an FLD-dependent manner. Consistent with a role in lipid regulation, lipidomic profiling has revealed that DIP2 mutants selectively accumulate specific DAG subspecies, suggesting that DIP2 regulates DAG homeostasis in neural tissues.

Recently, we identified several de novo variants in the human homolog DIP2A. The individuals present with developmental delay, seizures, and hypotonia. Notably, most of the variants cluster within the functionally critical FLDs, suggesting that they may disrupt the DAG/TAG balance and contribute to disease pathogenesis.

To investigate the pathogenicity of these human variants, we developed Drosophila models to test the functional conservation of human DIP2A. A DIP2 T2A knock-in (CRIMIC) revealed robust expression of DIP2 in neurons as well as subsets of glia, with particularly high expression levels in the MBs. Behavioral assays reveal that DIP2 mutants display defects in climbing and heat-induced stress responses (seizures), indicating impaired motor function and reduced neural stress resilience. These features parallel those observed in individuals carrying DIP2A variants.

We are currently evaluating the functional conservation of human DIP2A through rescue experiments testing whether human UAS-DIP2A constructs and patient-derived variants can restore function in DIP2 mutant flies. These experiments will help elucidate disease-relevant molecular mechanisms and determine whether the human variants represent loss-of-function or not. Parallel lipidomic profiling of rescued lines will assess how each human allele restores, or fails to restore, DAG subspecies balance.

445F *ABCA1* and *ABCA7* regulate glial lipid droplet formation and neuroprotection in Alzheimer's disease models

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, memory impairment, and behavioral changes. It is the leading cause of dementia worldwide. The pathophysiology of AD is marked by the accumulation of β-amyloid plaques and neurofibrillary tangles composed of hyperphosphorylated tau protein, leading to synaptic dysfunction, neuronal loss, and cerebral atrophy. Oxidative stress due to mitochondrial dysfunction further exacerbate neuronal damage and accelerate disease progression. Alzheimer's disease is increasingly recognized as a genetically complex disorder, influenced by a growing array of risk factors. Recent large-scale genome-wide association studies (GWAS) and meta-analyses have identified approximately 90 independent risk variants across 75 genomic loci associated with AD and related dementias, with more than 40 of these loci discovered within the past few years.

The ATP-binding cassette (ABC) transporters *ABCA1* and *ABCA7* are critical regulators of lipid efflux and homeostasis in neuronal and glial cells. These transporters facilitate the movement of phospholipids and cholesterol to apolipoproteins, thereby supporting membrane stability, lipid droplet dynamics, and cellular protection against oxidative damage. Our previous research has identified more than ten AD risk genes, including *ABCA1* and *ABCA7*, that are essential for the generation of lipid droplets in glial cells. We hypothesize that impaired lipid droplet formation in glia, particularly due to *ABCA1/7* dysfunction, may play a role in driving neurodegeneration observed in AD. We generated humanized *Drosophila* models in which we replaced fly orthologs of *ABCA1/7*, *Eato* and *ldd* respectively, with human *ABCA1* and *ABCA7* to investigate the functional impact of disease-associated genetic variants on glial lipid droplet formation and neurodegeneration. Our results indicate that both *ABCA1* and *ABCA7* are required in neurons for the formation of glial lipid droplets, and that loss-of-function variants in *ABCA1/7* significantly impair lipid droplet formation and cause neurodegeneration. This work advances our understanding of how conserved lipid transport pathways intersect with disease-associated genetic variation to influence neuron-glia interactions and maintain brain health. Future studies will identify the lipids transported by these proteins and compounds of therapeutic potential for carriers of *ABCA1* and *ABCA7* variants.

446F Repurposing FDA-Approved PDE4 Inhibitors to Rescue Mitochondrial Dysfunction in CHCHD10^{S59L}-associated ALS-FTD

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Mutations in *Coiled-coil-helix-coiled-coil-helix domain containing 10* (*CHCHD10*) have been linked to amyotrophic lateral sclerosis and frontotemporal dementia (ALS-FTD). Previous research from our lab, established that the pathogenic CHCHD10^{S59L} mutant disrupts mitochondrial dynamics, leading to mitochondrial fragmentation and respiratory dysfunction in both *Drosophila* and human cell models. These phenotypes were strongly modified by the PINK1/Parkin mitophagy pathway, suggesting that excessive mitophagy contributes to pathology. Here, we investigated whether FDA-approved PDE4 inhibitors could mitigate this mitochondrial toxicity via modulating the PINK1/Parkin pathway in cAMP-PKA dependent manner. We first validated that by activation of adenylate cyclase by forskolin prevented the accumulation of PINK1 and Parkin in CCCP-induced mitochondrial depolarization in HeLa cells. Forskolin treatment similarly rescued mitochondrial fragmentation caused by CHCHD10^{S59L}. Augmenting cAMP levels by inhibiting its degradation with FDA-approved phosphodiesterase-4 (PDE4) inhibitors produced comparable effects. They significantly reduced mitochondrial fragmentation, PINK1/Parkin accumulation, LC3 aggregation. Especially, mito-QC assays further confirmed decreased mitophagy flux in CHCHD10^{S59L}-expressing cells by PDE4 inhibitor treatment. Seahorse analyses revealed that forskolin and PDE4 inhibitors enhanced basal and maximal respiration and ATP production, indicating functional restoration of mitochondrial bioenergetics. In *Drosophila* expressing the homologous mutant C2C10H^{S81L}, treatment with forskolin or roflumilast ameliorated muscle degeneration, restored mitochondrial morphology, and improved ATP levels in indirect flight muscles. Notably, combined treatment with forskolin and PDE4 inhibitors, particularly apremilast produced additive or synergistic protective effects against both CCCP and CHCHD10^{S59L}-induced mitochondrial damage, even at submaximal doses.

Together, these findings demonstrate that elevating intracellular cAMP through coordinated stimulation of its synthesis and inhibition of its degradation effectively mitigates CHCHD10^{S59L}-induced mitochondrial dysfunction. Furthermore, FDA-approved PDE4 inhibitors, potentially combined with forskolin, could be repurposed for CHCHD10^{S59L}-linked ALS-FTD and related disorders, though modulation of the protective PINK1/Parkin pathway should be carefully considered.

447F Glypican-4 affects lifespan and axon sprouting in Alzheimer's disease models

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Alzheimer's Disease (AD) is a neurodegenerative disease that occurs in the aging population, yet one class of molecules that becomes altered in this disease are proteins most prominently known for their roles in developmental axon guidance and synaptogenesis such as Slit proteins and the heparan sulfate proteoglycan (HSPG) Glypican-4 (GPC4). Even Amyloid Precursor Protein (APP), the protein from which the hallmark amyloid fragment is derived, plays a role in axon guidance in early development and has even been shown to bind directly to Slit proteins. Moreover, GPC4, Slit2, and Slit3 all localize to amyloid plaques in AD patient tissue, suggesting a possibles roles in contributing to the growth of dystrophic neurites around these plaques. Here, we specifically focus on GPC4, which is often thought of for its role as an astrocyte-derived factor in synaptogenesis in early development. Interestingly, GPC4 expression is specifically increased in human microglia in AD and strongly correlates with plaques containing phosphorylated-Tau (pTau)-positive dystrophic neurites. These neuritic plaques are more highly associated with cognitive decline and circuit disruption than diffuse or dense core plaques. However, how glial GPC4 regulates neurodegeneration and AD pathology is poorly understood. The extensive genetic tools available in *Drosophila* allow us to easily manipulate GPC4 and related genes in glia, and observe the effects of altered expression in an AD context. In flies pan-neuronally expressing the first 42 amino acids of human Aβ (hAβ42), we found that knocking down the fly ortholog of GPC4 (dally-like protein, dlp) in glia extends lifespan, while overexpressing it leads to earlier death. Additionally, in animals expressing mutant human tau (hTauP301L) in photoreceptor neurons, these axons display hAβ42-dependent ectopic sprouting in an aging-dependent manner. Glial expression of human GPC4 (hGPC4) induces the same sprouting effect in aged animals; however, this phenotype is significantly exaggerated when Tau+ neurons are exposed to both neuronal hAβ42 and glial hGPC4, suggesting that GPC4 and Aβ42 act synergistically to regulate axon sprouting in aged animals. While axonal sprouting was reported in AD patient tissue over twenty years ago, the mechanisms regulating it and the consequences of this phenomenon are still not well understood. Our results integrate patient data with an *in vivo* animal model, and begin to shed light on the effects of glial GPC4 in AD where we found that it exacerbates phenotypes ranging from neuronal morphology to altered lifespan. Our work aims to elucidate the specific mechanisms of GPC4, its binding partners, and consequences of its functions in AD and neurodegeneration.

448F DNA replication in fat body links the NF-κB *Relish* to sickness sleep

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Sickness sleep occurs in *Drosophila* as a transient response to stress, including aseptic injury and infection. The NFκB Relish is required for injury and infection-induced sleep through its expression in fat body. Partial loss of *Relish* from fat body also reduces daily sleep. However, the molecular mechanisms by which Relish regulates both daily and sickness-induced sleep from fat body remain unclear. To address this question, we conducted RNA sequencing of fat bodies dissected from female flies subjected to aseptic injury, bacterial infection with *E. coli*, and untreated controls in wild type and *Relish*^{E20} null mutants. Comparisons were also made between wild type, *Relish*^{E20} null and heterozygous mutants in undisturbed conditions. We performed differential expression analysis between treatment groups and compared the results across genotypes. We hypothesized that a cross-section of differentially expressed genes between wild type, *Relish*^{E20} null and heterozygous mutants from both experiments would be relevant to its effects on sleep. We screened selected gene candidates for alterations in daily or sickness-induced sleep and found that genes involved in cell cycling significantly disrupted both daily and sickness-induced sleep when knocked down specifically in fat body. Using 5-ethynyl-2›-deoxyuridine (EdU) treatment, we found that infection induces DNA replication in fat cells distal to the injection injury site. Together, these findings suggest a role of *Relish* in DNA replication, maintenance, and repair in its regulation of daily and sickness sleep.

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449F The Protective Role of lncRNA NEAT1_1 Against ALS-FTD-linked *CHCHD10*^{S59L} toxicity in Drosophila

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are characterized by an interplay of pathogenic mechanisms, like mitochondrial dysfunction and TDP-43 proteinopathy. Mitochondrial dysfunction caused by *CHCHD10*^{S59L} mutation initiates the subsequent pathology, TDP43 proteinopathy. Although NEAT1_1 upregulation has been reported to ameliorate TDP-43 toxicity, it has not been studied whether NEAT1_1 can modify the specific toxic cascade initiated by *CHCHD10*^{S59L}.

In this study, we hypothesize that the constitutive short isoform of Nuclear Paraspeckle Assembly Transcript, NEAT1_1, can suppress the toxicity of *CHCHD10*^{S59L} by modulating TDP-43. We generated a Drosophila disease model expressing the Drosophila ortholog of CHCHD10, C2C10H with a corresponding disease-causing mutation, *S81L* (*C2C10H*^{S81L}) using eye-specific GMR-GAL4. To test the genetic interaction between NEAT1_1 and *C2C10H*^{S81L}, we expressed them together in the eye and compared them with various critical control groups. Our data indicate that the lncRNA NEAT1_1 expression ameliorates the *C2C10H*^{S81L}-mediated toxicity, and further mechanistic studies are required to understand the protective mechanism of NEAT1_1 on the mitochondrial toxicity of *CHCHD10*^{S59L}, potentially via TDP-43.

450F Impaired Glial Phagocytosis Leads to Neuroinflammation and Neurodegeneration in Aging Drosophila

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Neurodegenerative diseases are characterized by progressive and selective neuronal loss, and impaired glial function is an important contributor, yet the mechanisms underlying this process remain unclear. In Drosophila melanogaster, loss of phagocytic receptor Draper results in impaired clearance of apoptotic neurons, leading to age-dependent neurodegeneration. This study aims to uncover molecular mechanisms that drive neurodegeneration arising from impaired glial phagocytosis. To identify gene expression changes associated with neurodegeneration, single-nucleus RNA sequencing was performed on aged *draper*-deficient flies, revealing upregulated genes spanning immune, metabolic, and signaling pathways, and highlighting candidates not previously linked to neurodegeneration. To validate the effects of these genes on neurodegeneration, we performed RNAi knockdowns to determine if any of these genes can suppress neurodegeneration in *draper*-deficient flies. Through the screening, we found that knocking down *LPCAT*, *SPH93*, and *BomS3* significantly reduced neurodegenerative vacuoles, while knocking down *SPH93*, *pyr*, and *Tep4* improved survival outcomes in *draper*-deficient flies, suggesting that these genes may promote neurodegenerative processes when overactive. To further assess the sufficiency of these genes in driving neurodegeneration, we followed up by overexpressing them and scoring the resulting neurodegenerative phenotypes. In addition, behavioral assays, including climbing and olfactory assays, were performed to evaluate these genes' impact on neurodegeneration-associated behavioral performance, providing functional context for their roles in selective neuronal maintenance. Together, these findings identify genetic regulators of neurodegeneration and provide evidence for their roles in preserving neuronal health during aging.

451F Exploring dietary effects on renal stone formation in *Drosophila melanogaster*

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Kidney stones affect millions of people worldwide and can cause excruciating pain, obstruction, and infection. Most kidney stones are composed of calcium oxalate (CaOx). How the stones are formed is not fully understood due to the complexity of the human renal system, but dietary excess of oxalate, genetics, and environmental factors have been found to play a role. *Drosophila melanogaster* have a simplified renal system that consists of transparent Malpighian tubules, which filter and excrete, functioning similarly to the human nephron. Others have found that when flies are fed a diet supplemented with sodium oxalate, they readily form CaOx crystals in the Malpighian tubules. *Drosophila* are a compelling model in which to study renal stones due to their short life cycle, genetic tools, and conservation of disease-causing genes and key pathways in humans. We sought to explore dietary causes of CaOx stone formation in *Drosophila* renal tubules. We replicated the finding that a high-oxalate diet induces stone formation in *Drosophila* by imaging the Malpighian tubules of flies fed a sodium oxalate diet using polarized light microscopy. We tested feeding flies the sodium oxalate diet from one to four days and found that stones form after only one day of sodium oxalate dietary supplementation. Additionally, the number, perimeter, and area of stones are not statistically significantly different between 1, 2, 3, and 4 days of feeding flies the sodium oxalate diet. We also tried a different mounting technique to increase efficiency of our dissection and mounting protocol and found that the stone number and perimeter were not significantly affected by using this technique. Overall, we found that one day of sodium oxalate dietary supplementation is sufficient to induce CaOx stones in the Malpighian tubules, thus making the workflow more efficient for future experiments. Future work will test other dietary supplementation as well as study conserved transporters, which will help to gain a better understanding of stone formation and oxalate transport mechanisms to identify potential targets for prevention and treatment of renal stones.

452F *De novo* variants in *BRSK1* are associated with neurodevelopmental disorders

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The Texome Project supports the diagnosis of individuals who face financial or systemic barriers to genomic medicine by providing access to rare disease diagnostic procedures. Using the sequencing data from the Texome Project, we applied an AI model, AI-MARRVEL, to reanalyze unresolved cases and identified *BRSK1* as a gene that may be associated with a neurological disorder. We subsequently identified other individuals harboring rare heterozygous *BRSK1* variants through GeneMatcher. Affected individuals share developmental delay and variably present with epilepsy, anxiety, ADHD, and autism. All variants are predicted to be deleterious based on protein structural modeling and pathogenicity prediction tools. *BRSK1 (SAD-B)* encodes an AMPK-related serine/threonine kinase required for neuronal polarization and synaptic function. To determine the function of the variants, we utilized *Drosophila* to model the variants. We find that *Drosophila sff*, the orthologue of human *BRSK,* is expressed in neurons of the larval central nervous system (CNS) and adult brain. Loss of *sff* in flies causes a severe locomotor impairment, bang and heat sensitivity, and a short lifespan. We also observe a significant overgrowth of the third-instar neuromuscular junctions (NMJs) which is associated with elevated expression of Futsch, a microtubule associate protein. Expression of the human reference *BRSK1* cDNA rescues the behavioral as well as the Futsch phenotype. In contrast, three patient-derived alleles provide only a partial rescue and fail to normalize Futsch levels. We therefore propose that they are partial loss of function alleles. We also find that overexpression of reference *BRSK1* cDNA shortens lifespan, suggesting that both increased and reduced protein levels are critical for normal physiology. In summary, our data support a model that heterozygous loss of *BRSK1* lead to a neurodevelopmental syndrome with variable phenotypes that can include seizures, autism, microcephaly, intellectual disability and other neurological phenotypes. We are currently testing whether *sff* functions as an upstream regulator of *Futsch* and are investigating the underlying mechanism. Note that an established regulator of *Futsch*, *Fmr1*, is the most common genetic contributor to autism and other neurodevelopmental disorders.

453S Identification of an actin-mitochondria-glutamate pathway underlying epilepsy: digenic diagnosis of patients and treatment

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Epilepsy is one of the most prevalent neurological disorders with a complex etiology. Genetic studies have identified over 1,000 epilepsy-associated genes. Yet, more than 50% of individuals with a presumed genetic cause of epilepsy are still awaiting a genetic diagnosis, and anti-epileptic drugs are ineffective in more than 30% of cases. A major barrier to effective diagnosis and treatment is our limited understanding of the molecular mechanisms underlying epilepsy, particularly in complex cases involving two or more genes. While disruptions of genes that encode ion channels and synaptic proteins directly affect neuronal activity in a subset of epilepsy cases, there remain a large number of epilepsy-associated genes with no obvious connections to neuronal activity, including ~50 actin regulatory genes. Here, by studying the actin regulatory genes in the context of epilepsy, we identified an "actin-mitochondria-glutamate pathway". We demonstrate that reduced actin polymerization levels promote Dynamin-Related Protein 1 (DRP1)-mediated mitochondrial fission, which elevates reactive oxygen species (ROS) levels, leading to increased glutamatergic transmission and seizures. Reducing mitochondrial fission with the DRP1 inhibitor Mdivi-1, or suppressing ROS with N-acetyl-L-cysteine amide, significantly alleviates the seizure phenotypes in flies. Interestingly, we observed that trans-heterozygous loss-of-function (LoF) mutants for genes affecting this pathway exhibit obvious seizure-like phenotypes, suggesting that seizures may arise from a combination of two heterozygous genetic variants that affect this pathway. To apply our findings toward improving the diagnosis of digenic epilepsy, we assembled an epilepsy cohort of 200 individuals from Baylor Genetics who had not received a diagnosis based on clinical exome or genome sequencing. We analyzed their sequencing data by ranking the top risk variants using AI-MARRVEL, an AI-powered tool to prioritize possible disease-causing variants. This led to the identification of 17 individuals with epilepsy who carry two heterozygous LoF variants in genes known to regulate either actin polymerization or ROS. We evaluated the effects of the genetic variants in eight individuals by generating trans-heterozygous mutant flies for the orthologs, and found that five exhibited synergistic or additive effects leading to seizures. Overall, this novel "actin-mitochondria-glutamate pathway" may advance the identification of polygenic etiologies in individuals with seizures and highlight novel therapeutic targets.

454S Functional consequences of cardiomyopathy mutations in alpha-actinin-2

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Hypertrophic and dilated cardiomyopathies (HCM and DCM, respectively) are characterized by thickening (hypertrophic) or thinning (dilated) of the ventricular myocardium. Both HCM and DCM can lead to diastolic or systolic dysfunction and cause sudden cardiac death or heart failure. Recently, many variants in the ACTN2 gene, encoding for α-actinin-2, have been reported in patients with HCM and DCM. However, little is known about how these variants impact the function of the α-actinin-2 protein or the heart. Here, we investigated the functional impact of 11 missense variants that localize to the actin-binding domain (ABD) of α-actinin-2. Using actin co-sedimentation assays, we show that 10 of the 11 variants disrupt (increase or decrease) the binding of the α-actinin-2 ABD to actin. Further, circular dichroism studies show that all the ABD protein variants are well-folded, although some cause protein destabilization. To determine how the variants impact α-actinin function in vivo, we generated transgenic fly lines that express Drosophila α-actinin, with C-terminal fusion to mCherry, and with the human mutations introduced at conserved sites. Confocal imaging of α-actinin-mCherry in the cardiac fibers of fully intact, live larvae, shows that wild-type α-actinin-mCherry properly integrates into muscle Z-disks, based on its co-localization with Zasp66-GFP. However, some of the variants that altered actin binding in vitro showed reduced Z-disk integration. Further, we investigated the impact of the variants on beating and width of the larval heart chamber. Remarkably, most of the variants caused decreased heartbeat, and some resulted in increased diastolic or systolic heart chamber width. In summary, our biochemical and in vivo Drosophila data support that most of the variants are disruptive to α-actinin function in vitro and in vivo, supporting the pathogenicity of the variants.

455S The human antimicrobial peptide, LL-37, mitigates amyloid beta-induced deleterious effects on apoptosis, motor function, longevity, and gene expression in Alzheimer's disease model *Drosophila*

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Alzheimer's disease (AD) affects an estimated 7 million Americans, a number projected to double by 2060 according to the Centers for Disease Control and Prevention. Aβ42 peptides are thought to contribute to AD pathology due to their aggregation into soluble oligomers, fibrils and subsequent formation of plaques, with the aggregates showing associated neurotoxicity. *In vitro* studies have shown that the human antimicrobial peptide LL-37 can reduce Aβ42 fibril formation. This study investigates the potential rescuing effects of LL-37 *in vivo* using *Drosophila melanogaster* as a model organism while using genetic, biochemical, and behavior approaches.

Four transgenic *Drosophila* lines were used, expressing: Aβ42 alone, LL-37 alone, both Aβ42 and LL-37, and a non-expressing control, using either a pan-neuronal (elav-Gal4) or eye (GMR-Gal4) specific driver. Longevity assays and RNA profiling were conducted to assess phenotypic and molecular effects. Co-expression of LL-37 with Aβ42 resulted in a significant rescue of both eclosion rate and adult survival compared to Aβ42 expression alone. Conversely, LL-37 expression alone, like Aβ42 expression alone, had a detrimental effect on survival. RNA sequencing revealed that LL-37 co-expression reversed Aβ42-induced gene expression changes, particularly in genes related to proteolysis. Data from a Rapid Iterative Negative Geotaxis assay indicated that pan-neuronal LL-37 expression partially restores motor function deficits associated with Aβ42 expression. In flies expressing the peptides in developing eyes, using the GMR-GAL4 driver, staining for the apoptosis marker, DCP1, showed a significant decrease in apoptosis within dual expressing flies when compared to apoptosis levels seen in Aβ42-expressing flies. To assess whether dual expression can rescue the degenerative phenotype in eyes of Aβ42-expressing adult flies, scanning electron microscopy (SEM) will be used to visualize and compare external eye morphology across genotypes to evaluate potential phenotypic rescue.

Our findings suggest that LL-37 can mitigate AD-related pathology *in vivo* and highlight its potential as a therapeutic candidate in the treatment and prevention of Alzheimer's disease. The observed rescue supports the idea that LL-37 may interfere with Aβ42 fibril formation and also modulate downstream biological processes affected by amyloid toxicity, including changes in proteolysis and neurodegeneration

456S Exploring how the human μ-opioid (MOR) affects Notch signaling in *Drosophila*

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Opioid use disorder (OUD) is a debilitating condition with many causing factors, such as physical, psychological, and social struggles. OUD seems to be a chronic progressive disease but does respond to treatment. Despite many new developments in pharmacological treatments, relapse rates have remained high, highlighting a deeper understanding of OUD at a molecular level is needed to better help those suffering. In humans, multiple opioid receptors exist and play crucial roles in pain perception, including μ-opioid receptor (MOR), a G protein-coupled receptor (GPCR). The human MOR mediates most reward effects of opioid use by modulating neuronal excitability. Understanding the human MOR signaling could provide insight into tolerance and dependence witnessed in chronic opioid users. Drosophila melanogaster are powerful genetic models for studying many human diseases and disorders as they share remarkably similar genes, they have a high fecundity and are relatively inexpensive. Interestingly, however, Drosophila lack opioid receptors in their genome, making it difficult to utilize them in OUD research. However, since the downstream signaling pathway components of opioid receptors are conserved in Drosophila, we hypothesized that expressing human μ-opioid receptors in flies would generate observable phenotypes. We further hypothesized that these phenotypes could be used to identify genetic pathways regulating human μ-opioid receptors through the Drosophila genetic system. We used the GAL4/UAS system to express human μ-opioid receptors in various Drosophila tissues during development. Surprisingly, human μ-opioid receptors induced notched wing phenotypes in Drosophila. Here, we report how the Notch signaling pathway is pharmacologically and genetically linked to human μ-opioid receptors in Drosophila, presenting a novel approach for studying opioid receptor signaling.

457S Regulation of Neuroprotective Lipid Droplets in a Humanized Model of Alzheimer's Disease Reveals APOE as a Pivotal Determinant of Neurodegeneration

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Alzheimer's disease (AD) is the most common neurodegenerative disorder, affecting millions of lives of patients and caregivers. Effective therapeutic intervention for AD remains challenging due to an incomplete understanding of how genetic risk translates into cellular vulnerability for neurons. The APOE gene is the highest known genetic risk factor for developing AD with the ε4 allele driving disease risk. APOE functions in lipid transport including in the formation of glial lipid droplets (LD) in response to neuronal oxidative stress. We have generated humanized fly models expressing disease-associated variants in APOE ranging from predicted protective alleles (e.g., Christchurch) to predicted risk alleles (e.g., R145C, R150H, R251G). We aim to explore the function of these variants using our in vivo model to increase our understanding of AD etiology and to identify potential therapeutic avenues.

We performed lipid droplet analysis of humanized APOE variants associated with AD in fly retinas. We have identified loss-of-function variants, including APOE4, that impair LD formation in response to neuronal oxidative stress and accelerate retinal degeneration. Behavioral assays demonstrated parallel declines in locomotion and memory, directly linking LD dysregulation to neuronal decline. Future lipidomic and proteomic profiling are expected to reveal isoform-specific enrichment of cellular stress and lipid transport proteins, establishing molecular signatures of APOE4-driven neuron vulnerability. Because Drosophila permits rapid genetic screening, this model provides a powerful discovery platform for identifying suppressors of APOE-induced lipid stress and neurodegeneration. Our data supports a model in which LDs initially buffer toxic lipids but lose their protective capacity when APOE-dependent lipid shuttling fails. Importantly, this work defines a conserved metabolic mechanism by which APOE genotype modulates lipid-driven neuroprotection and reveals tractable genetic targets for restoring lipid droplet homeostasis in AD.

458S Investigating the Effects of Lion's Mane Mushroom and Silkworm Pupae on Amyloid Plaque Burden in the Mushroom Bodies of Drosophila Alzheimer's Models

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Alzheimer's disease (AD) is the most common form of dementia and currently affects over 55 million people worldwide, a number which is expected to triple by 2050. The disease is characterized by progressive cognitive decline and neuropathological hallmarks such as beta-amyloid plaques and intracellular tau tangles. Most approved drugs only provide relief from symptoms, but do not slow the progression of the disease. Preventative measures involving diet and exercise are increasingly emphasized but are not sufficient to fully prevent or significantly delay the disease. Given these limitations, there is a growing interest in natural bioactive compounds that could complement or inspire new therapeutic approaches.

Lion's mane mushroom (*Hericium erinaceus*) contains erinacines and hericenones, which stimulate nerve growth factor production and demonstrate neuroprotective, anti-inflammatory, and amyloid-reducing effects in models of Alzheimer's. Similarly, silkworm pupae (*Bombyx mori*) are rich in antioxidant proteins and peptides that protect against oxidative stress, protect the brain's communication system for learning, and improve memory performance in animal models of neurodegeneration.

Most AD research relies on mammalian systems, but Drosophila melanogaster offers a powerful and genetically controllable model for studying amyloid pathology. The mushroom bodies in the fly brain serve as centers for learning and memory and are especially vulnerable to amyloid-induced toxicity. This project aims to use Drosophila as an ideal model to investigate whether ingested nutraceuticals, such as Lion's mane and silkworm pupae, can reduce amyloid plaque burden and rescue memory functions.

Flies will be divided into groups: wild-type controls, untreated AD model, AD model with lions mane, AD with silkworm pupae powder, and AD model receiving a combination of both interventions. Bloomington fly media will be prepared, with treated groups receiving media with either silkworm pupae or lion's mane or both. Following dietary exposure, adult fly brains will be dissected and analyzed for amyloid plaque burden in the mushroom bodies. In parallel, behavioral testing will be conducted through olfactory conditioning tasks to determine whether supplementation improves learning and memory performance.

459S Phenotypic Characterization of Synthetic Amyloid Beta (Aβ) Mutations in a *Drosophila* Model of Alzheimer›s Disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder that affects more than 55 million people worldwide. A central hallmark of AD pathology is the buildup of amyloid beta (Aβ) plaques, which disrupt neuronal signaling and drive neurodegeneration. Using transgenic Drosophila models expressing wild-type (WT) Aβ pan-neuronally, our lab has observed AD-like phenotypes, including reduced lifespan, impaired locomotion, increased apoptosis, and altered gene expression. Importantly, early-onset familial AD is often associated with single amino acid substitutions in Aβ, highlighting the profound influence of subtle structural changes on Aβ aggregation and toxicity.

Prior in vitro studies from Dr. Jevgenij Raskatov's lab identified two Aβ42 side-chain modifications, Q15E and N27D (both deamidations), that slow Aβ42's aggregation kinetics and reduce its cytotoxicity. Based on these results, we generated transgenic Drosophila carrying the corresponding missense mutations. Using the GAL4-UAS system, we compared flies expressing WT Aβ42 with those expressing single and double-mutant variants to evaluate their effects on AD-related phenotypes in vivo.

Longevity assays showed that flies pan-neuronally expressing the Q15E variant of Aβ42 survived significantly longer than flies expressing WT Aβ42. A similar but less dramatic increase in longevity was seen for flies expressing the N27D variant and flies expressing a variant with both changes. Morphological analysis with scanning electron microscopy (SEM) of the compound eye revealed distinct differences among WT and mutant genotypes. Preliminary SEM results supported longevity results, showing a partial rescue effect associated with the missense mutations compared to WT Aβ42. Locomotor performance is being evaluated using the Rapid Iterative Negative Geotaxis (RING) assay to measure comparative decline in performance.

Together, these findings suggest that specific Aβ42 modifications can mitigate toxicity in vivo, with differential effects depending on the mutation. With this work, we hope to advance our understanding of the structural basis of Aβ42 toxicity and aggregation and provide insight into potential therapeutic strategies for Alzheimer's disease.

460S Utilizing a *Drosophila* model to Investigate Metabolic shift in Snyder-Robinson Syndrome

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Snyder-Robinson syndrome (SRS) is a rare X-linked developmental disorder caused by loss-of-function mutations in spermine synthase (SMS) which catalyzes the anabolic conversion of spermidine to spermine. Polyamines, including putrescine, spermidine and spermine play important roles in various cellular processes. Previous studies in our *Drosophila* SRS model and SRS patient cells showed that accumulation of spermidine and its catabolic byproducts reactive oxygen species (ROS) and aldehydes compromise mitochondrial function, thereby contributing to disease pathology. To further characterize the effects of dysregulated polyamine homeostasis on cellular metabolism, we conducted a metabolic analysis of SRS patient derived lymphoblastoid cells using the Biolog Phenotype Mammalian Microarray (PM-M) technology. Incubation with different compounds such as carbon energy sources, amino acids and hormones was followed by measurement of NADH production. The results revealed that specific amino acids or dipeptides significantly induce energy metabolic shift in SRS cells. To investigate whether amino acid specific metabolic shift is an adaptation to or an adverse effect of disease, we employed an in vivo SRS *Drosophila* model. Dietary supplementation with the amino acids or their dipeptides was followed by lifespan and climbing analyses to assess overall survival and neuromuscular performance as indicators of energy metabolic changes. Interestingly, we observed sex-specific differences in response to supplementation. Furthermore, different feeding paradigms show significant differences suggesting a complex effect of amino acid induced energy metabolism shift in SRS. This study validates energy metabolism shift as a major pathomechanism of SRS and lays the foundation for developing therapeutic treatment targeting amino acid metabolism.

461S Crossspecies multiomics mapping of APC/KRASdriven metabolic networks

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Colorectal cancer (CRC) is the third most diagnosed malignancy and the second leading cause of cancer-related deaths worldwide. Over 70% of CRCs harbor activating mutations in EGFR/KRAS and WNT/APC pathway genes, which regulate intestinal stem cell (ISC) proliferation and differentiation. While these oncogenic pathways are well characterized transcriptionally, their metabolic interactions and cooperative roles in tumor progression remain poorly understood. Our preliminary data show that EGFR signaling promotes ISC self-renewal through an anabolic program that diverts carbohydrates to biosynthetic pathways while using fatty acid oxidation (FAO) to sustain ATP production. Evidence also suggests that WNT/APC signaling drives similar metabolic changes. Here we show that co-activation of WNT/APC and EGFR/KRAS signaling reprograms cellular metabolism, creating cancer-specific vulnerabilities distinct from either pathway alone. To test this, we employ an integrated multi-omic strategy—combining transcriptomic, metabolomics, lipidomics, and proteomics—across *Drosophila* models and patient-derived organoids (PDOs). This approach maps metabolic changes under normal and oncogenic conditions involving EGFR/RAS and WNT/APC pathways. In *Drosophila*, ISC-derived cell clones carrying Apc^{Δ}, Ras^{G12V}, or dual Apc^{Δ}; Ras^{G12V} mutations reveal that dual-hit clones show enhanced proliferation, altered morphology, and cancer-like phenotypes, unlike single hits. Transcriptomic and metabolomic analyses identified distinct differentially expressed genes (DEGs) and metabolic profiles in the dual-hit state, particularly in mitochondrial, lipid, and nucleotide biosynthetic pathways. Integrated multi-omics analysis further identified unique metabolic modules reflecting synergistic reprogramming. Parallel studies in patient-derived cancerous (CRC) organoids show similar patterns: compared to normal control gut organoids, CRC organoids displayed altered morphology, transcription, and metabolism, including reprogrammed lipid and amino acid metabolism and enhanced glycolytic and mitochondrial activity. Following prioritization by evolutionary conservation and multi-omic integration, ~40 candidate genes (e.g., *r, mt:ND4L, eloF, Ak1*) will be functionally tested via RNAi or inhibitors in Apc^{Δ} Ras^{G12V} *Drosophila* ISC clones and *APC/KRAS* colon organoids to assess tumor-suppressive effects. Validated effectors will be evaluated in mouse and xenograft models to identify targetable metabolic dependencies. Together, these findings demonstrate that concurrent APC and KRAS mutations establish a unique synergistic metabolic program driving aggressive CRC. By integrating multi-omics and cross-species models, this study is finding conserved, cancer-specific metabolic effectors as promising therapeutic targets in colorectal cancer.

462S Investigating whether the human antimicrobial peptide, LL37, can ameliorate the neurodegenerative phenotypes induced in *Drosophila* by expression of the human amyloid alpha peptide.

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Alzheimer's disease (AD) is a progressive neurological disorder caused by neurotoxic protein aggregates, including amyloid-beta aggregates, leading to gradual cognitive decline and, in some cases, loss of motor function. Amyloid-β ($A\beta_{1-42}$) forms when the amyloid precursor protein (APP) is cleaved by a series of secretases, which can then aggregate, eventually forming amyloid plaques. Amyloid-α (Aα) (aka P3 or $A\beta_{17-42}$) is an alternative product formed by APP cleavage and has been shown, like Amyloid-β ($A\beta_{1-42}$), to be amyloidogenic *in vitro*. Experiments in transgenic *Drosophila* in our lab examine Aα's potential involvement in the neurodegeneration that is characteristic of AD.

To assess the effects of Aα on neuron function, longevity, and climbing assays were conducted on flies pan-neuronally expressing Aα, $A\beta_{1-42}$, or both peptides using the Appl-Gal4 driver line. Scanning electron microscopy (SEM) and RNA sequencing were used to determine Aα's structural and molecular effects on the developing eye structure and gene regulation, respectively in flies expressing these peptides under the GMR-Gal4 driver. The results of these assays indicated that $A\beta_{1-42}$ and Aα have deleterious effects on locomotive ability, overall lifespan, on ommatidia and bristle structures in the adult eye, and on gene expression, with the most significant degenerative effects seen in flies co-expressing $A\beta_{1-42}$ and Aα. In addition, expression of Aα or $A\beta_{1-42}$ in developing eye neurons, under the GMR-Gal4 driver, led to increased levels of neuronal apoptosis in eye imaginal discs as compared to controls. All these data suggest that Aα exacerbates the effects of $A\beta_{1-42}$ and has similar but less severe neurodegenerative effects when expressed on its own.

Our current work expands on these findings by investigating the role of Aα and its potential interaction with the human antimicrobial peptide LL37. Previous results from our collaborator, Annelise Barron at Stanford, show that the human antimicrobial peptide LL37 interacts with $A\beta_{1-42}$. In our lab, we have shown that coexpression of LL37 with $A\beta_{1-42}$ ameliorates the deleterious effects of $A\beta_{1-42}$. We hypothesize that interaction with LL37 may also modulate Aα aggregation and, as a result, affect the severity of AD-like pathology and progression induced by Aα.

463S A specific *WARS2* variant is associated with Parkinsonism

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WARS2 encodes the mitochondrial form of tryptophanyl-tRNA synthetase, an enzyme that links the amino acid tryptophan to its corresponding transfer RNA (tRNA) inside mitochondria, an essential step in mitochondrial protein synthesis. Biallelic pathogenic variants in *WARS2* usually cause a very severe, early-onset developmental and epileptic encephalopathy (DEE). Interestingly, a specific variant, p.W13G, when inherited with a second severe loss of function *WARS2* allele, is associated with parkinsonism that shows a clinical response to levodopa treatment. Here we describe four individuals carrying the *WARS2* p.W13G variant in trans with a severe loss of function allele. All four exhibit tremors consistent with *WARS2*-related parkinsonism and improvement upon levodopa therapy. Patient-derived fibroblasts revealed reduced WARS2 protein levels and mildly decreased ATP production. Interestingly, transmission electron microscopy (TEM) of these fibroblasts shows expansion of the endoplasmic reticulum (ER), altered mitochondrial morphology, and accumulation of lipid droplets. Additionally, lipidomic profiling uncovered elevated levels of ceramides and sphingolipids, lipid species that are implicated in Parkinson's disease.

To explore the functional impact of these variants, we are modeling *WARS2* variants in *Drosophila melanogaster*. The Drosophila ortholog (*TrpRS-m*) is predominantly expressed in neurons, and its knockdown leads to progressive climbing defects (motor decline), heat sensitivity (heat induced seizures), and bang sensitivity (mechanical seizures) phenotypes. Interestingly, flies with neuronal *TrpRS-m* knockdown also show increased lipid droplets in surrounding glial cells and reduced visual response amplitudes.

We hypothesize that the p.W13G variant may disrupt mitochondrial targeting of the WARS2 protein, causing its mislocalization to the ER, which triggers ER stress and secondary lipid imbalance. Ongoing work using humanized fly models expressing *WARS2* patient variants aims to dissect how these molecular changes contribute to Parkinsonism. Together, our findings highlight that WARS2-associated parkinsonism arises not only from defective mitochondrial translation but also from disrupted lipid metabolism and ER stress, revealing a new link between mitochondrial dysfunction and Parkinson's disease mechanisms.

464S Targeted modifier screen of spinocerebellar ataxia dendritic arbor defect using a neuronal β-spectrin protein interactome

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Spinocerebellar ataxia type 5 (SCA5) is an autosomal dominant neurodegenerative disease caused by mutations in the *SPTBN2* gene that encodes β-III-spectrin. Beta-III-spectrin is a cytoskeletal protein that is expressed predominantly in the cerebellum and is essential for cerebellar Purkinje neuron dendritic arborization. A SCA5 L253P missense mutation, localized to the actin-binding domain (ABD) of β-III-spectrin, causes a 1000-fold increase in actin binding. We previously showed that expression of fly β-spectrin containing the human L253P mutation causes a pronounced decrease in dendritic arborization of larval sensory neurons. The goal of this project is to establish neuronal protein interactomes for wild-type and L253P β-spectrin, and to use this data to guide a targeted genetic modifier screen for modulators of the SCA5 arbor phenotype. We expressed a functional wild-type β-spectrin with C-terminal GFP tag, broadly in neurons using the *elav-Gal4* driver. β-spectrin-GFP protein, or GFP by itself, was isolated from adult head extracts using GFP magnetic beads, and samples analyzed by tandem mass spectrometry for unbiased protein identification. By this approach, we established a neuronal β-spectrin interactome consisting of approximately 171 proteins. The interactome is enriched in proteins known to associate with β-spectrin, such as α-spectrin, actin, adducin (hts) and neuroglian. In addition, many novel interactors are highly enriched, such as SERCA and CAMKII involved in calcium signaling. Further, we established a fly line constitutively expressing fly L253P β-spectrin and optimized conditions for the modifier screen. We are using new AI-based Image-Pro software, and working with Media Cybernetics, to develop a protocol to greatly accelerate dendritic arbor analyses. We will present our progress performing the SCA5 arbor modifier screen targeting components of the β-spectrin interactome with RNAi, in conjunction with new methodology for high throughput neuron morphology analyses.

465S NAD+ synthase NMNAT confers protection against DPR neurotoxicity in a *Drosophila* model of ALS

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Hexanucleotide repeat expansions (GGGGCC, G4C2) in the C9ORF72 gene represent the most common genetic cause of familial amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD). Among the multiple pathological mechanisms implicated in C9-ALS/FTD, toxicity from dipeptide repeat protein (DPR) generated via repeat-associated non-AUG (RAN) translation is thought to drive neurodegeneration through cytoplasmic aggregation and mislocalization of the nuclear proteins and nuclear transport defects. Despite these observations, the detailed processes contributing to the disease are not fully understood. Existing therapies show only modest efficacy in delaying motor function deterioration and limited effectiveness in early stages of the disease, highlighting an urgent need for new therapeutic discoveries. Our previous studies have established the robust neuroprotective capacity of nicotinamide mononucleotide adenylyltransferase (NMNAT), a key NAD+ synthesis enzyme with dual functions in NAD+ metabolism and in protein homeostasis. Here, using a *Drosophila* model of C9-ALS, we reveal that nuclear, but not cytoplasmic NMNAT isoforms is neuroprotective against glycine-arginine (GR) induced neurotoxicity. Specifically, co-expression with a nuclear NMNAT isoform suppresses, while cytoplasmic NMNAT exacerbates GR-induced morphological and functional neurodegeneration. These findings highlight an isoform- and compartment-specific interplay between DPR toxicity and NMNAT, offering new mechanistic insights into ALS/FTD pathophysiology and informing the development of targeted therapeutic strategies.

466S Tumor induced peripheral biotin deficiencies precede cachexic wasting

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Cachexia is a chronic systemic disease that affects up to 80% of cancer patients. This condition is described by significant body fat loss, muscle loss, anorexia and early satiety, however it is not reversible by supplementary nutrition. Despite its prevalence, the onset and underlying initiating mechanisms of cachexia remain poorly characterized. Recently, *Drosophila melanogaster* has emerged as a valuable model for investigating cachexia. In examining pre-cachexic muscles, we have identified that one of the earliest changes in muscles is altered mitochondrial shape and distribution. Mitochondria have high levels of biotin accumulation due to its role as a cofactor of multiple mitochondrial enzymes. Thus, streptavidin, due to its high biotin binding affinity is an effective mitochondrial marker independent of membrane potential. Surprisingly, we have found that streptavidin fails to accumulate in mitochondria of cachexic muscles, despite other mitochondrial markers still indicating the presence of mitochondria. We have further found a corresponding biotin accumulation in the tumors. Together, these results suggest a novel metabolic connection between muscles and pro-cachexic tumors. This interaction significantly precedes occult muscle wasting, and involves a tumor-induced biotin deficiency in the muscle.

467S DEG/ENaC channel PPK1 is required for wound-induce chemical-allodynia

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Animals rely on nociceptive sensory systems to detect and respond to potentially damaging stimuli. While physical or UV irradiation damage to the epidermis leads to hypersensitivity of nociceptors through TrpA1 channels (Im and Galko 2011), the mechanisms of chemical associated hypersensitivity are unknown.

Exposing *Drosophila* larva to high concentrations of hydrochloric acid induces nociceptive behaviors (Lopez-Bellido et al. 2019). Acid sensitive ion channels (ASIC) are proton-gated and belong to the degenerin/epithelial sodium channel (DEG/ENaC) family. In mammals, ASIC channels and TrpA1 contribute to acid sensing. In *Drosophila*, mutation of the DEG/ENaCs *ppk19* and *ppk30* have been found to disrupt acid nociception independently of *ppk1* (Jang et al. 2022). We find that *ppk1* is required for chemical-allodynia (sensitivity to normally sub-threshold acid concentrations). In contrast to thermal-allodynia, *dTrpA1* and *painless* are dispensable. This suggests there may be a wound-initiated mechanism that sensitizes nociceptors to acid stimulus. Recently, the wound signal Vulnusin was identified to activate the PPK1/PPK26 complex for immediate nociceptive behavior from puncture wounding (Guo et al. 20205). Our ongoing work explores the role of Vulnusin in sensitizing nociceptors to initiate chemical-allodynia.

Exposing larvae to hydrochloric acid also damages the larval epidermis. Physical injuries that breach the larval cuticle (puncture wound) induce chemical-allodynia. In contrast, non-cuticle breaching injuries (pinch wounds) induces thermal-allodynia but fails to elicit chemical-allodynia. This suggests that compromising the permeability of the cuticle may be required for chemical-allodynia. Because acid exposure may also compromise the cuticle, we investigated the effects of acid-induced tissue damage on acid-nociception. We find that acid exposure does not induce hypersensitivity. Instead, sequential acid exposure results in a loss of nociceptive behavior (hypoalgesia). Ongoing work aims to determine the role of epidermal damage to the loss of nociception after acid exposure.

We find that acid damage induces a unique loss of nociceptive sensitivity, and that wound induced allodynia is sensitized to chemical stimulus in a *ppk1* dependent but *dTrpA1* independent mechanism. Uncovering the pathways that can lead to allodynia may reveal conserved mechanisms that may potentially inform new approaches for pain relief and therapies for chronic pain.

468S Neuroprotection via glial lipid droplet formation requires endocytosis and mitochondrial complex I function in neurons and in glia

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Alzheimer's disease (AD) is a prevalent neurodegenerative disease that affects cognition and life expectancy of those diagnosed. There is no cure for this disease, but researching its pathology and mechanisms may lead us to find better therapeutic avenues for treatment. The causes of AD are complex and dozens of genetic risk factors for disease have been identified to date. Using a neuronal ROS-induced glial LD formation model, we screened AD risk genes for their role in glial LD formation. Our efforts identified, among others, mitochondrial complex 1 proteins NSUFD7 (ND-20), NDUFS3 (ND-30), and endosomal size regulator protein RABEP1 (RBPN-5) as critical LD genes. We further characterized these genes by investigating their cell-type specific role in the brain using the GAL4/UAS expression system. We found a significant decrease in LD formation when each of these genes are lost in either neurons or in glia compared to controls. These findings implicate these genes as critical lipid droplet factors and provide a mechanism by which they impact genetic risk for the development of AD. Future studies will further interrogate the cell-type specific role for each gene in protecting the brain from degeneration due to oxidative stress.

469S Cocaine Increases Dopamine Signaling in the adult *Drosophila melanogaster* Mushroom Body

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Cocaine-related deaths have been on the rise since 2011.[1] The National Survey on Drug Use and Health reported in 2023 that 1.3 million of the 5 million people who reported using cocaine in that year had a substance use disorder.[2] One of the impacted neurotransmitters is dopamine, which is involved in the pleasure and reward systems of the brain and contributes to the high one feels after drug use. Drosophila melanogaster has a nervous system with similar neurotransmitters and proteins responsible for release and reuptake as humans; thus, cocaine should work through a similar mechanism in humans and flies. Additionally, Drosophila melanogaster is a simple model that is both cost and time effective. The goal of this study is to understand how cocaine changes dopamine release and reuptake in the Drosophila mushroom body. In this study, the mushroom body region of an adult Drosophila melanogaster brain is targeted for the detection of dopamine via Fast Scan Cyclic Voltammetry (FSCV). FSCV is an electrochemical technique that uses a 7 µm carbon fiber microelectrode to detect electroactive neurotransmitters. In dissected adult brains, the release of dopamine is stimulated by puffing on 10 pmol of acetylcholine using a small capillary. Measurements of stimulated dopamine release with acetylcholine were conducted before and after cocaine was added. As expected, the introduction of cocaine to the Drosophila melanogaster brain resulted in a longer clearance time of dopamine due to diminished reuptake. The t50, or time it takes to clear dopamine from its peak concentration to 50% of the initial dopamine concentration, increases from 8.6 seconds without cocaine to 14.4 seconds in the presence of cocaine. Repeated stimulations, with cocaine present, interrogate the effect of repeating these stimulations every 1 minute show that dopamine depletes more when cocaine is present. This depletion is also seen when stimulations are repeated every 10 minutes, but synthesis contributes more to maintaining dopamine function on this time frame. These findings demonstrate the feasibility of using FSCV to monitor real-time dopamine dynamics in the Drosophila brain. This system could serve as a valuable platform for future investigations of cocaine's effects on synaptic transmission and addiction-related neurobiology.

470S The Atossa-Porthos pathway regulates neuronal survival in an Alzheimer's disease model of Drosophila melanogaster

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Alzheimer's disease (AD) is the most common form of dementia, and is hallmarked by the accumulation of toxic amyloid beta aggregates and hyperphosphorylated tau tangles. The initial appearance and progression of AD coincides with mitochondrial dysfunction, and dysregulation of mitochondrial respiration is an early feature of AD. It is hypothesized that restoration of disrupted oxidative phosphorylation can help preserve neuronal activity and prevent further disease progression. Our lab has identified the Drosophila gene Atossa (Atos) as a key activator of oxidative phosphorylation and ATP production in macrophages. We have found that Atos shares homology and mitochondrial regulatory capacity with human AtosA, a gene with four AD-associated SNPs. Atos and AtosA are broadly expressed in the Drosophila and human brain, respectively. To test the potential relevance of AtosA in AD, we utilized a well-established Drosophila model of the disease, expressing the aggregating non-soluble form of human Amyloid-β in the retina which induces necrosis. We observed that knocking down Atos alongside Amyloid-β expression increased retinal neural necrosis four-fold, while Atos knockdown on its own had no effect. In macrophages Atos upregulates Porthos, a DEAD-box RNA helicase, to spur ribosome biogenesis and the translation of mRNAs with short 5'UTRs, many of which enhance mitochondrial function and OxPhos. Atos appears to again work through Porthos in the neuronal context to provide resilience against Amyloid-β; concomitant Porthos knockdown in Drosophila neurons increases Amyloid-β-induced retinal necrosis and excitingly, Porthos over expression decreases such death. We are currently examining how Atos impacts amyloid beta aggregation and tau accumulation in the brain, along with its effects on preserving cognitive function. Our work argues that Atos and Porthos are previously unrecognized cellular actors that play crucial roles in maintaining neuronal survival in the face of toxic Amyloid-β aggregation; these findings raise the possibility that the human orthologs, AtosA and DDX47, could serve as useful targets for therapeutic intervention in AD.

471S Anchor knockdown in the Insulin producing cells decreases ethanol sensitivity and increases ethanol preference

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Alcohol misuse is a leading cause of premature death and disability, and it contributes to over 200 diseases and injury-related health conditions worldwide, highlighting the importance of identifying associated risk factors. An efficient model to characterize molecular mechanisms of alcohol behaviors is Drosophila melanogaster, considering the high conservation of ethanol-related behaviors between fruit flies and mammals. In both systems, the also highly conserved mechanistic target of rapamycin complex 1 (mTORC1) has been implicated in aspects of ethanol-related behavior. Here, we study a modulator of mTORC1 signaling, anchor, and explore its role in ethanol-related behavior, including ethanol-induced sedation and preference. While never studied in the context of ethanol, behavior, or the brain, anchor is highly expressed in neuronal populations, such as the insulin-producing cells (IPCs), which modulate response to ethanol-induced sedation.

In order to investigate whether anchor in the IPCs alters sensitivity to ethanol and ethanol preference, we use an IPC anchor knockdown model. We find slower sedation rates in anchor knockdown flies, demonstrating a lower ethanol sensitivity compared to genetic controls. Interestingly, we also find that IPC anchor knockdown flies have higher ethanol preference compared to genetic controls. Findings suggest that anchor expression in the IPCs is directly related to ethanol sensitivity and preference, developing understanding of how IPCs influence multiple dimensions of ethanol-related behavior.

472S Activation of BMP signaling and transcriptional target Trio modify ALS models in *Drosophila melanogaster*

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by motor circuit dysfunction. We have previously shown that upregulation of the bone morphogenetic protein (BMP) signaling pathway results in cell-specific rescue of defects in locomotion, survivability, and neurotransmission associated with a Drosophila *dSod1*^{G85R} knock-in ALS model. To better understand the extent to which the BMP rescue is generalizable across different causes of ALS, we examined the ability of activated BMP signaling to restore motor function, neuronal morphology, and survival in a second knock-in ALS patient mutation, *dSod1*^{A4V}, as well as in a motoneuron-driven *C9orf72(G4C2)* repeat expansion ALS/FTD model. In both models, we saw rescues in locomotion, survivability, and neurotransmission with activation of BMP signaling.

Knock-in *dSod1* ALS models allow us to also probe the cell specificity of BMP signaling rescue. To date, we have found that the activation of BMP signaling non-autonomously, as well as cell autonomously in neurons improves survivability and locomotion, but not when expressed in muscles or glia. Similarly, we found that the cell autonomous activation of BMP signaling in neurons through directed expression of the constitutively active Sax receptor yields consistent and robust suppression of degeneration phenotypes. These data suggest that improvement in motor function and survival is achieved when BMP signaling is increased specifically in neurons.

Trio, a guanine exchange factor that regulates actin dynamics in the neuron, is a transcriptional target of BMP signaling. We found that targeted overexpression of Trio in cholinergic neurons shows a potent rescue of failed eclosion exhibited by *dSod1*^{G85R}. Consistent with this rescue, our data shows an increase in bouton number at the pharate neuromuscular junction. Additionally, our adult onset *dSod1*^{A4V} model exhibits differential severity in male vs female. Interestingly, trio expression shows a sex preferential rescue in climbing defects. We also find that overexpression of Trio in a motoneuron-driven model of *C9orf72(G4C2)* repeat expansion ALS shows a significant rescue in lifespan, demonstrating that Trio, like BMP signaling, has the ability to rescue ALS-induced defects across models. Further studies will focus on uncovering the underlying mechanism responsible for Trio-based suppression of neurodegeneration.

473S A modifier screen reveals relationship between tau and nucleoporins

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Neurodegenerative diseases such as Alzheimer's Disease and Parkinson's Disease devastatingly affect a patient's quality of life, causing severe cognitive and motor impairments in over 50 million people worldwide. While amyloid beta plaques and tau neurofibrillary tangles are hallmark features of AD, the mechanism behind these pathologies leading to neurodegeneration and disruption of other cellular functions are unclear. Recent studies suggest a link between these pathogenic proteins and the disruption of nucleocytoplasmic transport at nuclear pore complexes (NPCs), with tau directly interacting with nucleoporins (proteins in NPCs) to lead to tau mislocalization and disruption of NPC function. Preliminary data in mice show that mammalian genome contains over 30 nucleoporins, thus it remains unclear which specific nucleoporins are responsible for this interaction with tau. This prompts further investigation to see which nucleoporins are important. To determine nucleoporins that act as genetic modifiers of tau induced neurotoxicity, we performed a targeted RNAi base screen in *Drosophila melanogaster*. The tauopathy model was generated using panneuronal driver to express 2N4R isoform of human MAPT (tau), and the tau flies were crossed to over 80 RNAi lines targeting individual nucleoporin orthologs. Rescue of neurodegeneration will be measured by lifespan and negative geotaxis (climbing) assay. We developed a novel automated system for climbing assay to efficiently measure neuronal dysfunction in flies across multiple metrics. I will present candidate nucleoporin genes that have shown modified neurotoxicity when knocked out.

474S Lipid metabolism as a potential mediator of sleep disturbances in *Drosophila* models of neurodegeneration

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Alzheimer's disease (AD) is a devastating progressive neurodegenerative disease that leads to memory loss and is becoming increasingly common with limited disease-modifying treatments. Sleep disturbances are frequently seen prodromally and throughout AD and other neurodegenerative diseases, where pathological features—including amyloid-beta (Aβ), tau, and TDP-43—shorten and fragment sleep, and chronic sleep disruption accelerates neurodegeneration in humans, mice, and Drosophila. Despite growing evidence for the bidirectional relationship between sleep and neurodegeneration, the underlying mechanisms remain poorly understood. We hypothesize that lipid metabolism alterations could be the missing link mediating the relationship between sleep and neurodegeneration. Our lab recently demonstrated the essential role of sleep in clearing glial lipid droplets (LDs) that accumulate following wake to mitigate oxidative damage in neurons. Furthermore, many top AD genetic risk factors identified through GWAS are involved in lipid metabolism, and early lipid alterations—such as lipid peroxidation and dysregulated sphingolipid metabolism—are implicated in AD onset and progression. We find that Drosophila models of AD recapitulate mammalian sleep loss phenotypes and have increased lipid droplets (measured by BODIPY staining) and elevated lipid peroxidation (measured by MDA staining). We aim to further dissect the mechanistic relationship between lipid alterations and sleep disturbances in AD flies, leveraging the strong genetic tools available in Drosophila.

475S Suppression of dRUFY Rescues Synaptic and Motor Deficits by Restoring Lysosomal Distribution in a Drosophila FUS-ALS Model

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive loss of motor neurons. Mutations in the RNA-binding protein Fused in Sarcoma (FUS), such as the juvenile-onset *FUS.P525L* variant, lead to toxic cytoplasmic protein aggregation. Using an unbiased CRISPR knockout screen in HEK293T cells, we identified RUFY3 (*dRUFY* in Drosophila) as a novel genetic modifier of FUS-ALS. While RUFY3 has no known disease-linked mutations in patients, its loss significantly reduced FUS.P525L protein aggregation. RUFY3/dRUFY is an adaptor protein that facilitates retrograde lysosome transport by linking Arl8-positive vesicles recruited by the BORC complex to dynein-dynactin motors via the effector Syd (Sunday Driver). We found that overexpressing *FUS.P525L* in motor neurons resulted in severe larval locomotion impairments, enlarged neuromuscular junction (NMJ) bouton size, and reduced synaptic strength. Notably, we found that motor neuron-specific *dRUFY* knockdown (KD) significantly rescued these behavioral and physiological deficits. Mechanistically, we found that LAMP1-positive lysosomes are depleted at the NMJ in FUS-ALS larvae, a defect reversed by *dRUFY* suppression. Further, knockdown of the BORC subunit *BORCS7*, which recruits Arl8 to lysosomes, rescued motor deficits. Conversely, *Arl8* KD on its own – which decreases the movement of lysosomes to the NMJ – phenocopied FUS-induced impairments. These results suggest that FUS-ALS pathology involves excess retrograde pull of lysosomes from the NMJ, or a failure in anterograde delivery of them to the NMJ. Together, these data indicate that FUS-ALS disrupts lysosomal homeostasis and that suppressing *dRUFY*-mediated retrograde transport restores axonal distribution to maintain synaptic integrity, highlighting this transport axis as a potential therapeutic target for ALS.

476S Unraveling the Synergistic Toxicity of GGCCTG and Dipeptide Repeats in a SCA36 *Drosophila* Model

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Objective:

To determine the relative contributions of hexanucleotide RNA repeats (HRR) and dipeptide repeats (DPR) to neurodegeneration in spinocerebellar ataxia type 36 (SCA36).

Methods:

We generated Drosophila SCA36 models expressing a series of NOP56 (GGCCT)n repeat-related constructs to dissect HRR- and DPR-mediated toxicity. Patient-derived fibroblasts were compared with model flies for their cellular phenotypes. Individual DPR and HRR transgenes were evaluated for neurotoxicity in SCA36 flies.

Genetic suppression of the Spi4 ortholog and pharmacologic inhibition of transcription using 6-azauridine (6-AZA) were also tested.

Results:

SCA36 flies reproduced cellular defects seen in patient fibroblasts. Among individual DPRs, Proline-Glycine (PG-DPR) caused the most prominent motor and sensory dysfunction. HRR transgenes induced adult-onset, age-and length-dependent neurodegeneration. Constructs engineered to express only HRR or only DPR produced attenuated phenotypes, indicating that both HRR and DPR contribute to SCA36 pathogenesis. Knocking down Spt ortholog or treating with 6-AZA exacerbated neurodegeneration in both flies and patient cells.

Conclusions:

Both HRR and DPR drive SCA36 neurotoxicity, with PG-DPR exerting the strongest toxic effect. The Drosophila models provide a robust platform for screening HRR- and DPR-targeted therapies. However, SpI4-related transcription-suppressing strategies may worsen degeneration, underscoring the need for careful evaluation of mechanism-based interventions for SCA36.

477S Drosophila disease model of RUNX protein mutants using live cell protein and transcription imaging

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The RUNX (Runt-related transcription factors) family are important master regulators responsible for hematopoietic and osteoblastic lineage determination. Mutations of RUNX1 and RUNX2 are associated with leukemia and cleidocranial dysplasia in humans. However, we lack understanding of the molecular mechanisms connecting genotype to phenotype, specifically, how disease-associated mutations alter protein function and consequently disrupt downstream gene expression programs in vivo. The DNA binding domain of RUNX is highly conserved across family members and species, and many disease-associated mutations from leukemia and cleidocranial dysplasia patients map to this region. By taking advantage of this high degree of conservation, we use Drosophila Runt, a homolog of RUNX1 and RUNX2 in mammals, as a model system. In Drosophila we analyze how the spatio-temporal behavior of Runt changes with the introduced mutations. We used confocal microscopy and genome editing to simultaneously track and quantify target genes' expression and Runt protein during early embryogenesis. Endogenous Runt was fused to an anti-eGFP antibody (llamatag) to track its position and concentration throughout the embryo. To examine how Runt's role as a transcription factor is compromised by the introduced mutations, we used the MS2/MCP system to observe and quantify mRNA production of Runt target genes during embryogenesis. For this study we are looking at Ftz, Tollo, and Slp1 all endogenously tagged with 24xMS2 stem loops. We have generated a Runt mutant which replaces the last amino acid of the DNA binding domain with a stop codon. This mutant is associated with familial platelet disorder and acute myeloid leukemia in humans. Our wild-type line recapitulates the characteristic 7-stripe Runt pattern at nuclear cycle 14. On the other hand, our mutant line shows no Runt stripe formation at all, while there exists residual Runt expression uniformly across the embryo throughout nuclear cycle 14. Furthermore, mutant flies are homozygous lethal. With this project we hope to reveal at a molecular level how the disease-associated mutations of the highly conserved Runt lead to several disease phenotypes starting from the embryonic stage.

478S Screening FDA-approved compounds that attenuate seizure expression in a *Drosophila* model of Dup15q syndrome

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Epilepsy is a major co-morbidity in the autism spectrum disorder duplication of chromosome 15q syndrome (Dup15q syndrome), with seizures often reported to be pharmacoresistant. In Dup15q syndrome, supernumerary copies of the E3 ubiquitin ligase gene *UBE3A* are thought to account for many of its neurological phenotypes. Indeed, in flies, overexpression of the ortholog *dube3a* recapitulates many aspects of Dup15q syndrome. Using an automated video tracking system and a tethered fly electrophysiological preparation, we have previously found glial-restricted expression of *dube3a* (*repo* > *dube3a*) leads to spontaneous seizures that are exacerbated by high temperature (~38 °C). Neuronal *dube3a* expression (*nsyb* > *dube3a*) did not give rise to overt seizure phenotypes.

Here, we describe a high-throughput screening protocol using video tracking data to identify compounds that attenuate seizures in the glial *dube3a* expression model. Based on fly locomotion trajectories, we developed machine-learning approaches to quantify behavioral states, including walking and locomotion pauses observed in control flies, along with immobilization, uncoordinated movements and convulsions primarily observed in *repo* > *dube3a* flies. As a proof-of-concept, we monitored the effect of 10 selected FDA-approved compounds previously reported to improve behavioral phenotypes in the model. The effect of each of these compounds on electrophysiological phenotypes was assessed in parallel, using the tethered fly preparation.

Of the drugs tested, serotonin and minoxidil led to the greatest behavioral and electrophysiological improvements, while other compounds had modest but significant effects. Furthermore, the throughput of the video tracking system indicates efficient screens of large compound libraries are feasible. Our approach may be generalized to the large collection of fly epilepsy models to identify potential avenues for therapeutic intervention.

479S Investigating the Role of Oxidative Stress in Traumatic Brain Injury in *Drosophila melanogaster*

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Traumatic brain injuries (TBI) occur from a severe impact to the brain causing damage and are a leading cause in morbidity and mortality, leading to structural and biomolecular dysfunction in the body. Many mechanisms of human TBI can be modeled using *Drosophila melanogaster*. Previous research shows that a TBI disrupts sleep, and that sleep helps clear reactive oxygen species (ROS), protecting against oxidative stress. We wanted to investigate whether oxidative stress is detrimental to TBI outcomes and whether it represents a key mechanism underlying injury progression and recovery. To investigate this question we modified the oxidative stress levels in flies genetically and pharmacologically to see how it impacted short- and long-term outcomes of a TBI. We also measured expression of oxidative stress response genes and found that members of the glutathione-S-transferase (GST) family were differentially expressed acutely and in long term after injury. Future research will investigate the GST gene family and how related stress response pathways interact with TBI. Increasing the understanding of the role of oxidative stress in TBI could help develop new TBI treatment or recovery methods.

480S Reduction of endogenous Uba5 partially rescues Zika virus NS4B-induced neurodevelopmental defects

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In healthy organisms, ubiquitin-like activating enzyme 5 (UBA5) responds to ER stress, regulates protein homeostasis, and is essential for embryonic development via the UFMylation pathway. Variants in the UBA5 gene are known to cause conditions such as developmental and epileptic encephalopathy, seizures, and microcephaly or reduced head size. Zika virus (ZIKV) infection during pregnancy can cause similar serious birth defects with severe microcephaly. ZIKV non-structural protein 4B (NS4B) plays a role in viral replication, membrane rearrangement, and acts as an immune suppressant. Zika virus NS4B physically interacts with UBA5 in human cells, suggesting that NS4B might obstruct UBA5 function during development to cause developmental defects. Using *Drosophila melanogaster,* we investigated the ZIKV-NS4B and UBA5 interaction during neurodevelopment. We found that while expression of NS4B alone results in pupal lethality and reduced brain volume, co-expression of UBA5 with NS4B shifts the lethal stage to late embryogenesis, suggesting UBA5 expression enhances NS4B-induced phenotypes. Conversely, reduction of endogenous Uba5 activity partially rescues NS4B brain growth defects, such as dividing cell count in the central brain and overall brain volume. We predict that NS4B increases activity in the UFMylation pathway via UBA5, resulting in neurodevelopmental defects.

481S The role of Centrocortin in neurodevelopment

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Modulation of the c-Myc oncogene by the paraneoplastic antigens CDR2 and CDR2L contributes to paraneoplastic cerebellar degeneration (PCD), an immune-mediated disorder marked by Purkinje neuron degeneration. We previously identified an embryonic role of Centrocortin (Cen), the sole *Drosophila* ortholog of CDR2/CDR2L in maintaining mitotic fidelity. However, the molecular mechanisms linking Cen to neuronal integrity and oncogenic signaling remain elusive. Here we report the neuronal function and molecular targets of Cen using custom *Drosophila* constructs and proteomics analyses. We demonstrate that depletion and neuron-wide over-expression of Cen showed significantly increased disruption in mushroom body, a neuronal structure analogous to the human hippocampus. We also observe locomotor defects in adult *Drosophila*, as measured by negative geotaxis assay (NGA). Moreover, expression of a *Cen* transgene rescues mushroom body defects in a cell-autonomous manner. We further find that Cen dysregulation alters Myc expression levels in *Drosophila* larval brain extracts and reduces Myc in proliferative neural stem cells (NSCs), progenitors of neurons and glia, as a consequence of reduced NSC number, while the proliferative state of NSCs remains unchanged. Proximity interaction analyses in the early embryo indicate that Cen interacts with factors involved in cytoskeletal organization, genome stability, and neuroanatomical development. Further work in *Drosophila* brain not only a conserved cytoskeletal element and genome stability proteins, but also sensory perception and locomotor behavior proteins. Collectively, these findings establish Cen as a key regulator of neuronal structure and function. The interaction of Cen with cytoskeletal regulators supports its role in neurodevelopment and points to a mechanistic link between mitotic integrity, oncogenic signaling, and neurodegenerative processes. Our study highlights Cen as a valuable genetic model for PCD research.

482T Dissecting the Molecular and Circuit Architecture of Courtship Behavior in *Drosophila* Species and Hybrids

Colleen Palmateer, Kelsey C Morris, Scott Nolting, Jackson Mast, Michelle N Arbeitman Biomedical Sciences, Florida State Univ

Elucidating the mechanisms that drive behavioral evolution is important to both further our understanding of speciation and to provide insights into how the potential for behaviors are specified. Male courtship behaviors are rapidly evolving traits that play a significant role in pre-mating isolation across species. To examine the genetic basis underlying species differences in courtship behavior, we generated hybrid males by crossing two strains of *D. melanogaster* females with *D. simulans* Lhr males. This design enabled us to assess how genetic background influences the expression of species-specific courtship behaviors and whether these behaviors are specified in a quantitative manner. Additionally, this approach allowed us to evaluate the role of the female courtship target in shaping male behavioral responses. In both single-pair and mate-choice preference assays, hybrid males exhibited behavioral plasticity, modulating their courtship strategies depending on the species of the female. Notably, the *D. melanogaster* maternal strain significantly influenced the behavioral repertoire of the hybrids. To investigate potential neural correlates of these behavioral differences, we examined fru-expressing neuronal subsets in hybrid males. The projection patterns observed were highly similar to those in *D. melanogaster*, suggesting conservation of circuit architecture despite the hybrid genetic background. To gain insight into the molecular mechanisms driving species-specific behaviors, we identified male-specific Fruitless (Fru^M) targets in *D. melanogaster* and *D. simulans* using the CUT&Tag approach. This analysis revealed a core set of conserved Fru^M target genes, as well as species-specific targets. Given the role of pheromone signaling in pre-mating isolation, we focused on chemosensory receptors exhibiting species-specific Fru^M binding. Several such receptors were identified. To functionally assess the role of these receptors, we performed a genetic screen in *D. melanogaster* using a genetic intersectional approach to silence neurons co-expressing f*ru P1* and specific chemosensory receptor genes. In courtship preference assays with both *D. melanogaster* and *D. simulans* females, we identified three additional olfactory receptor neuron subtypes that co-express *fru P1* and modulate species-specific courtship behavior. We used the Trans-Tango approach to evaluate their second-order olfactory projection neurons, revealing their targets in higher-order processing centers. This study identifies new molecular mechanisms underlying how the potential for behavior is established and provides insights into the substrates for evolution of these behaviors.

483T Visualization of the AANATL7 protein in the male accessory gland through generation of a *AANALT-7::mCherry* gene fusion using a co-CRISPR HDR gene conversion approach

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The male accessory gland has been shown to be critical to a number of steps involving reproductive behavior in Drosophila. Secondary cells within the accessory glands contain substances critical to post-mating responses (PMRs) of females, as the loss of these cells negatively affect PMRs (1). N-acetylhistamine (NAH), a metabolite of histamine, is present in secondary cells (2) and is synthesized by the enzyme Arylalkylamine NAcetyltransferase-Like-7, encoded by the *AANATL-7* gene (3). *AANATL-7* is expressed in main cells of the accessory gland, but not in secondary cells that contain NAH (4). To understand where AANATL7 functions, the mCherry sequence was fused to the carboxy-terminus of AANATL7, to create an AANATL7::mCherry gene fusion. Using a co-CRISPR/Cas9-mediated homology-directed repair (HDR) approach (5), 84 G_0 flies were generated from *nos-Cas9* embryos that were injected with a donor DNA plasmid containing the AANATL7::mCherry gene fusion. sgRNA-generating plasmids were co-injected, targeting both the *AANATL-7* and *ebony* genes to identify G_0 broods which had a CRISPR-induced *ebony* mutation, enhancing the likelihood of identifying an HDR event between the donor plasmid and *AANATL-7*. Injected G_0 adults were isolated and mated individually to *ebony*^{2} flies. Genomic PCR analysis of male progeny from isogenic strains established from these *ebony* flies was done to detect the *AANATL-7::mCherry* converted gene using primers outside of the donor DNA sequence. *AANATL-7::mCherry* gene conversion was identified in fly lines derived from 2 *ebony*-producing G_0 flies. One G_0 fly (GV-#3) generated 48% *ebony* mutant progeny in its brood and yielded 4/45 *ebony* sub-lines that contained the *AANATL-7::mCherry* gene fusion. A second G_0 fly (GV-#35) generated 70% *ebony* mutant progeny in its brood and yielded 16/32 *ebony* sub-lines that contained the *AANAT-7::mCherry* gene fusion. Examination of accessory glands dissected from *AANATL-7::mCherry* flies using confocal microscopy revealed a strong red fluorescence in male accessory gland main cells consistent with mCherry fluorescence. Importantly, histochemical staining for N-acetylhistamine (NAH) in these *AANATL-7::mCherry* male flies confirmed that secondary cells contained NAH, demonstrating that the AANATL7::mCherry fusion did not disrupt AANATL7 function. These results demonstrate successful CRISPR-mediated HDR gene conversion of *AANATL-7*, providing a powerful tool for future studies concerning its role in male accessory gland physiology as well as in other tissues that AANATL7 is expressed in, including the CNS (4).

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484T A gene encoding a phospholipase A2 (*tecu*) in *Drosophila melanogaster* plays a role in modulating retinal electrical activity.

Laura Alejandra A Lujano Pérez, Juan R Riesgo-Escovar UNAM

Drosophila melanogaster exhibits sophisticated visual behaviors mediated by photoreceptors organized into approximately 800 ommatidia per compound eye. These photoreceptors project to the optic lobe neuropils and from there, interneurons project to higher visual centers, such as the optic glomeruli, central complex, and mushroom bodies, where distinct circuits encode color, brightness, phototaxis, and motion detection. Phototactic responses can be evaluated through Benzer's countercurrent assay and electroretinogram (ERG) recordings. The ERG measures extracellular field potentials generated by the combined activity of photoreceptors and their laminar postsynaptic partners, capturing a sustained receptor potential that reflects photoreceptor depolarization and ON/OFF transients corresponding to synaptic responses in L1–L3 neurons. Together, these approaches provide powerful tools to link genetic and synaptic mechanisms with visually guided behaviors in *D. melanogaster*. Phospholipases A2 (PLA2s) have been proposed to play key roles in phototransduction. Traditionally, PLA2s were considered housekeeping enzymes required for the turnover of unsaturated fatty acids in membrane phospholipids, but they are now recognized as signaling molecules and second-messenger mediators. Moreover, the ratio of phospholipids to lysophospholipids influences membrane dynamics, regulating curvature and vesicle fusion. In this study, we investigated the role of the *tecu* gene, which encodes a putative secreted PLA2, in visual processing through countercurrent assays and ERG analyses. We used the *tecu* mutant line 13256 from the Bloomington Drosophila Stock Center, with w^{1118} flies as controls. Seven-day-old flies were used for both assays. Mutant males showed a significantly reduced response index to 100-lux light stimulation (0.6917 ± 0.1566 rad) compared with *yw* controls (0.9536 ± 0.1020 rad). No significant differences were detected in females. ERG recordings revealed a reduction in the time to two-thirds recovery ($t_{2/3}$: 24.77 ± 6.396 ms) in mutant males compared with *yw* controls (36.41 ± 5.895 ms, $p < 0.05$). Females also showed shorter recovery times (mutant: 22.42 ± 4 ms vs. control: 45.23 ± 12.74 ms, $p < 0.05$). These findings suggest that *tecu* contributes to maintaining proper visual processing in *D. melanogaster*, likely through mechanisms affecting photoreceptor and lamina synaptic transmission, highlighting a potential role of PLA2 activity in regulating visual physiology. Founding: UR307 UNAM.

485T Treat yourself: How neurons modulate food perception and impact aging

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In the words of Ernestine Ulmer, "Life is uncertain. Eat dessert first." While many people would prefer to live the dessert-first lifestyle, tips for healthy aging and longevity instead promote eating a nutritious and balanced diet. However, research suggests that the role of food perception on lifespan is complex. For example, previous work has shown that modifying a fly's perception of its food environment by exposing diet-restricted animals, which normally live longer, to a nutrient-related odorant is sufficient to limit the lifespan-extending effects of restriction without affecting food intake. Therefore, to better understand how the perception of food's tastiness impacts lifespan, our research focuses on how neurons modulate the pleasurable perception of food and how these neurons affect aging.

We tested neural populations that were previously identified as being involved in pleasure based or hedonic feeding, a "need-free" feeding drive that is evoked by the incentive salience of food. By measuring the effect of optogenetic stimulation of targeted neuronal populations on proboscis extension during food presentation, we discovered that subsets of the α'/β' lobes of the mushroom body acutely modulate taste perception and that select protocerebral anterior medial dopaminergic neuronal (PAM DAN) populations do as well. Notably, these same PAM DAN subsets also modulate aging when activated throughout a fly's lifespan. This suggests that neurons that modulate food perception can also mediate long-term, aging effects. In the future, healthy aging and longevity tips may include ways to change one's frame of mind towards their food in addition to diet composition.

486T Sex-specific roles of the diuretic neuron and its receptors in regulating aggression in *Drosophila*

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Aggression is a fundamental behavior evolutionarily conserved across the animal kingdom. It enables animals to secure resources, defend territory and compete for mates. Crucially, the fruit fly *Drosophila* expresses a mammalian CRH-like neuropeptide, diuretic hormone (DH) 44, which regulates stress responses, feeding behaviors and metabolic homeostasis. As a powerful model, the fly offers quantifiable behavioral assays, as well as genetically tractable neural pathways and neuromodulatory systems to investigate the primary neural mechanism of DH44-dependent sexually dimorphic aggression. A major unanswered question in neuromodulatory biology is that how DH44 signaling is divided across its two receptors, R1 and R2, to generate sex-specific aggression in *Drosophila*.

Manipulation of DH44 neurons bidirectionally modulated aggressive behavior, indicating that DH44 signaling is a key driver of aggression. However, this effect did not map uniformly across sexes, suggesting that receptor-specific pathways underlie these differences. To identify the circuitry responsible for this divergence, we independently activated or silenced DH44 R1 and DH44 R2-expressing populations. R1 manipulation altered aggression in both sexes, whereas R2 had selective effects that emerged only under specific manipulations, revealing a sex-dependent dimorphism between the two receptors. These findings indicate that DH44 neurons influence aggression broadly, while the downstream mechanisms may rely on distinct receptor pathways in males versus females.

Together, our data reveal that sex-specific organization within the DH44 signaling system shapes the emergence of aggression. Our work uncovers a previously uncharacterized receptor-level logic within a conserved neuromodulatory pathway and establishes a foundation for understanding how internal state signals are routed through divergent neural circuits to generate sex-specific behavioral outputs.

487T Multiple neural circuit elements of *Drosophila* courtship are repurposed from larval life during metamorphosis

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As juvenile animals mature, they acquire new innate behaviors such as mating and aggression, accompanied by extensive remodeling of the nervous system. In many species, this process involves the addition of newly born neurons that integrate into circuits controlling adult behaviors. However, juvenile neurons have been documented to recycle and reprogram for new functions in adulthood. The molecular mechanisms that enable such neuronal reprogramming remain largely unknown.

We recently identified a population of neurons in the Drosophila larval abdominal ganglion, called the DDAG neurons, that express the *Tlx*-like nuclear receptor *dissatisfaction* (*dsf*). During metamorphosis, a subset of these neurons acquires sexual identity through expression of the sex-determination gene *doublesex* (*dsx*). These *dsf*- and *dsx*-co-expressing neurons subsequently undergo apoptosis in males, but in females they remodel and repurpose for roles in adult mating behaviors. The transformation of these neurons from larval to adult female-specific roles offers a system to dissect the mechanisms governing neuronal reprogramming during maturation.

Here, I will present our work using single-cell RNA sequencing to molecularly categorize these neurons in larvae and adults. This approach has enabled us to precisely match individual adult DDAG neurons to their larval counterparts. We discovered that the DDAG neurons in adults consist of five anatomically distinct groups that contribute to different aspects of mating behaviors in mated and unmated females. These five groups of neurons are derived from a subset of three anatomically and molecularly distinct groups of larval neurons.

These data allow us to now investigate (1) how the larval counterparts of the DDAG neurons contribute to behavior, and (2) the transcriptional program that repurposes juvenile DDAG neurons during metamorphosis. Our findings provide new insight into neuronal plasticity during maturation and illustrate how shared neural substrates can be sculpted into distinct circuits whose functions depend on sex and life stage.

488T Buoyancy increases sensitivity to chemical nociception

Jaime A Arroyo, Vanessa Pando, Jacob Jaszczak Biology, New Mexico State University

One major challenge in spaceflight is proprioceptive adaptation, which is the ability of your body to sense its own position and coordinate movements. When astronauts are in microgravity, they experience altered sensory feedback due to the absence of gravitational forces on their bodies. This results in motor coordination impairments that significantly slow or limit completion of mission tasks (Weber et al. 2017). Astronauts also report increased heightened pain experiences (Sauer et al. 2023). To prepare for these impairments, astronauts practice mission critical tasks under buoyant conditions in large pools. Modeling weightlessness in a controlled laboratory setting presents challenges and therefore the effects of weightlessness on the proprioceptive and nociceptive neural circuits are poorly understood.

We have developed an experimental system to study the effects of buoyancy on nociceptive sensory circuits in *Drosophila* larva. Exposing *Drosophila* larva, that are crawling on a substrate, to high concentrations of hydrochloric acid induces nociceptive behaviors (Lopez-Bellido et al. 2019). We find that larvae will also exhibit acid induced nociceptive behaviors when they are suspended in a liquid droplet. We have begun comparing how larvae respond to acid stimuli if they are crawling (terrestrial) or suspended (buoyant). In a concurrent study, we find that when larvae experience acid damage they lose terrestrial nociceptive sensitivity to acid stimuli (hypoalgesia). In contrast, larvae that experience acid damage and are tested in the buoyant condition do not lose nociceptive sensitivity to acid stimuli. Both terrestrial and buoyant conditions require the activity of the nociceptive C4da neurons. We hypothesize that the increase in sensitivity of larvae in the buoyant condition arises from a change in proprioceptive sensation. Previous studies have found that mechanical sensory inputs can decrease nociceptive sensitivity in larvae through inhibition of second-order neurons (Pan et al. 2023). Our ongoing work aims to determine whether inhibition or activation of proprioceptive circuits can decrease the nociceptive hypersensitivity of buoyant larvae. This system could be an effective model for understanding how the neurological processes of proprioception modulate nociceptive sensitivity.

489T Evolution of behavioral dynamics underlying mechanosensory oviposition preference in *D. suzukii*

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The agricultural pest *Drosophila suzukii* has evolved a unique egg-laying behavior, marked by a preference for ripening fruit, which has aided its significant ecological expansion. Underlying this novel behavior is the evolution of multiple sensory systems, including mechanosensation, which enables oviposition on firmer substrates. However, the understanding of *Drosophila*'s mechanosensation-based egg-laying is limited. Assessment of oviposition of *D. suzukii*, as well as its two sister species *D. biarmipes* and *D. subpulchrella*, across varying mechanosensory environments found that *D. suzukii* has evolved heightened sensitivity to substrate stiffness as well as a greater tolerance of firmer substrates. We evaluated egg-laying in a decision-making context by conducting two-choice experiments. Surprisingly, a preference for laying eggs on the firmer of two options was not robust and varied based on the comparison of substrates. High-resolution behavior tracking of *D. suzukii* during decision-making trials found that flies exhibit preferential, sex-specific locomotive behavior, which is highly correlated with oviposition. We used survival analysis of egg-laying bouts as a function of time and fly locomotion to develop a statistical framework for the study of oviposition decision-making. Our model accounted for a potential interactive effect between substrates, which would suggest flies make direct relative comparison between options. However, this was only identified as significant in one experimental setting, where oviposition preference was greatest. In others, locomotive behavior was identified as the significant predictor of oviposition. These results offer an alternative perspective to the idea that *Drosophila* oviposition decisions are made using relative value comparison of available options, but that inherent substrate qualities may modulate oviposition to elicit preference. Our study provides valuable insight into the study of the evolution of complex, quantitative behaviors.

490T Unraveling aspartame induced anxiety: Insights from Drosophila Melanogaster

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The use of artificial sweeteners has become increasingly prevalent in modern diets over recent decades, driven by rising concerns about sugar intake and metabolic health. Well-known to aid in weight loss and the management of blood sugar levels in diabetics, many of these sugar substitutes, like aspartame, are shown to negatively affect neural circuitry and are linked to the onset of anxiety disorders. While anxiety research has traditionally relied upon mammalian models, this study aims to take advantage of the *Drosophila* model, which offers a genetically tractable and highly conserved system to study the molecular mechanisms underlying these behavioral changes. To evaluate aspartame-induced anxiety-like behaviors, we conducted the wall-following (WAFO) and Struggle Response in Immobilized Fly (STRIF) assays after flies were exposed to a 0.05% aspartame diet for 7 days. With increased serotonin signaling known to be associated with the onset of anxiety-like symptoms, we then used quantitative PCR (qPCR) to evaluate the expression of the neuronal serotonin transporter, dSERT. Through this multidisciplinary approach, we seek to uncover the neurobiological and genetic underpinnings of how artificial sweeteners can impact brain function and behavior; while validating *Drosophila* as a powerful model for studying anxiety.

491T Influence of Glyphosate and Roundup on *Drosophila melanogaster* Mating and Courtship Behavior

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Glyphosate, a widely used herbicide found in many weed killers, is used to eliminate unwanted leaves around crops. This raises concerns because it can impact our health, and the health of affected environments. Previous results from our lab indicate that glyphosate-based herbicides affect survival (particularly of larvae), reproduction (ovary size and # offspring) and behavior (feeding, activity, geotaxis) of flies. This study tests the hypothesis that Roundup Super Concentrate (RSC), a GBH, affects reproductive behavior of D. melanogaster, specifically courtship and mating. In this experiment, one female and one male fruit fly, reared on the same or different treatments for a period of 7 days, were transferred into each of the ten chambers of a mating wheel without anesthesia to preserve natural behaviors. Flies from different treatment groups, control, glyphosate at 2 g/L and 5 g/L, and RSC at 2 g/L and 5 g/L, were placed in separate chambers. Throughout the experiment, we carefully recorded male courtship behaviors, observing each pair of flies once per minute for 15 minutes, specifically noting following, singing, licking, and attempted mating. We documented successful mating as an indication of female receptivity, a vital reproductive dynamic. After the observation phase, each pair was transferred into a vial containing control food for an additional 14 days, and we monitored the vials to assess reproductive output. This was quantified by counting the number of pupae and adult offspring produced. Unlike other behavioral assays conducted in our lab, treatment (Control, glyphosate, RSC) of either sex did not affect courtship or copulation frequency. However, both glyphosate- and RSC-exposed females produced fewer daughters and sons than control females, with no difference between glyphosate and RSC. These reproductive outcomes suggest a concerning trend; exposure to glyphosate-based herbicides may detrimentally affect the health of insect populations in agricultural areas, but not through neural/behavioral disruption. This highlights the important need to reconsider the implications of GBH use in our ecosystems.

492T Roundup Super Concentrate Exposure Decreases Activity of *Drosophila melanogaster*: Potential mechanisms

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Glyphosate-based herbicides (GBHs) kill weeds and leaves around produce. GBHs elicit great concern because food residues may affect our health and the health of the environment. This study examines whether Roundup Super Concentrate (RSC), a GBH, affects different mechanisms leading to changes in level of activity. The first experiment looks at activity levels, using fruit flies, *Drosophila melanogaster*. We expose flies to 10, 5, or 2 g/L RSC, 10g/L glyphosate, 2g/L POEA, a combination of 5g/L glyphosate and 2g/L POEA, or control medium before using a *Drosophila* Activity Monitor to record how often the flies cross a light beam in each 24-hour trial. We tested 20 individual flies of each age, 3, 5, and 7 days, for both males and females for each treatment. In addition, some flies were exposed to the 10 g/L RSC treatment for two days before being released to the control medium to assess whether the effects of exposure are permanent or whether their activity levels can recover. We found that exposed flies exhibited less activity, especially females and especially at higher concentrations. Released flies only partially recovered, which might indicate that RSC toxicity occurs through multiple mechanisms. Exposure to glyphosate and POEA, alone or in combination, does not affect their activity levels, indicating that other ingredients in the formulation are responsible for this effect. Next, we looked at whether RSC 10g/L affected protein levels as a corollary of nutritional state. Activity data was collected, and then flies were homogenized for protein analysis using a Pierce BCA assay kit. There was no correlation between activity levels and protein when exposed to RSC 10g/L. However, flies whose protein level was experimentally modified by adding bakers yeast to the surface of the medium or removing nutritional yeast from the medium, did differ in protein content while activity was affected by RSC but not protein treatment. To examine the role of the neuromuscular system in RSC toxicity, we measured negative geotaxis in group assays where all flies are tapped to the bottom of the chamber and the number who cross the midpoint within 10 seconds recordeed. Exposed flies exhibited both less geotaxis and lower activity levels. This suggests that RSC does not reduce activity by impacting protein content of the flies, but it's mechanism of toxicity likely involves the nervous system. Reduction in activity is important for animal fitness because it impacts the ability to find food, find mates, and avoid predators.

493T Embryonic Blm-deficiency leads to sleep and circadian disruption in adult *Drosophila*

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In *Drosophila melanogaster*, the absence of maternally loaded Blm protein during early embryonic development leads to DNA damage and high embryonic lethality. How this early DNA damage affects physiological function in surviving adults is unknown. We investigated whether DNA damage caused by Blm-deficiency disrupts sleep and circadian rhythms. To test our hypothesis that early embryonic DNA damage affects sleep and circadian regulation, we collected adult progeny from *Blm*– mothers crossed to *Blm*+ fathers and from the reciprocal cross, *Blm*+ mothers crossed to *Blm*– fathers. In both crosses, surviving progeny were heterozygous for *Blm*, but one set developed with maternally loaded Blm protein (from *Blm*+ mothers) and one without (from *Blm*– mothers). Using continuous activity monitoring, we measured sleep and circadian patterns under 12 hours light:dark cycles as well as both constant darkness and constant light. Both male and female flies from *Blm*– mothers showed disrupted sleep patterns with increased daytime sleep and reduced nighttime sleep during normal light:dark conditions and atypical circadian rhythms (arrhythmic or outlier periods) in constant dark and constant light conditions. In constant darkness, these flies exhibited sleep deficits and atypical circadian rhythms, consistent with weak internal circadian regulation. In constant light, flies from *Blm*– mothers slept more than controls, possibly because reduced sensitivity to light cues diminished light›s normal sleep-suppressing effect. These findings suggest that Blm-deficiency-induced DNA damage during early development has long-lasting effects on the systems regulating adult sleep and circadian behavior.

494T Exploration of Robo3 Function in Hemilineage 14A during Postembryonic Development in the Ventral Nerve Cord

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Drosophila melanogaster goes through two phases of neurogenesis. The embryonic phase establishes the larval central nervous system (CNS); the postembryonic phase, from 2nd instar to late pupal stage, establishes the adult CNS. Axon guidance molecules have been extensively researched in the embryonic stage for their role in establishing the larval CNS, but how they organize the adult CNS is not well-understood.

The ventral nerve cord (VNC) of Drosophila is analogous to the mammalian spinal cord, performing motions as simple as kneeling and as complex as take-off. Hemilineages are the fundamental unit of the VNC; they are defined as neurons that arise from the same neuroblast, have similar transcription factor identity, and express the same neurotransmitters (glutamate, GABA, or acetylcholine). Hemilineages provide a framework for understanding circuit formation during development. We lack clarity for how hemilineages express axon guidance molecules during the pupal stage to form the neuronal circuitry present in a healthy adult. The Roundabout (Robo) family mediates axon repulsion from the midline and organizes axons into lanes which differ in their distance from the VNC midline. To understand the role of axon guidance molecules in the pupal stage, I analyzed Robo3 expression in a hemi-lineage restricted manner.

We first looked at Robo3 protein expression at 24 hours after puparium formation (APF), finding that Robo3 was expressed at this time stage. Interestingly, its expression pattern appeared restricted to certain regions. We next analyzed whether *robo3* was expressed in all cells or only a subset. We accomplished this by using a *robo3-GAL4* driving expression of tdTomato (RFP). At 24 hours APF, a majority of neurons in the VNC expressed Robo3. When stained against Robo3, part of the expression pattern strongly resembled the projections of hemilineage 14A. Curious, we stained against Robo3 in our 14A driver. We found that Robo3 protein expression broadly overlapped with hemilineage 14A. Based on this strong overlap, we next hypothesized that, in the absence of *robo3*, 14A axon architecture would be disturbed. Interestingly, when robo3 is completely lost, 14A axon architecture is still intact in the adult. This implies that *robo3* is redundant in hemilineage 14A. In future experiments we will examine Robo1 and Robo2 protein expression at 24 hours APF, and then test whether Robo1 or Robo2 compensate for the loss of *robo3* during the development of hemilineage 14A.

495T A role for proprioceptors in efficient olfactory navigation

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Proprioception is the sensory process encoding the body position and movement of an organism. Proprioceptors are specialized cells that sense displacement and movement of the body. The class I md neurons ddaE and ddaD of *Drosophila melanogaster* larvae function as proprioceptors. The dendrites of the class I md neurons project dendrites to each hemi-segment of the larval body wall and undergo deformations as the peristaltic muscle contractions travel along the longitudinal axis of the body during crawling. Mutations in *Drosophila transmembrane channel-like (Tmc)* prevent Ca^{2+} responses of the ddaE and ddaD neurons during movement, indicating that TMC is required for neuronal activation during crawling. Given TMC's role in proprioceptor activation during crawling, we wondered whether loss of TMC would impair larval navigation. To test this hypothesis, we turned to an olfactory navigation assay. Larvae will perform a combination of head casts, turns, runs and stops to navigate towards an attractive odor, i.e., food. As they head cast, larvae sweep their head left to right to identify the direction of greater concentration and subsequently turn their bodies to initiate a run in that heading before stopping and repeating the sequence. Our results demonstrate that TMC mutant larvae navigate along an olfactory gradient with diminished efficiency. TMC mutant larvae require a greater amount of time to reach an attractive odor source, performing large c-bend head-casts which extend all the way to the posterior region of their bodies, rarely observed in control larvae. These results suggest that early stages of navigation are likely impaired, particularly the association between head casting and olfactory inputs. The inability to coordinate these sensory inputs potentially accounts for most of the delay in reaching an odor source efficiently.

496T Dynamic microbial associations alter chemosensation in *Drosophila melanogaster*

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Vector-borne diseases remain a growing global challenge as environmental change expands insect populations and their interactions with humans. Chemosensation—the ability of insects to detect and respond to odors—drives host-seeking, foraging, and mating behaviors that underlie disease transmission. Recent studies show that gut microbes influence chemosensory receptor function and behavioral valence, but most focus on stable endosymbionts rather than transient, environmentally acquired species. Understanding how these transient microbes modulate olfactory signaling could reveal novel, eco-friendly tools for insect control.

This study investigates how three transient gut microbes—*Serratia marcescens*, *Enterococcus durans*, and *Pseudomonas aeruginosa*—influence odor-guided behavior and receptor activity in *Drosophila melanogaster*. I hypothesize that these microbes secrete metabolites or signaling molecules that modulate transcriptional or neuronal activity within the olfactory system, thereby shifting behavioral attraction or avoidance. Adult Y-maze and larval two-choice assays test odor preference toward 10^{-1} 1-octanol diluted in DMSO across three genotypes: wild-type, $Orco^{-}$ (odorant receptor co-receptor deletion), and $Ir25a^{-}$ (ionotropic receptor co-receptor deletion).

Preliminary results reveal significant differences in odor response between axenic and microbe-associated flies. Axenic $Ir25a^{-}$ adults display aversion to 1-octanol, while $Orco^{-}$ mutants show attraction, suggesting distinct receptor-class contributions to odor valence. Reintroducing *S. marcescens* partially restores attraction in $Ir25a^{-}$ flies, indicating microbial modulation of ionotropic signaling. Early larval assays reveal weaker or inconsistent responses, possibly due to developmental immune barriers limiting microbial colonization.

Collectively, these findings suggest that transient microbes dynamically shape olfactory-driven behavior through receptor-specific pathways. By uncovering how microbiota alter chemosensory signaling, this research advances our understanding of interkingdom communication and provides insight into new, microbiome-informed strategies for disrupting disease-vector host-seeking behavior.

497T Microbial quorum-sensing metabolites modulate insect olfaction and vector competence

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Insects impose a major burden on global health and agriculture, causing an estimated $70 billion in annual crop losses and over one million deaths through vector-borne diseases such as malaria, dengue, and Zika. *Aedes aegypti* is a principal vector of these pathogens, yet insecticide-based control is failing due to resistance and ecological harm. The insect microbiome—microbes inhabiting salivary glands, midgut, and appendages—has been linked to vector competence, but the mechanisms connecting microbial activity to insect behavior remain unclear.

Symbionts such as *Serratia marcescens, Asaia bogorensis, Pseudomonas aeruginosa, Acinetobacter calcoaceticus, and Chryseobacterium spp.* influence transmission, development, and immune modulation. These bacteria produce quorum-sensing (QS) molecules—diffusible signals that coordinate microbial behavior—which may also interact with host chemosensory systems. Because mosquitoes locate hosts primarily through olfaction, microbial QS metabolites could alter expression of olfactory receptors, reshaping host-seeking and biting behavior.

I hypothesize that QS secretions from these taxa, particularly those inhabiting the *A. aegypti* maxillary palp, modulate transcription of olfactory co-receptors such as ORCO and IR25a. To test this, I combine microbiological, molecular, and neurogenetic approaches across *A. aegypti* and *Drosophila melanogaster*. We validated the presence of *S. marcescens* in Drosophila via PCR and are isolating additional microbial taxa from environmental and mosquito sources. Early co-culture assays show *S. marcescens* and *P. aeruginosa* dominate under nutrient-limited conditions, suggesting they may persist and influence signaling within the palp.

Microbial secretomes and purified QS fractions will be screened using luciferase reporters under ORCO and IR25a promoters to identify metabolites that induce chemosensory gene expression. Active fractions will undergo LC-MS/MS analysis, while transcriptomic and behavioral assays will establish links between microbial signals, receptor regulation, and host-seeking responses.

This work reveals how bacterial metabolites reprogram insect olfaction and influence vector competence. By identifying microbes and QS molecules that modulate chemosensory sensitivity, it highlights new microbiome-based strategies for disrupting disease transmission without reliance on conventional insecticides.

498T Investigating the Contribution of Morphological Divergence to Behaviour Using Artificial Intelligence.

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Male genital morphology plays a critical role in mating success and reproductive isolation, yet its behavioural and fitness consequences remain poorly understood. In fruit flies, the closely related species *Drosophila simulans* and *D. mauritiana* (which diverged less than 250,000 years ago) exhibit differences in the size and shape of their genital structures. Males of *D. simulans* have smaller claspers (paired grasping structures with mechanosensory bristles) and larger posterior lobes than *D. mauritiana* males. At the same time, females differ in the length of the vaginal plate.

Previous research in our lab identified the gene *tartan* as contributing to differences in male clasper size and female vaginal plate length between these species. We used CRISPR/Cas9 to generate *D. simulans* males with *D. mauritiana*-like claspers and females with *D. mauritiana*-like vaginal plates. We examined how these morphological changes affect mating behavior and reproductive outcomes by conducting high-throughput mating-behavioral assays and applying computational approaches to track and quantify mating behaviors, such as copulation latency, copulation duration, and the mobility of the copulating pair.

Males with *mauritiana*-like claspers mated less often, took longer to initiate copulation, and had shorter copulation durations compared to *simulans*-like males. Despite these apparent behavioural disadvantages, females that mated with males carrying mauritiana-like claspers produced more progeny. Moreover, when paired with *mauritiana*-like vaginal plates, these males showed improved mechanical fit and copulation stability, partially restoring mating performance. Together, these results suggest that subtle, coordinated changes in both male and female genital morphology can alter copulatory and postcopulatory interactions, with trade-offs among mating success, behavioral compatibility, and fertilization efficiency likely contributing to the co-evolution of male and female genitalia and to reproductive divergence between species.

These findings suggest that mauritiana-like clasper morphology influences both the mechanics of copulation and postcopulatory sperm use, revealing an unexpected trade-off between mating success.

499T Neuromodulation of Social Behavior and Aging in *Drosophila*

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In nature, social animals create groups to increase productivity and protect against predators. For humans, social interaction is known to be essential for physical and mental health as well as longevity. However, the underlying neural and physiological mechanisms that govern this crucial relationship remain poorly understood. Previous work in our laboratory has established that, in the fruit fly *Drosophila melanogaster*, social interactions modulate health and aging through neuropeptide signaling pathways that are evolutionarily conserved. For example, we found that social interaction with living and dead conspecifics acutely influences stress resistance and fat storage while regulating lifespan in a manner that involved serotonin signaling. In males, social interactions involving sensory perception of female pheromones causes rapid and reversible NPF-dependent decreases in fat stores, starvation resistance, and lifespan.

My research, using *Drosophila melanogaster* as a model, investigates how genetic and environmental factors shape social behaviors and modulate lifespan. I have found that flies exhibit reproducible social tendencies and that these tendencies are modulated by aging as well as genetic and environmental perturbations. Social spacing increases with aging and with inhibition of mechanosensory pickpocket1 (ppk1) neurons, whereas optogenetic stimulation of neurons that produce brain insulins increases pairwise social interactions. In parallel, I have also found that increased social density significantly reduces lifespan and that this effect is reversed by environmental enrichment and by silencing of insulin-producing neurons. Ongoing research aims to elucidate how insulin signaling and environmental enrichment mediates social effects on aging and to identify novel neural pathways and environmental contexts that shape the relationship between social experience and longevity. By leveraging cutting-edge behavioral tracking and neuromodulatory techniques, this work provides insight into the conserved mechanisms by which social interactions regulate aging and offers potential targets for interventions that promote healthy lifespan.

500T Characterization of sleep and impacts of sleep deprivation on sensorimotor behavior in tethered flight across Drosophila species

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To generate contextually appropriate behavior, organisms must prioritize certain sensory cues, ignore background noise and generate a behavioral output based upon those assignments. This relies on discriminating, identifying, and assigning subjective value or valence to salient signals, even amidst a noisy sensory backdrop. The neuronal circuits performing these assessments must be flexible enough to accommodate different internal and external states, yet be sufficiently stereotyped to reproduce adaptive behavioral responses. In other words, a given input might produce a variety of outputs depending on the current internal or external state of the animal, and gaining an understanding of how these processes work is the focus of our work. We use sleep as a model for investigating how changes in internal state are integrated and encoded to influence the perception of sensory stimuli and resulting generation of contextually appropriate behavior. Additionally, to begin to understand how environmental pressure might shape dynamic decision-making we take a comparative approach, examining Drosophila species from varied ecological habitats. We expand upon recent findings, using the Drosophila Activity Monitor (DAM) to characterize sleep of cosmopolitan and desert flies, that revealed an increased sleep drive in desert-dwelling Drosophila mojavensis species (Yano et al., 2024), by characterizing additional species. We also take a parallel approach, using FlyVISTA (Keleş et al., 2025) to generate deep phenotypes of sleep across species from varied ecologies. Following baseline sleep characterization using DAM and FlyVISTA, we examine the impacts of sleep deprivation across species by measuring effects on sensorimotor output, via modulation of saccade dynamics, and odor tracking behaviors in tethered flight. These studies set the foundation for further comparative research investigating the underlying circuit mechanisms that confer flexibility, robustness, and resiliency across habitats with temperate and extreme environmental pressures.

501T easyClock: a user-friendly tool for circadian rhythm analysis and visualization

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Circadian rhythms are conserved across species, from *Drosophila* to humans, and their precise characterization is essential for understanding behavioral and physiological oscillations. Modern circadian research increasingly relies on high-resolution behavioral and multi-omics data. To address the need for a standardized, versatile, and easy-to-use analytical tool, we developed easyClock, a standalone desktop software designed to streamline circadian rhythm analysis and visualization. This software features:

(1) one-step installation on Windows and macOS;

(2) a fully graphical, code-free user interface;

(3) comprehensive rhythmicity detection methods covering unimodal/bimodal patterns, Cosinor-like curves, and other diverse waveform structures;

(4) enhanced robustness for handling outliers, autoregressive noise, and non-stationary oscillations.

(5) built-in support for summarizing group comparisons and inter-variability; and

(6) visualizations including time-series plots, actograms, and regression model fits.

As a demonstration, we applied easyClock to re-analyze a *Drosophila* time-series transcriptomic dataset, illustrating its ability to detect rhythmic gene expression across large datasets. Overall, easyClock provides a validated, open-source platform that enables circadian researchers—regardless of coding background—to perform high-quality analysis locally and efficiently.

502F Experience-dependent modulation of Drosophila feeding behavior

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From flies to mammals, the taste system plays a crucial role in making feeding decisions. Attractive tastes such as sugar promote feeding, while aversive tastes such as bitter and high salt concentrations suppress feeding. In addition to using taste input to regulate immediate feeding behavior, flies integrate sequential taste cues when making feeding decisions. Previous studies from our lab found that feeding responses are enhanced by recent exposure to sugar taste and suppressed by recent exposure to bitter taste, and this modulation lasts for seconds to minutes. In ongoing work, we are investigating the neural mechanisms underlying this modulation. First, we are testing whether experience-dependent modulation of feeding responses occurs only between specific combinations of taste inputs, which would require cross-talk between specific taste pathways, or whether any stimulus may create a general “pro-feeding” or “anti-feeding” state that modulates future responses to any other stimulus. Second, we are using optogenetic manipulations to investigate whether neurons that regulate immediate feeding responses also modulate future feeding responses. Finally, we are using calcium imaging to determine which node(s) of the sensory-motor circuit for feeding responses are modulated by recent taste experience to enable flexible behavior. Together, these studies provide insight into the neural mechanisms underlying experience-dependent modulation of behavior.

503F Discovery and Development of Anxiolytic Agents from Tropical Marine Algae using *Drosophila melanogaster* as a Behavioral Model

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Based on reports from the World Health Organization (WHO) and the National Institute of Mental Health (NIMH), one third of the population is affected by anxiety disorders. These are often treated with benzodiazepines and antidepressants, which can lead to tolerance and dependence. This highlights the need for safer and more effective anxiolytic therapies. Marine natural products, particularly from tropical brown algae, offer promising alternatives due to their bioactive metabolites with neuroprotective properties. *Stypopodium zonale* has emerged as a potential source of such compounds. Our research focuses on studying the anxiolytic effects of natural products derived from tropical marine algae from the coastal waters of Puerto Rico, using the fruit fly *Drosophila melanogaster* as a model organism. We aim to chemically characterize algae extracts, conduct anxiety-related behavioral tests in *D. melanogaster*, and propose a mechanism of action underlying the observed anxiolytic effects. By identifying the proteins that are upregulated or downregulated after acute exposure, we seek to uncover how these extracts alter protein networks and influence anxiety pathways. The methodology involves exposing fruit flies to algae extracts through both chronic (from oviposition to adulthood) and acute (6 hours) treatments. Following exposure, anxiety-like behavior is assessed using Open-Field (OFT) and Dark Light Box (DLB) tests, where wall-following behavior — *Drosophila’s* natural tendency to stay close to the arena’s edges— is quantified. A Wall-Following Decrease Index (WFDI) is calculated to measure reductions in wall-following behavior, based on the distance the flies travel away from the walls toward the center of the arena. Rapid Iterative Negative Geotaxis (RING) assays were performed as a counter-assay to confirm that the algae extracts do not affect locomotor activity. In the DLB test the latency time measures how long it takes for *Drosophila melanogaster* to enter the dark compartment for the first time, where a shorter latency indicates lower anxiety. The time spent in each area reflects anxiety levels: spending more time in the illuminated zone suggests less anxiety, while less time indicates greater anxiety. Recent data has shown that an organic extract of *Stypopodium zonale* has anxiolytic effects, comparable to the effect of diazepam, a benzodiazepine used as our positive control. Statistically significant anxiolytic effects were also obtained for *Dictyota cervicornis and Padina boergesenii*, although no anxiolytic effects were observed for *Lobophora variegatta*, *Ulva*, *Symploca*, and *Sargassum sp.* species. Our research represents an unprecedented approach to anxiolytic drug discovery as it improves our understanding of Puerto Rico’s marine algae and their natural product’s chemo-diversity.

504F Genetic variation in oviposition and feeding preference under copper stress reveals divergent behavioral responses across *Drosophila melanogaster* recombinant inbred lines

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Behavioral resistance functions as an adaptive aspect of sensory physiology and evolution, when organisms alter food consumption or oviposition to avoid adverse conditions. Such behaviors may arise prior to or together with physiological tolerance, influencing evolutionary adaptations to environmental stressors. We studied copper-induced alterations in reproductive and feeding behaviors among recombinant inbred lines (RILs) derived from the Drosophila Synthetic Population Resource (DSPR). We analyzed the variation across recombinant inbred lines (RILs) in two vital traits that influence survival in contaminated environments: oviposition decision making and feeding preference. Females from 88 RILs were assayed in custom made oviposition chambers offering a choice between control medium and 10 mM $CuSO_4$. Preference indices varied widely (ANOVA: F_{87}^{5}, $_{263}$ = 10.9, $p < 0.0001$), ranging from strong avoidance (PI ≈ −0.9) to mild attraction (PI > 0). These values were highly repeatable across replicates and yielded high broad-sense heritability ($H^2 \approx 0.88$) based on RIL means. Parallel high-throughput microplate feeding assays quantified feeding preference for 2 mM copper in 197 RILs. Both sex and genotype strongly influenced feeding response (Sex: $F_1 = 350$, RIL: $F_{196} = 14.3$, both $p < 0.0001$), with males and females displaying partially correlated strategies ($r = 0.74$). Additionally, feeding preference also showed high heritability ($H^2 \approx 0.87$) and a significant QTL was detected on chromosome 2R, providing a targeted region to search within for possible candidate genes. Despite shared ecological context, oviposition and feeding responses were only weakly correlated across lines ($R^2 = 0.02$), suggesting that different genetic mechanisms regulate copper detection in gustatory vs reproductive contexts. These findings show a range of behavioral strategies from extreme avoidance to relative tolerance, suggesting diverse evolutionary responses to metal contamination. QTL mapping and genomic annotation are being used to identify possible copper decision-making candidate genes. Future research will expand the scope of oviposition assays to cover more RILs and use specific RNAi knockdown to confirm the function of candidate genes. Eventually, combining behavioral, physiological, and molecular information, this study will reveal the mechanisms behind the interaction and co-evolution of different facets of copper resistance, thus leading to a general understanding of the basis of adaptation in metal-contaminated environments.

505F Examining Nutrient-Dependent Plasticity of Sleep-Wake Rhythms in *Drosophila*

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Normal sleep and circadian rhythms during early life are important for brain development. Indeed, disruptions to circadian rhythms early in life can lead to long-term effects on adult behaviors, neuronal morphology, and circadian physiology. Although the molecular mechanisms encoding cellular rhythms are well understood, little is known about how rhythmic behaviors first emerge. Our previously published work showed that sleep-wake rhythms develop in early 3rd instar *Drosophila* larvae (L3) due to the formation of a circuit between the DN1a clock neurons and Dh44 neurons in the *pars intercerebralis* (clock output center). Additionally, our published work demonstrate that formation of this circuit is due to changes in the metabolic state as larvae raised on low nutritional conditions (1.2% glucose) or forced to adopt constant feeding strategies do not develop sleep-wake rhythms. Here, we examine how the nutritional environment influences DN1a-Dh44 circuit development across the lifespan. We show that the loss of sleep-wake rhythms in low nutrient environments is nutritionally plastic. Larvae raised on low glucose until early L3 and then moved to regular sugar diets (8% glucose) for 4 hours show a recovery of sleep-wake rhythms with an increase in sleep duration at night. Our preliminary data indicate that larval Dh44 glucose metabolism genes are necessary for this behavioral plasticity, suggesting that Dh44 neurons themselves may sense the nutritional environment to drive sleep-wake rhythm development. Finally, we examine the effects of low glucose diets on adult sleep timing and investigate the potential roles of the adult DN1a-Dh44 circuitry in this process. Together, our data provide new insights into how sleep-wake rhythm circuits develop in the context of changing nutritional cues. Future work will examine neural circuit formation and plasticity under changing nutrient conditions.

506F Investigating how chronic hunger modulates physiology and aging in *Drosophila*

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Neural states—and the motivational drives that they create—unify our understanding of how different environmental cues have profound effects on aging, across species. Previous work has shown that cues about danger, food, time, and social environment elicit perception-dependent neural states that modulate aging. Research in our lab investigating feeding-related brain states using the fruit fly, *Drosophila melanogaster*, has established that the perception of hunger induces a neural and motivational state that drives changes in physiology and aging. We have found that flies experiencing chronic hunger exhibit altered feeding behavior and increased lifespan. However, we lack a complete understanding of the neuronal and molecular mechanisms underlying hunger-driven neural states and their effects on aging. Furthering our understanding of this relationship may uncover important details about the aging process and strategies to delay or eliminate the onset of age-related diseases.

My research aims to understand how chronic hunger is neuronally and molecularly encoded in the brain, and how it works to modulate physiology and aging in *Drosophila melanogaster*. My work focuses on interrogating two different models of hunger in the context of feeding and aging. In a dietary model of hunger, we have shown that diets low in branched-chain amino acids (BCAAs) acutely increase feeding and hunger compared to diets high in BCAAs, but that over time this relative difference disappears. Similarly, we have found that optogenetic activation of hunger-producing R50H05 neurons increases feeding and hunger compared to controls, but with chronic activation we observe a decrease in this effect. These results suggest that chronic hunger leads to physiological changes resulting in a decrease in feeding over time, and we propose that these changes reflect an adaptation to chronic hunger, which may have consequences beneficial to the health of an organism. Importantly, we have also found that flies experiencing chronic hunger—either through low BCAA diets or R50H05-activation—extend lifespan, suggesting that chronic hunger exerts beneficial, modulatory effects on aging. Together, these results support a working model in which chronic hunger drives homeostatic and physiological adaptations that slow aging. Ongoing work will test this model at the neuronal and molecular levels, with a particular focus on the role of serotonergic signaling.

507F Leveraging fungal metabolites for sustainable biocontrol of spotted-wing *Drosophila*

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The management of agricultural pests remains a critical global challenge, growing increasingly urgent as the world's population surpasses 8.2 billion and food demand intensifies. Among the most destructive emerging threats to fruit production is *Drosophila suzukii* (spotted-wing *Drosophila*, SWD), an invasive species that uniquely infests ripe, market-ready fruit. Unlike other *Drosophila* species that target decaying fruit, *D. suzukii* oviposits in intact fruit, causing punctures and tears that promote microbial infection and rapid spoilage, leading to substantial economic losses.

Entomopathogenic fungi (EPF) represent a promising alternative to chemical insecticides, yet their field efficacy remains constrained by environmental instability. To advance their potential as biocontrol agents, we isolated 12 fungal strains from soil and plant material collected in highly predated berry farms across New Jersey. These isolates are currently being identified and tested in behavioral assays to assess their effects on *D. suzukii* physiology and behavior.

Additionally, we have identified a fungal exudate—originally identified from *D. melanogaster* fecal material—that significantly reduces *D. suzukii* survival. Ongoing work aims to determine whether this effect is mediated through behavioral avoidance, altered feeding, disrupted mating, or reduced oviposition.

We hypothesize that fungal strains differ in their ability to infect and modulate host behavior and reproduction under varying environmental stressors, and that specific metabolic byproducts drive these differences. This research will inform the development of more robust, environmentally sustainable fungal biocontrol strategies targeting *D. suzukii* and other major agricultural pests.

508F The Effect of Circadian Rhythm on Social Experience Acquisition and Retention

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The ability to rapidly comprehend and process information from the surrounding environment is a crucial skill for social animals. Prior research has shown that chronic, but not acute, social isolation reduces sleep in *Drosophila*, but the kinetics of this process and the detailed changes in sleep architecture remain less characterized. Here, we first replicated previous findings that social isolation decreases daytime sleep and further demonstrated that this reduction is later compensated by an increase in nighttime sleep. We also analyzed additional sleep parameters to better understand the effects of social isolation on sleep profiles. To examine the interaction between social experience and light-dark cycles, we socially and individually housed fruit flies under normal light-dark cycles, constant light, and constant dark, and assessed their social experience acquisition and retention level. The results indicate that social experience acquisition is most robust under normal light-dark cycles, but remains significant under other conditions. Building on these findings, we asked about the transcriptional difference between socially housed and individually housed flies. We found that CREB activity in the Mushroom Body of the socially housed flies was significantly higher than that of their individually housed counterparts. Notably, the CREB activity ratio between socially and individually housed flies exhibits a peak during nighttime that corresponds to the period when the two groups differed in nighttime sleep. Taken together, these results unveil the relationship between social experiences and circadian regulation of sleep.

509F Developmental stage-specific genetic manipulations of the neuropeptide *Pdf* result in sexually dimorphic phenotypes in rhythmic behavior.

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Circadian Clocks synchronize animal physiology and behavior with cyclic environment changes and regulate these processes on a 24-hr. cycle in the absence of external cues. The *Drosophila melanogaster* circadian network comprises of a highly interconnected network of ~240 neurons which rely on synaptic and peptidergic mechanisms to regulate behavioral rhythms. The small ventral lateral neurons (s-LNv) are considered the main pacemaker cells and release the neuropeptide Pigment Dispersing Factor (PDF) which acts as a major synchronizing factor and an output of the network. Males with null mutations in *Pdf* or its receptor, *PdfR* as well as *Pdf* CRISPR knockout in clock neurons show mostly arrhythmic sleep-wake cycles and increased sleep levels. However, recent studies from our lab have shown that female sleep-wake rhythms are less affected by the loss of *Pdf*. Since sexual dimorphism in the nervous system largely originate during development, and to distinguish between sex-specific roles of PDF in early development versus adults, we performed *Pdf* knockdown experiments in males and females throughout development or only as adults using the TARGET system. Our results suggest stage and sex-specific roles of *Pdf* in the regulation of behavioral rhythms. *Pdf* knockdown constitutively across development and adult stages results in significant loss of rhythmicity, short period in sleep-wake cycles, and increased sleep, although females were less affected than males. This is also accompanied by anatomical defects in the s-LNv neuronal projections, including misrouting, as previously reported. In contrast, adult-specific *Pdf* knockdown did not result in s-LNv neuronal terminal misrouting or affect period of sleep-wake rhythms in either sex but decreases behavioral rhythmicity only in males. Interestingly, development-specific knockdown of *Pdf* results in misrouting of s-LNv termini in both sexes and different behavioral outcomes in each sex: loss of rhythmicity in males and shortening of period in females. We also 'feminized' the *Pdf*- expressing neurons in males by expressing *transformerF* and found that clock parameters such as daily anticipation of light-dark transitions and the period of rhythms in males resemble those of female flies. Our results suggest that *Pdf* regulates distinct rhythmic properties in development versus adulthood, with this regulation differing between the sexes, and sexual dimorphism in circadian parameters can be partially attributed to the cell-intrinsic properties of the PDF-expressing neurons.

510F Investigating the Novel Role of the Activated Cdc-42-Assosciatied Tyrosine Kinase (ACK1) in Learning and Memory in *Drosophila melanogaster*

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In this study, the role of *Drosophila* activated Cdc-42 kinase (DAck) in learning and memory was investigated. DAck is the *Drosophila* homolog of the activated Cdc-42-associated tyrosine kinase (ACK1). ACK1 has been characterized for its anti-apoptotic function, leading to cell proliferation and differentiation in cells, and its role in lung, prostate, pancreatic and breast cancer. ACK1 has also been reported to have high expression levels in the brain, particularly in the hippocampus, neocortex and cerebellum. However, the function of ACK1 in the brain has not yet been characterized. Taking advantage of an appetitive associative olfactory learning assay, the *Drosophila DAck*^{86} null allele was used to research the role of DAck in learning and memory. Results indicate that *DAck*^{86} mutant larvae have statistically significantly lower preference scores, indicating a compromise in learning ability. Naive odor and sugar preference were assayed indicating that olfactory and gustatory senses were not affected by the mutation. These results suggest a role for DAck in learning and memory in *Drosophila*.

511F Fruit flies learn to interact differentially with inanimate objects of varied properties through repeated physical interactions

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Few studies have reported that fruit flies possess the cognitive or physical capacity to actively manipulate or interact with inanimate objects. This limitation has hindered the use of fruit flies as a model for investigating the biological principles underlying animal-object interactions. However, the scarcity of evidence supporting the ability of flies to manipulate an object may be due to a technical hurdle in keeping a freely moving fly engaged with an object reliably enough for the interaction to be quantitatively analyzed. We overcame this challenge by developing a new method to artificially promote fly-object interactions by guiding flies to repeatedly approach a target object, based on a technique our lab recently developed to artificially control the navigational behavior of freely-moving fruit flies (Iwasaki et al., 2025). The current study reveals that fruit flies, in fact, make sophisticated decisions about how to interact dynamically with an object through an array of previously unknown sequences of actions ("interaction motifs"). For instance, a fly may sometimes decide to grab a spherical object ("ball") with its forelimbs and use its whole body to pull it forward ("ball pull"), but at other times, the same fly may instead hop onto the ball and "walk" along the side of the ball to roll it around ("ball walk") or jump away from the ball immediately after contact ("jump"). Whenever a fly encounters a ball, it must decide which interaction motif to use to engage with the ball. Importantly, the flies learned about the physical properties of the balls (i.e., mobility or immobility) through repeated physical interactions and interacted preferentially with immobile balls. Through targeted genetic silencing, we identified a network of neural populations (hΔA, hΔJ, hΔM, hΔK, hΔI) in the central complex that flies use to interact differentially with objects based on their mobility. This suggests that these neural populations impact the neural computations necessary to direct flies toward objects with different properties for physical interaction. To our knowledge, this is one of the first studies to present fruit flies as a model for investigating the biological mechanisms underlying object manipulation, an area of research that holds significant potential for revealing how the nervous system enables animals to explore and learn about objects through physical interaction and adjust their behavior accordingly.

512F Investigating the larval functions of remodeled mating neurons in *Drosophila*

Kelly Kim, Kara Miller, Troy Shirangi Biology, Villanova University

We previously identified a sexually dimorphic population of abdominal interneurons in adult *Drosophila* females, called the DDAG neurons, that express the *Tlx*-like nuclear receptor, *dissatisfaction* (*dsf*), and the sexual differentiation gene, *doublesex* (*dsx*). These neurons exist in adults as an anatomically diverse population consisting of five subtypes, A–E, that influence a variety of mating behaviors in females. Interestingly, the DDAG neurons are born in the embryo and exist in larvae among a population of segmentally-repeating mature, differentiated *dsf*-expressing neurons in the abdominal ganglion. During early metamorphosis, a subset of these neurons acquires *dsx* expression, apoptose in males and remodel in females for functions in mating. Transcriptomic approaches have allowed us to identify the precise larval identities of each adult DDAG subtype. With this information, we are now determining the larval functions of each DDAG subtype. Here, we present our progress addressing this goal using optogenetic activation and silencing behavioral experiments and functional Calcium imaging with driver lines that precisely target subsets of *dsf*-expressing larval abdominal interneurons. These experiments will test the hypothesis that the DDAG neurons are reprogrammed from a population of interneurons with functions in larvae.

513F Comparative analysis of DAM2 and Zantiks systems for monitoring drosophila activity and circadian rhythms

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We compared two automated activity monitoring systems for studying sleep and circadian rhythm in Drosophila melanogaster. The Drosophila Activity Monitor 2 (DAM2) system records fly activity based on infrared beam crossings per unit of time. This experiment aimed to compare this established approach to the Zantiks system, which uses live video tracking to quantify movement as distance traveled per unit of time. We hypothesized that live video tracking might provide complementary behavioral metrics compared to beam-crossing, and determine whether beam-crossing detection misses activity that occurs away from the single-beam detector in the center of the vial, such as localized movements within a monitoring tube. Male flies aged 5 or 30 days were monitored in each system for five days under 12-hour light:12-hour dark (LD), followed by seven days in constant darkness (DD). Zantiks distance data were converted to activity counts per time bin to enable direct comparison with DAM2 data. Analyses assessed activity and sleep during LD and circadian rhythm during DD across age groups. Both systems detected age-related behavioral changes, including altered activity levels, sleep fragmentation, and circadian rhythm disruption. Critically, age-related behavioral differences between young and aged flies were consistently identified by both platforms, leading to comparable biological conclusions. These findings demonstrate that both monitoring systems effectively detect relevant phenotypic differences in Drosophila. Future analyses will examine native Zantiks distance metrics to determine whether continuous spatial tracking offers distinct advantages over discrete beam-crossing detection for quantifying activity, sleep, and circadian rhythms.

514F Investigating the role of larval abdominal interneurons, A26g, in eclosion behavior

Tejal Sasha Shanker[1], Kara Miller[2], Troy Shirangi[2] [1]Biology, Villanova University, [2]Villanova University

Recent work in our lab identified a segmentally-repeating population of interneurons in the abdominal ganglion of the larval nerve cord called the A26g neurons, whose function is unknown. Single-cell RNA-sequencing analyses have shown the A26g neurons can be uniquely identified by the co-expression of the genes *teyrha-meyrha* (*tey*) and *dissatisfaction* (*dsf*). During early metamorphosis, ABD-B+ A26g neurons at abdominal segments A5–A8 acquire sexual identity with the expression of the *doublesex* (*dsx*), apoptose in males, and remodel for functions in female mating behaviors. Here, we show that ABD-A+ A26g neurons at A1–A4 persist from larval to early adulthood in both sexes. Within 24 hours after eclosion, this anterior set of A26g neurons disappear. Using the cell death inhibitor, P35, we show that these cells are lost due to apoptosis during the first 24 hours of adult life. Given the timing of their cell death, we hypothesize that these neurons play a role in eclosion, which we are currently testing. Experiments currently in progress seek to formally test this hypothesis. This work provides new insights into the function of these anterior A26g neurons and contributes to the overall pursuit of exploring innate behavior and the developmental transitions of neural circuits.

515F Deep sleep architecture is selectively altered in hyper-aggressive *Drosophila melanogaster*

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Sleep is conserved across the animal kingdom and is critical for the regulation of social behaviors. Sleep disturbances manifest in myriad behavioral dysregulations, including agonistic interactions. Sleep and aggression are deeply interlinked behaviors, but the underlying neural and molecular mechanisms connecting them remain poorly understood. Recent discoveries of structured sleep states in *Drosophila* provide a unique opportunity to assess state-specific regulation of aggression, and provide a framework to better understand the shared relationship. By using two independently selected hyper-aggressive fly lines and quantifying sleep architecture across discrete sleep states, we explored associations between sleep and aggression. Only one of these states distinguished both hyper-aggressive lines from their respective controls. Across both hyper-aggressive lines, selected via two completely different selection methodology, a consistent relationship was found: significantly increased latency to nighttime deep sleep was associated with hyper-aggression. Given the role of serotonin in sleep and aggression, we measured central brain serotonin using MALDI-TOF MS and found significantly elevated levels in the central brain of hyper-aggressive flies. When serotonin levels were artificially elevated through pharmacological manipulations, the delayed sleep-onset phenotype for nighttime deep sleep was recapitulated in wild type flies. Together, our findings suggest that deep sleep, and specifically its timing, is selectively interlinked to aggressive internal states in *Drosophila*, potentially via serotonergic signaling. These results offer a shared molecular node in the aggression-deep sleep axis and position *Drosophila* as a tractable model system to dissect the developmental, neural, and molecular pathways linking social behavior and sleep.

516F In the Mood for *Yellow*: Understanding how *yellow* impacts female mating behavior in Drosophila melanogaster

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The pigmentation gene yellow offers a classic yet still unresolved example of how a single gene can link morphology and behavior in Drosophila melanogaster. Early genetic studies by Bastock (1956) showed that yellow mutations reduce male courtship success. Subsequent work established that the loss of yellow in non-neural doublesex-expressing cells, particularly in sex combs, reduces melanization and alters structure, hindering males' ability to grasp females during copulation. While these studies revealed yellow's role in male-specific morphology, they left unexplored whether yellow also shapes the female side of mating. Interestingly, yellow mutant males and females mate successfully with one another, suggesting possible compensatory changes in females.

This raises a key question: which female tissues depend on yellow-mediated pigmentation to support mating, and do yellow mutant females exhibit sensory or structural adaptations that accommodate less effective male grasping? Drosophila females possess mechanosensory bristles across the abdomen, genitalia, and thorax, which could interact with male sex combs and detect tactile cues during copulation. Understanding how yellow influences these female structures may uncover overlooked mechanisms by which pigmentation genes contribute to sexual communication.

Using behavioral assays and genetic perturbations, I compared courtship dynamics across all combinations of wild-type and yellow mutant males and females. Yellow mutant females display altered copulation latency, suggesting that female-specific yellow expression influences mating cues or structural traits that mediate male courtship success. Tissue-specific knockdowns using doublesex- and fruitless-GAL4 drivers indicate that yellow is required in doublesex-positive tissues of females to support normal mating, paralleling its role in males. Ongoing hybridization chain reaction (HCR) staining across pupal and adult stages reveals localized yellow expression in female genital and abdominal tissues, consistent with potential roles in cuticular structure.

By uncovering how yellow functions in females, this work expands the classical narrative of yellow's behavioral function from male-limited morphology to bidirectional sexual communication. More broadly, it illustrates how pleiotropic pigmentation genes integrate molecular, structural, and behavioral layers of mating behavior and evolution in Drosophila.

517F Comparative analysis of sexually dimorphic neural substrates involved in Alcohol neuroadaptation in *Drosophila*

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Understanding the neural architecture of Drosophila melanogaster has been accelerated thanks to the efforts of the Flywire Consortium, which has recently completed mapping of the Drosophila connectome. These efforts have made available electron micrograph data sets to the entire scientific community. This tool allows the tracing of neurons and contains information regarding synaptic count, neurotransmitter profiles, and mapping of synaptic partners. Although these data sets are extremely useful, they were all sampled from female fly brains. Because of this, assessing sex dimorphisms on the neuronal architecture of the fly brain was not possible. With the recent release of the full connectome data set for the male central nervous system, comparative connectomics can be performed. Previous research has shown a difference in alcohol tolerance development when comparing males versus females when it comes to behavior. A potential explanation for this difference in tolerance development could be linked to differences in neuronal connectivity. In D. melanogaster, there have been many neuronal populations and substrates that have already been associated with alcohol tolerance. Of particular interest are the DN1a neurons, which have been implicated in the development of alcohol tolerance. Here, we report the use of different data sets to address potential sexual dimorphism by looking at contrasting aspects of connectivity of DN1a neurons. For the identification of the same cells, we made use of the Codex, a platform where all the data sets converge and allow for the matching of specific neurons onto different connectomes. Comparative analysis was performed using the raw data for each connectome, and a filter for synaptic count was applied for connections above five synapses. We focused on differences in populations, number of synapses, and neurotransmitter profiles these neurons receive input from. This study can provide new insights into the sexually dimorphic regions that control behavior and serve as a pre-screen for neural circuits.

518F A New Mutant, no-day-sleep (*nds),* Exhibits a Specific Loss of Day Sleep

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Sleep is a critical and evolutionarily conserved process found across the animal kingdom, from invertebrates to mammals. *Drosophila* exhibit a bimodal distribution of inactivity, with periods of daytime and nighttime sleep, similar to human cultures before the invention of artificial light. The distinct roles and mechanisms regulating day- and nighttime sleep, however, are still unknown. Here, we introduce a new mutant, no-day-sleep (*nds*), which exhibits a 72% reduction specifically in daytime sleep. *nds* mutants also have a reduced lifespan, suggesting that daytime sleep is critical for survival. The current study aims to characterize the nature of this sleep phenotype, and identify the gene, cells, and circuitry impacted in the *nds* mutant. We show that the reduction in daytime sleep in *nds* mutants is derived from both a decrease in the number of sleep bouts as well as a reduction in the average duration of each sleep bout. While the total amount of nighttime sleep is not impacted in *nds* mutants, their nighttime sleep is characterized by a reduction in sleep bout number with an increase in bout duration, possibly indicating a deeper, more consolidated sleep at night as a consequence of the loss in daytime sleep. Reduced daytime sleep in *nds* mutants remains, however, plastic, as we have found that factors such as aging and temperature still affect daytime sleep similar to wild-type. Interestingly, the *nds* sleep phenotype was strictly sex-specific, present only in females and absent in males. Previous work has shown that mating induces a decrease in daytime sleep in wild-type females, which is initiated by the transfer of Sex Peptide (SP) found in male seminal fluid into the female reproductive tract during mating. Activation of the sex peptide receptor on sex peptide sensitive neurons (SPSNs) leads to silencing of SPSNs and downstream sex peptide abdominal ganglion neurons (SAG) neurons and consequently, a reduction in daytime sleep. Thus, we hypothesize that the *nds* mutation likely silences the SPR pathway, thereby mimicking the mated state. In support of this hypothesis, we show that inhibiting the SPR pathway or activating SPSNs and SAG neurons in the *nds* mutant background is sufficient to restore daytime sleep. With the recent identification of the *nds* gene, we are now testing how the *nds* protein functions to maintain signaling in the SPR pathway. This unique mutant also provides a novel opportunity to identify distinct behavioral and physiological roles of daytime versus nighttime sleep.

519F The Puratrophin-1-like (Pura) RhoGEF is necessary for developmental timing, locomotion, and the circadian rhythm patterns in *Drosophila*

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Circadian rhythm is a highly conserved mechanism that governs physiological and behavioral processes across the animal kingdom. While the core genetic components underlying the development and regulation of the circadian rhythm have been identified, recent studies suggest that members of the Rho family may modulate neuronal activity involved with the circadian rhythm. Here, we investigate behavioral changes in larvae and adult *Drosophila melanogaster* upon the loss of *puratrophin-1-like* (*pura*), a RhoGEF recently reported to regulate Rho1 oscillations in clock neurons. Using the ImageJ, Fiji plugin TrackMate, and DAM monitor software, we analyzed larval size, larval locomotion, and adult sleeping behavior. The *pura* mutant larvae exhibit both reduced overall size and reduced locomotion speed compared to wild-type controls at all instar stages. Larval mutants also show an abnormal wavy trachea, with the phenotype being most prominent in the third instar stage. Adult flies lacking *pura* exhibit lower levels of locomotion and disrupted sleeping patterns compared to wild-type flies. Future research directions include determining whether Pura expression patterns are altered by the circadian rhythm, uncovering the location and timing of Pura expression in neural cells, and identifying the transcriptional mechanisms by which pura is regulated.

520F Investigating the role of H3Q5 monoaminylation in glial regulation of visual neurotransmission

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Beyond their classical synaptic functions, certain monoamine neuromodulators have been shown to localize to the nucleus, where they can covalently modify histones and regulate gene expression. This emerging post-translational modification is known as Histone 3 (H3) monoaminylation, and it involves the covalent attachment of monoamine neurotransmitters like serotonin, dopamine, or histamine to glutamine (Q) residues on H3, a process enzymatically catalyzed by Transglutaminase 2 (TGM2). Monoaminylation has been previously shown to regulate gene expression and play critical roles in neurodevelopment, neuroplasticity, and behavior in rodents. Interestingly, H3 monoaminylation has not only been observed in neurons but also been detected in glia cells that take up these neuromodulators. Given that histamine is the key neurotransmitter in Drosophila photoreceptor signaling, Drosophila provides a tractable model to specifically investigate the functional role of histaminylation in the fly visual system. In this study, I focus specifically on the role of glia in histamine recycling and its impact on visual neurotransmission. I use the GAL4/UAS binary expression system to overexpress H3.3 variants with amino acid substitutions at the monoaminyl-modifiable H3.3Q5 residue, converting Q to alanine (A) or asparagine (N). Using repo-GAL4 as a pan-glial driver, I evaluated the contribution of glial H3 monoaminylation to neural function. To assess visual neurotransmission, I employed electroretinography (ERG), which records (1) light-evoked depolarization from photoreceptors as well as (2) hyperpolarization and depolarization of postsynaptic neurons at the on- and off-set of 1 second-long light stimulus, respectively. The postsynaptic responses in particular reflect histaminergic synaptic transmission. Our preliminary results show that flies overexpressing H3.3-Q5A or H3.3-Q5N variants exhibit reduced amplitudes of on- and off- post-synaptic responses relative to flies overexpressing H3.3-WT or non-H3 controls. This reduction suggests that impaired synaptic transmission may stem either from reduced H3.3 histaminylation or from structural perturbations to the H3 tail that compromise chromatin dynamics. Elucidating how histone monoaminylation in glia influences visual system neurotransmission promises to uncover novel epigenetic mechanisms that shape neural circuit function and histamine signaling in vivo.

521F Lineage-Based Neural Circuit Organization of the *Drosophila* Larval Brain: Developmental, Structural, and Functional Insights Revealed by the Connectome

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Pancrustacean nervous systems exhibit remarkable stereotypy, with conserved neuronal morphologies and projection patterns that persist across developmental and species boundaries. We used these developmental "anchor points" to establish a unifying framework for comparative connectomics using the *Drosophila* first-instar larval central brain connectome (~4,000 neurons, ~550,000 synapses). We assigned every neuron to its developmental lineage, distinguishing differentiated and undifferentiated classes, and morphologically clustered them to define neuron types. Differential projection and innervation patterns of these neuron types delineated neuropil compartments, yielding a systematic, hierarchical nomenclature grounded in lineage origin, morphology, and connectivity — directly alignable to adult *Drosophila* connectomes and emerging cross-species datasets. Embedding these anatomically defined groups into a low-dimensional latent space uncovered connectivity modules. This enabled functional inferences of previously undescribed neurons based on their developmental origins and network contexts. Furthermore, we developed a self-supervised graph-representation learning model that uncovered geometric constraints governing neuronal morphologies. Finally, comparative analysis of lineage organization between larval and adult connectomes identified conserved axonal tract scaffolds that endure pupal remodeling, demonstrating that early-established lineage-based layouts provide a stable reference frame for adult brain assembly. Thus, we assemble a comprehensive atlas documenting every developmental lineage, sensory afferent population, and neuropil compartment in the larval brain. By integrating high-resolution connectomics and developmental analysis, this resource provides a framework for dissecting conserved principles of circuit architecture and modifications underlying functional diversity across developmental stages and evolutionary divergence.

522F Social experience alters behaviors by reprogramming the Fruitless pathway and circadian state in *Drosophila*

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From flies to humans, social experience shapes a range of cognitive and behavioral processes. Previous studies have shown that group housing suppresses many behaviors, such as courtship, aggression, and feeding, in *Drosophila melanogaster,* and also resets the circadian state. Here, we focus on courtship suppression induced by group housing. To determine the mechanisms by which social experience modulates courtship behaviors, we performed bulk tissue RNAseq and single-cell RNAseq from cells expressing FruitlessM (FruM) and DoublesexM (DsxM), two transcription factors that label interconnected neural circuits for socially driven behaviors, from grouped or isolated male brains. These revealed that social isolation alters *fru* and *dsx* levels throughout the brain. Knocking down *fruM* in different *fruM*-positive neuron subpopulations in the brain has diverse effects on social experience-dependent changes in courtship. Furthermore, group housing increases the expression of *stripe* (*sr*) and *Hormone receptor-like* (*Hr38*) genes, which encode neural activity-induced transcription factors, in most neurons within social circuits. We found that knocking down *sr* in *fruM*-positive neurons effectively eliminates the impact of social experience by increasing courtship in group-housed males. Importantly, social experience also alters the expression of FruM/DsxM putative target genes regulating circadian states throughout the brain. Disrupting the function of multiple circadian genes diminishes the effect of group housing on courtship. Our findings suggest that group housing/social enrichment suppresses courtship by reprogramming the circadian arousal state, whereas courtship-elevating effects of social experience rely on unique influences of FruM expression and function in different neurons within social and clock circuits. These results are significant as they point to modulation of circadian arousal state as a possible central strategy for mediating the pleiotropic effects of social experience on organismal responses.

523S A descending neuron supports context-dependent modulation of nociceptive behaviour in *Drosophila* larvae

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The ability to respond to imminent threats is critical to an organism's survival. While these defensive responses are usually construed as robust reflexes, organisms must be able to integrate context cues and past experiences to maximize their chances of survival. Here, using the nociceptive system of *Drosophila*larvae, we study the mechanisms that underlie context-dependent modulation of nocifensive behaviours, i.e. the defensive behaviours used to escape threats. Previously, we identified that an octopaminergic feedback loop supported the sensitization of larvae following noxious experiences. Now, we identify a distinct mechanism that contributes to experience-dependent nociceptive hypersensitivity and likely allows for modulation of nociceptive behaviour through multisensory integration. Upon reviewing connectome data, we identified a descending neuron (SeIN138) that is both upstream and downstream from nociceptive neurons and contact-chemosensory neurons. At the behavioural level, we find that SeIN138 suppresses nocifensive behaviour (i.e. rolling) when activated along nociceptive neurons. Consistent with our behavioural assays, we find that SeIN138 is less active following sensitization of the larva due to previous nociceptive experience. Further, chronic activation of SeIN138 leads to a sensitization of larvae to noxious stimuli through a reduction in SeIN138's sensitivity. Surprisingly, silencing this neuron leads to a reduction of the larva's response to harmful stimuli and impairs its ability to adaptively tune its nociceptive behaviour as a function of noxious experience. Together, our findings uncover a circuit motif for context-dependent descending control of nociception, where dysregulation drives nociceptive hypersensitivity. More broadly, this work advances our understanding of how experience and sensory context shape defensive behaviours, offering insights into conserved principles of pain modulation and maladaptive sensitization.

524S TRPV1-Independent and Sex-Specific Capsaicin Signatures in *Drosophila*

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Capsaicin, the major phytochemical in chili peppers responsible for the spicy sensation, triggers sex-specific dietary behaviors in *Drosophila*, which lacks the canonical transient receptor potential vanilloid subtype 1 (TRPV1). Here, we demonstrate that first-order gustatory sweet-sensing Gr64f$_+$ neurons and their expressed receptors mediate capsaicin taste that undergoes feeding habituation. Single-nucleus RNA-seq brain signatures reveal IPC activation through *Dsk* and glial *Clk*-mediated responses, along with longevity-associated transcriptional programs, suggesting a shared desensitization pathway. In females, dopaminergic activation coincides with enriched synaptic, TGF-β, and AstA signaling within learning and memory centers. In males, elevated *foxo*, together with activation of WNT, NPF, and Dh31 signaling axes, drives rapid habituation, accompanied by antagonistic *Capa* expression and modulation of energetic pathways. We also tested the gero-protective potential of capsaicin acting through TRPV1-independent mechanisms, observing significant lifespan extension in both sexes, together with improved negative geotaxis (climbing) and increased starvation resistance in aged flies. Collectively, these results establish *Drosophila* as a model for dissecting TRPV1-independent capsaicin chemosensory mechanisms and underscore the critical role of sex in shaping chemosensory responses and longevity outcomes.

525S Unraveling the Novel Genetic Connection Between Insomnia and Cognitive Impairment

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Insomnia affects up to one-third of the global population and is strongly associated with cognitive impairment, including deficits in learning, memory consolidation, and executive function. Despite this clinical overlap, the genetic mechanisms linking insomnia to cognitive impairment remain poorly defined. Recent GWAS studies identified human candidate genes associated with insomnia, including candidate genes that show strong associations with cognitive impairment. However, the functional relevance of these genes and their mechanistic contribution to cognitive impairment have not been experimentally defined. To dissect the molecular pathways linking insomnia susceptibility to cognitive impairment, we an innovative utilized *Drosophila* models with conserved sleep regulation and memory/learning circuits. By using mushroom body specific driver (OK-107-Gal4), we knocked down *Drosophila* orthologs of human insomnia-linked genes. Our initial results identified four candidate genes—*Mnt, Rapgap1, AtpSynC, and CG32264* whose knockdown caused significant impairments in aversive olfactory memory. These findings were observed consistently in both 3-week-old flies and were exacerbated in 6-week-old flies, suggesting age-dependent vulnerability. Collectively, these findings suggest that insomnia-associated genes modulate sleep behavior and disrupt neurobiological pathways including synaptic plasticity and inflammatory signaling, that are essential for memory integrity. Defining the mechanistic links provides a foundation for identifying early biomarkers of cognitive impairment and developing targeted strategies to prevent insomnia-induced memory impairment.

526S Gut microbiome contributes to behavioral phenotype and memory dysfunction in *Drosophila* model of Alzheimer's Disease

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Objective: Alzheimer's disease (AD) pathology spread beyond tau and amyloid protein aggregation with the involvement of systemic factors like neuroinflammation, gut-brain axis, and circadian disruptions. Current study aims to understand how gut microbiome shaping AD pathology through modulating these systemic factors.

Methods: Transgenic *Drosophila melanogaster* expressing AD associated genes and their mutants (*HTau*, *HTau*^{R406W}, phospho *Tau*, a*β*42 and *APP*) were crossed with glial cell specific driver (*Glaz-GAL4*) carrying flies. Progeny flies were maintained under CC (flies with intact microbiome) and AA (microbiome depleted using antibiotics) conditions throughout the experiments. Using qPCR microbiome, inflammatory markers and apoptotic genes were analyzed. Protein expression changes, lipid accumulation and synapsin were quantified by western blot, and immunofluorescence assays. Behavioral phenotypes such as sleep/circadian activity measured using *Drosophila* activity monitoring system (DAMS), olfaction memory and shock avoidance measured using T-maze for fly, and locomotion analyzed using geotaxis assay apparatus.

Results: *Lactobacillus* and *Acetobacter* abundance altered in AD flies under CC, whereas AA flies showed 90% reduction in gut microbiome, along with upregulated neuroinflammation (*Upd, Dome, Hopp, Stat92e*) and cell death (*Hid, Reaper*). AA flies accumulating lipid droplets, reduced synapsin, elevated Tau, pTau, a*β*42, ubiquitinated proteins, and Ref2p proteins suggesting impaired autophagy mechanism, despite increased pAMPK in the fly brains. In addition, disrupted sleep/circadian rhythms, memory ascertain the importance of gut microbiome in AD flies. We observed gene and sex dependent changes in locomotory performance.

Conclusions: Gut microbiome mitigating AD progression by controlling inflammatory responses, lipid and protein homeostasis, preserving circadian and cognitive functions. Whereas microbiome depletion aggravating neurodegeneration supports the importance of gut-brain axis as a critical modulator of AD pathology. It highlights importance of targeted microbiome interventions that provide therapeutic benefits in AD condition.

527S Acetylcholine and KCl induced endogenous glutamate release adult *Drosophila* brain

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Glutamate is a major neurotransmitter present in flies. It acts as an excitatory neurotransmitter at the neuromuscular junction while acting as inhibitor in the olfactory system. In *Drosophila*, both ionotropic and metabotropic receptors are present. However, there have been limited methods to understand real-time glutamate signaling in the adult Drosophila brain. iGluSnFR is a fluorescent sensor used for monitoring changes in glutamate levels in the synapse. Based on a bacterial protein, it produces fluorescent changes when extracellular glutamate binds to the protein. Thus, iGluSnFR can be used to study glutamate dynamics in the fly brain.The ventromedial protocerebrum (VMP) lobe, specifically the right VMP was the region of interest for our studies due to ease in implanting the capillary. This region has glutaminergic Projection Neuron (PN) network for pacemaker neurons concerning the circadian rhythm.

The initial working of the sensor was confirmed using glutamate as the stimulant. The experiments were conducted on a homozygous OK371-Gal4; iGluSnFR line. The adult fly brain was dissected, and the analyte was injected using a capillary connected to a pneumatic ejector and the concentration was varied by loading the capillary with different stock solutions. The capillary was inserted in the right ventromedial neuropil region of the brain, where the sensor was brightly expressed and easier to access. Puffing on 52 fmol of glutamate increased the DeltaF/F to 5.0. To study the endogenous glutamate release, acetylcholine (a fast excitatory neurotransmitter) and potassium chloride (KCl) were used as stimulants with a concentration ranging from 1M to 0.1mM. The DeltaF/F_0 against time plots were analyzed by calculating the area under the curve as the peak intensities did not change but the time course did with increased concentrations. The glutamate uptake inhibitor DL-TBOA increased the time course of glutamate signaling. These studies show that iGluSnFR is useful for understanding glutamate dynamics in the fly brain and could be useful for understanding how drugs and genetic mutations change glutamate release.

528S Electrochemical detection of serotonin in *sert* mutant *Drosophila* larvae

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Drosophila melanogaster is an excellent model to study the dynamics of neurotransmitters in diseases such as depression and Parkinson's. 5-Hydroxytryptamine (5-HT), or serotonin, is an electroactive neurotransmitter associated with neurological diseases such as depression. Neurological diseases can occur from dysregulation of serotonin transporters (SERT) not allowing for the reuptake of serotonin to occur efficiently. Selective serotonin reuptake inhibitors (SSRIs) are the most prescribed antidepressants, blocking SERT to increase the amount of circulating serotonin. Fast- scan cyclic voltammetry (FSCV) is a method to detect 5-HT that measures fast changes of neurotransmitters in the brain. Optogenetics is used for precise release of serotonin, leading to more accurate measurements of reuptake time. The goal of this study is to understand the effect of *sert* on the dynamics of serotonin release. Two mutant fruit fly lines, *dSERT*^{16} and *dSERT*$^{\Delta 3.9}$, were tested to determine the effect these genetic mutations have on the dynamics of serotonin release and reuptake. *dSERT*^{16} is a partial loss of function mutant with the promotor region of the DNA removed that codes for *dSERT. dSERT*$^{\Delta 3.9}$ is a complete loss of function mutant, with removal of 3.9 kb of the d*SERT* coding sequence. *dSERT*$^{\Delta 3.9}$ slows reuptake of serotonin significantly, and the serotonin does not go back to baseline with t_{50} unable to be calculated after 30s. *dSERT*^{16} also slows reuptake, but not as substantially, and signals eventually return to baseline. Further tests were performed involving the wild type and mutants with the addition of the SSRI escitalopram. The hypothesis is that escitalopram will have a larger effect on the wild type serotonin reuptake compared to both mutants. Future directions of this project include how other smaller mutations in the *sert* gene influences overall serotonin reuptake and SSRI efficacy.

529S miR-10 Knockdown Reveals Cell-Type-Specific Regulation of Obesogenic Behavior

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Our lab previously showed that paternal exposure to a Western diet (WD), high in fats, salt, and sugars, alters protein and miRNA expression in Drosophila melanogaster offspring brains, along with increased food intake (Murashov et al., 2023). MiR-10 was identified through miRNA-target enrichment analysis as a key potential regulator of proteomic changes in the brains of offspring from WD-fed fathers. However, whether miR-10 directly mediates these effects and how it acts within specific brain cell types, particularly neurons and glia that control feeding and reward, remain unknown.

Consistent with this prediction, qPCR revealed a six-fold increase in miR-10 for WD-fed fathers and a two-fold increase in WD offspring ($p<0.05$). Using the Gal4/UAS system, we used RNAi-mediated knockdown of miR-10 in dopaminergic and serotonergic neurons (Ddc-Gal4), as these neurons are involved in food and reward-seeking behavior. Using a consumption/excretion assay, we found that knockdown of miR-10 led to a nearly two-fold increase in male food consumption ($p<0.0001$). Because miR-1006 shares an identical 3p seed sequence with miR-10-3p, we generated double knockdowns (miR-10 + miR-1006) to eliminate potential compensatory effects.

Behavioral analyses, including consumption/excretion, FLIC, locomotor, and passive avoidance, were conducted using neuronal (Elav-Gal4) and glial (Repo-Gal4) drivers with knockdown UAS. Knockdown of either miR-10 or miR-1006 in all neurons resulted in two-fold increased consumption ($p<0.001$), whereas glial knockdowns of both miRNA showed decreased feeding and locomotion ($p<0.05$, $p<0.01$), but impaired short-term memory in passive-avoidance assays ($p<0.01$). These phenotypes are consistent with WD offspring, suggesting miR-10 modulates consumption behavior through both neuronal and glial cells contributing to obesogenic behavioral traits, including hyperphagia, reduced sleep, and impaired learning and memory.

To probe potential downstream targets, we assessed Mei-P26 expression, a TRIM-NHL RNA-binding protein predicted to interact with miR-10-3p/miR-1006-3p (Agarwal et al., 2018). Overexpression of Mei-P26 in Ddc neurons phenocopied the double miRNA knockdown in consumption/excretion assays ($p<0.05$), suggesting miR-10/1006 regulate obesogenic behavior through Mei-P26-dependent pathways. Together, these findings identify a novel miRNA–Mei-P26 regulatory pathway that links paternal diet to inherited metabolic and behavioral traits.

530S Sleep Deprivation and Neurodegeneration in Drosophila melanogaster: Investigating the Effects of Practical Sleep Loss Paradigms on a Post-Translational Model of Alzheimer's Disease

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Sleep disruption is increasingly recognized as a contributing factor to neurodegenerative disease, yet the mechanisms by which sleep loss influences Tau pathology remain unresolved. This study uses a post-translational Drosophila tauopathy model to examine how acute and chronic blue-light–induced sleep deprivation affects Tau accumulation and its regional distribution within the brain. Replication of Blue-light exposure treatments has been validated as an effective and reproducible method for reducing sleep in this system. Preliminary immunohistochemical analyses indicate qualitative increases in total Tau and phosphorylated Tau (AT8) following sleep deprivation, with notably elevated signal in the mushroom body calyx, a region involved in learning and memory. These observations suggest that sleep loss may exacerbate Tau burden in synaptically active or metabolically demanding circuits. Ongoing work is focused on refining staining consistency, improving image quantification, and incorporating autophagy markers (Atg8a, Ref(2)P) to evaluate whether disrupted proteostasis contributes to the observed accumulation. Although data collection is in progress, current results establish a promising foundation for identifying cellular pathways linking sleep deprivation to tauopathy and for future experiments assessing autophagy as a potential modulatory mechanism.

531S GWAS of microbiome-dependent sucrose feeding in the *Drosophila*

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High sugar consumption is a major factor in global obesity. In the US, 75% of adults are considered overweight/obese. The microbiome has been implicated as a potential modulator of host nutritional behaviors, including feeding and food preferences. Previous work from our lab and others have shown that the gut microbiome can influence fly feeding preference for protein and sugar, yet how host genetic background might interact with the microbiome to modulate feeding preference remains unclear. In this study, we conducted a dye-based feeding assay to compare sugar feeding preference between conventional and axenic flies of different genetic backgrounds - the *Drosophila* Genetic Reference Panel (DGRP). Results showed that the DGRP exhibit variation in the microbiome-mediated sucrose consumption and was also sexually dimorphic. A GWAS test for the sucrose consumption identified 202 polymorphisms for female and 90 polymorphisms for male DGRP flies, which are found in genes such as *Rugose*, *OtopLa*, *Vkor*, *sm*, etc. Our results indicate a microbiome-mediation of sucrose consumption in *Drosophila*, which may be regulated by fly genetics.

532S AstCC: An arthropod somatostatin is a resilience factor in Drosophila wing expansion

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Somatostatins are important inhibitory peptides in mammals. Their receptors are highly conserved in Bilateria, including Drosophila, which has two somatostatin receptor orthologs encoded by the genes AstC-R1 and AstC-R2. These receptors bind peptide ligands that lack obvious homology to somatostatins and are encoded by the genes Allatostatin C (AstC) and CC (AstCC). Functional characterization of AstC and AstCC signaling is limited, but in Drosophila and crabs both peptides are expressed in abdominal neurons that co-express Crustacean Cardioactive Peptide (CCAP) and Bursicon. The latter two hormones play critical roles in ecdysis, the regulation of which is an important model for understanding neuromodulatory control of CNS function and behavior. Here we characterize AstCC signaling in the fly and report its behavioral role in wing expansion, a critical component of the adult ecdysis sequence.

Using an AstCC-specific antibody, we have mapped the central nervous system distribution of AstCC across Drosophila development. We confirm co-expression in many CCAP neurons during the larval and pupal stages except for one pair of non-CCAP cells in the central brain. In pharate adults, AstCC is prominently expressed in the subset of CCAP neurons that co-express bursicon, including those of the abdominal ganglion (B_{AG} neurons) and the subesophageal ganglion (B_{SEG}), which have previously been implicated in wing expansion. AstCC null mutants are viable and expand their wings under normal conditions, but they largely fail to do so when confined. In addition, they exhibit abnormal behavior. Instead of perching and expanding their wings, they persist in eclosion-related behaviors such at ptilinum extension, rarely seen in controls.

AstC-R1 (R1) knock-out animals phenocopy AstCC null mutants and are unable to expand their wings when confined. In addition, we find that optogenetic activation of AstC-R1-expressing neurons suppresses wing expansion, while silencing them promotes it. We conclude that AstC-R1-expressing neurons mediate the inhibitory effect of confinement on wing expansion and that their inhibition by AstCC—likely released by bursicon-expressing neurons—counters this influence. In summary, our study demonstrates that AstCC is a resilience factor in wing expansion, that overcomes environmental inhibition by suppressing the preceding motor program required for eclosion.

533S Regulation of feeding initiation and maintenance by Mushroom Body

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Feeding behavior in *Drosophila melanogaster* requires the integration of internal hunger signals with external sensory cues. The mushroom body (MB) and its associated neurons are well known for their roles in learning, memory, and decision-making, where food often serves as a reward and internal states shape the valence of food-related cues. However, the precise role of the MB in innate feeding behavior remains poorly understood, although some mushroom body output neurons (MBONs) have been shown to modulate the proboscis extension response—a behavioral measure of feeding initiation. The MB receives information about taste and feeding context largely through multisynaptic pathways, and many MB neurons, including Kenyon cells (KCs) and MBONs, express high levels of the insulin receptor (InR). Together, these core MB components—including KCs, dopaminergic and octopaminergic inputs, and the APL and DPM neurons—represent a central node in the neural network governing feeding motivation, though their specific contributions to feeding initiation and maintenance are unclear.

Using the Fly Liquid-Food Interaction Counter (FLIC), which quantifies individual sip events in real time, we examined how manipulating MB-associated neurons influences feeding on sucrose and hydrolyzed yeast. Neuronal activity was modulated using the Gal4-UAS system with *dTrpA1* for activation and TNT for inhibition. Among the neurons tested, the anterior paired lateral (APL) inhibitory neuron showed the strongest effects: APL activation suppressed sucrose feeding, whereas inhibition enhanced feeding—particularly in females—and markedly increased yeast consumption, suggesting that APL normally restrains feeding motivation. In contrast, activation of the DPM neuron, which releases serotonin and sustains motivational states, enhanced feeding on both sucrose and yeast, while inhibition reduced feeding, indicating that DPM promotes feeding drive. Manipulation of 22B (OA-VPM3/VPM4) and dopaminergic neurons targeted by Th;58E02-Gal4 did not alter overall feeding but revealed a preference for yeast, suggesting roles in nutrient selection rather than motivation. Finally, we explored the contribution of insulin receptor signaling within these neurons and will present data highlighting sex differences in feeding regulation and the MB's integrative role in sugar and yeast preference.

534S Neto/Neuropilin activates and amplifies Semaphorin/Plexin signaling to drive homeostatic plasticity

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Rapid homeostatic plasticity is essential for survival, enabling neuronal circuits to withstand destabilizing forces and restore function during critical challenges such as predator evasion or toxin exposure. Semaphorin/Plexin signaling is central to presynaptic homeostatic potentiation at central synapses and at neuromuscular junctions across species. However, our understanding of this pathway has remained incomplete, as secreted Semaphorins bind weakly to their Plexin receptors and require additional co-receptors for efficient signaling. Using the *Drosophila* neuromuscular junction system, we identify Neuropilin and Tolloid-like protein (Neto-α), an auxiliary subunit for ionotropic glutamate receptors, and the tyrosine kinase Abelson (Abl) as critical components of the Sema2b/PlexB signaling pathway that drive homeostatic potentiation and stabilize synaptic strength. Neto-α functions as a Neuropilin-like co-receptor that cooperates with Sema2b to relieve PlexB autoinhibition and activate signaling. In parallel, Neto-α recruits Abl, which functions as a cytosolic amplifier to enhance pathway output and thus enable a rapid, robust homeostatic response. By combining signaling initiation and amplification within a single molecular assembly, Neto-α ensures fast and efficient homeostatic compensatory responses to perturbations. This evolutionarily conserved signaling module, Neto (or Neuropilin)/Sema/Plex/ Abl, which integrates pathway activation and amplification, likely operates in cellular contexts beyond neural function, including in tumorigenesis.

535S Optimization of *Drosophila* Social Memory Training Parameters Using a Neural Network–Based Curve Fitting

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Long-term memory (LTM) formation in *Drosophila* typically relies on repetitive training protocols often designed through trial-and-error. Identifying optimal strategies remains challenging due to the complex interactions between training duration, intensity, and spacing. In this study, we applied the **Complex System Response (CSR)** framework, driven by neural network-based curve fitting, to systematically optimize training parameters for social memory in *Drosophila*.

Using an automated laser tracking system (ALTOMs), we compared classical and operant paradigms. While CSR improved classical training efficiency, it yielded a breakthrough in operant conditioning, achieving a 24-hour **Restraining Index (RI) of 63.28**—the highest reported score in *Drosophila* social memory assays. Our findings revealed distinct parametric requirements for short-term versus long-term memory formation, where long-term optimized protocols produced a characteristic delayed improvement in performance.

To explore the underlying neural basis, we integrated **single-cell RNA sequencing** and spatial mapping, revealing that optimized operant training significantly upregulates the immediate early gene **Hr38** across multiple neuronal populations, including αβ- and γ-Kenyon cells, dopaminergic neurons, and Mi15. This suggests that data-driven behavioral optimization enhances memory by recruiting a broader ensemble of memory-relevant circuits. Our work demonstrates a scalable strategy to link behavioral enhancement with molecular and anatomical circuit engagement, offering new insights into the biological constraints of learning.

536S Complex Interplay of Hormonal, Neuronal Responses by Gut-Brain Axis to a Deficit in Essential Amino Acids

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A deficit in protein can trigger a nutrient-specific appetite; however, the mechanism underlying this process remains poorly understood. Here, we found that a set of distinct neuronal and systemic mechanisms is activated in the microbiome-gut-brain axis to induce an essential amino acids (EAA) specific appetite. In Drosophila, EAA deprivation increases the expression of CNMa in the gut enterocyte, which activates enteric neurons and ellipsoid body neurons in the brain to promote the intake of EAAs. By contrast, CNMa inhibits the activity of sugar-sensing DH44 neurons, thereby reducing sugar intake. Protein deprivation in mice also results in an increased appetite for EAAs that occurred independently of FGF21 and required spinal pathway. Together, we propose the fundamental mechanisms underlying the maintenance of EAA homeostasis across species.

537S An α_1 antagonist shows age-dependent efficacy for alcohol use disorder

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Alcohol Use Disorder (AUD) is a condition that affects approximately 10% of the US population and has significant detrimental costs to individual health, economic productivity, and public safety. Despite these costs, treatment for AUD is limited to very few drugs. Recent preliminary clinical studies have shown that blocking a_1-noradrenergic receptors using the a_1 antagonist doxazosin reduces alcohol consumption. However, how a_1 antagonists impact alcohol consumption, or how efficacy changes in aging patients (an at-risk group for AUD that have lower a_1 receptor expression) is not fully understood.

We found that the a_1 *Drosophila* ortholog *Octopamine receptor in mushroom bodies* (OAMB) exhibits reduced transcript and protein expression as organisms age, providing an effective model to investigate age-dependent efficacy for doxazosin in treating AUD. Quantification of alcohol consumption in young and aged flies that were treated with doxazosin or vehicle alone revealed a significant decrease in alcohol consumption in young doxazosin-treated flies relative to vehicle-only controls that was not observed in aged doxazosin-treated flies. Furthermore, RNAi-mediated gene knockdown of OAMB using either a pan-neuronal driver (*nsyb*-GAL4) or a split-GAL4 driver specific for mushroom body intrinsic neurons (MB010B) significantly increased alcohol consumption in young flies, indicating that 1) the efficacy of doxazosin in reducing alcohol consumption is dependent on a_1 receptor expression, which decreases with age, and 2) doxazosin acts on a_1 receptors within brain regions important for learning and memory to reduce alcohol consumption. These results provide evidence for the use of doxazosin in treating AUD and lay the groundwork for future clinical studies that emphasize a more personalized approach to the treatment of AUD.

538S Chronic gut inflammation leads to hemocyte dysregulation and sleep deficits in Drosophila

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Inflammatory bowel diseases (IBDs) are characterized by chronic gut inflammation, immune and metabolic dysregulation, and are frequently associated with sleep disturbances that may reflect disease activity, however, the cellular and molecular mechanisms underlying sleep deficits in IBD remain unknown. Here, we show that chronic gut inflammation induced by dextran sulfate sodium (DSS), or bleomycin disrupts both sleep and gut homeostasis in *Drosophila* and leads to increased expression of inflammatory cytokines and growth factors from the gut. The transcription factor *ETS2* is a downstream effector of epidermal growth factor receptor (EGFR) signaling and has been identified as a key regulator of macrophage inflammatory responses in IBD. We find that *pointed (pnt)*, the *Drosophila* homolog of human ETS1/ETS2, is upregulated in the gut following DSS exposure. In hemocytes—macrophage-like immune cells in *Drosophila*—overexpression of *pnt* recapitulates the sleep deficits observed in DSS-inflamed flies. Conversely, knockdown of *pnt* in hemocytes or expression of a *pointed* null allele increases sleep. These findings reveal a new role for the gut-immune-brain axis in modulating sleep during gut inflammation through the transcription factor *pnt/ETS2*.

539S Evolution of behavioral adaptations to temperature in *Drosophila* species from diverse habitats

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From boreal forests to hot deserts, species in the genus *Drosophila* have colonized a wide range of thermal niches. This broad distribution has been facilitated in part by the evolution of specific behavioral adaptations to temperature. Despite their importance, we know little about the molecular and cellular changes driving the diversification of behavioral responses to temperature in any animal. Our work so far examined the evolution of temperature preference behavior using Drosophila species from distinct thermal environment but characterized by very different phylogenetic relatedness: *D. melanogaster* (*melanogaster* group), *D. persimilis* (*obscura* group), *D. mojavensis* and *D. mettleri* (*repleta* group).

More recently, we began to survey behavioral responses to temperature in a broad set of species with a particular focus on the *repleta* species group, a highly diverse group extensively used in work related to ecological adaptation and speciation. Phylogenetic and biogeographical analysis indicate that this group started to diversify in the mid-late Miocene in south America. From there, *repleta* species have colonized central and north American regions and distinct thermal habitats. Hence, this species group represents a natural experiment in the adaptation to different thermal environments.

Our results so far reveal how, within *repleta*, evolutionary tuning of molecular heat receptors and diversification of central temperature processing circuits both participate in generating species-specific temperature responses. Our data also suggests that distinct mechanisms can drive the diversification of specific behaviors (from thermal preference to sleep regulation) in different species even within the same thermal habitat.

540S Investigating mating-induced ovulation circuit in *Drosophila melanogaster*

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Egg laying in female *Drosophila melanogaster* is controlled by a distributed neural circuit integrating mating status, central decision neurons, and peripheral motor execution. Previous work has identified a SPSN-SAG-pC1-oviDN/oviEN neuronal circuit that mediates mating-induced oviposition; however, it is unclear whether the same neuronal circuit regulates mating-induced ovulation. To address this question, we used the previously published tools to activate pC1 neurons, which were previously showed to reduce the egg laying capacity of mated females. In contrast, we did not observe obvious egg laying defect as previously reported. We went on to activate SAG and SPSN neurons to test whether this can replicate previous report. Unfortunately, activation of SAG and SPSN neurons did not affect mating-induced egg laying in our experimental setting. Furthermore, we went on to knock down sex peptide receptor (SPR), a receptor in SPSN neurons for relaying the mating signal to post mating behavior response, in all neurons or globally. We still did not observe any reduction in egg laying after mating. We are seeking help to explain our inability to reproduce the reported phenotypes. Nonetheless, we were able to reproduce the phenotype that inactivation of oviENs using tetanus toxin (TNT) resulted in a significant reduction in egg laying. We went on to carry out the ovulation assay by measuring the ovulation time. We found that oviEN-inactive females have normal ovulation. The defective egg laying is due to the oviposition defect. Therefore, oviENs do not involved in mating-induced ovulation. Future work is required to figure out the entire circuit for mating-induced ovulation.

541S Walking dead: Identifying the drivers of locomotor rhythms in *E. muscae* infected flies

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Behavior is driven by the activity and communication of interconnected neurons. Insect models allow for fine-grained analysis of both neuronal activity and species-specific behaviors. *Drosophila melanogaster* is susceptible to infection by the behaviorally manipulative fungus *Entomophthora muscae*. On their last day of life post-infection, infected flies exhibit an aberrant burst in locomotion called “summiting” prior to dusk. The Elya lab recently identified another behavioral aberration: infected flies fail to exhibit their stereotypical circadian locomotor peak on their last morning of life. Given the acute nature of this loss of fly locomotor rhythms, we suspect that *E. muscae* may drive host circadian dysregulation as the fly enters its final day of life. Here, we describe our efforts to investigate the mechanisms underlying the loss of the host’s morning locomotor peak and that the summiting behavior at death is independent from the similarly timed evening locomotor peak. We show that Infected moribund flies, but not flies that die from other manipulations, have irregular period oscillations in the last 48 hours of life. We also show that, while evening peaks change in free-running conditions for several circadian mutant flies, the timing of summiting remains consistent. These results highlight how *E. muscae* impacts circadian locomotor behavior at several timepoints.

542S Anesthesia-triggered dopaminergic bursts cause active forgetting: a paradigm shift in understanding cold-shock amnesia

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Retrograde memory loss, the inability to recall events preceding amnesia onset, is a well-documented consequence of anesthesia. Traditionally, forgetting has been considered a passive process, but over the last decade, *Drosophila* research has challenged this view by demonstrating that forgetting is a highly active, well-regulated biological process. Our work builds on this and makes a remarkable and unexpected discovery that anesthesia itself triggers a robust, widespread burst of activity in dopaminergic neurons (DANs), which appears to actively induce forgetting. This finding challenges the long-held assumption that cold-shock anesthesia induces memory loss as a mere consequence of biochemical inactivation, probably by enzymatic inhibition and reshapes our understanding of retrograde amnesia.

Using calcium imaging, we found that both cold-shock and CO_2 anesthesia elicit strong activation in PAM and PPL1 DAN populations. These responses vary in magnitude across subtypes—for example, the PPL1-γ1pedc neuron shows a significantly weaker response—yet they all are consistently time-locked to the onset of anesthesia. This calcium activity is accompanied by robust synaptic release of dopamine as evaluated by a dopamine reporter. In addition, pharmacological experiments show the response is input-driven, as it is completely abolished by Na^+ channel blockers and dampened by nAChR antagonists. Surprisingly, partial inhibition of Na^+ channels not only delays the DAN calcium response, but also delays anesthesia onset itself, suggesting a causal link between anesthesia state and neuronal activity.

Even more striking, we found that this anesthesia-triggered activation is not limited to dopaminergic neurons. Although not all, many other neurons, including Kenyon cells and certain GABAergic MBONs, also show significant activation, though responses are not uniform across all cell types.

Functional imaging of flies trained under the microscope further supports a direct link between anesthesia and memory trace elimination. Imaging of flies trained under the microscope indicate that olfactory memory traces observed by LTD in MBON-g1pedc>a/b, MBON-g2a'1 and MBON-a3 can be abolished by a short cold-shock under the microscope. Interestingly, the memory trace observed in MBON-g1pedc>a/b is slightly more resistant to cold-shock, matching the activity pattern of the corresponding PPL1-g1pedc.

Behaviorally and crucially, we show that anesthesia-induced amnesia can be prevented by blocking activity in PAM and PPL1 neurons during anesthesia using optogenetic and therogenetic tools.

Together, these findings reveal a previously unrecognized active mechanism by which anesthesia induces forgetting, mediated by rapid dopaminergic signaling. This paradigm-shifting discovery redefines how we think about anesthesia-induced amnesia and the biology of forgetting.

543S Abdominal sensory neurons modulate sleep and sickness responses in *Drosophila melanogaster*

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Sleep has been studied largely from a brain-centric perspective, and the contribution of peripheral sensory neurons to sleep regulation remains poorly understood. Here, we describe a circuit linking wake-promoting peripheral abdominal sensory neurons to sleep-promoting regions in the *Drosophila melanogaster* central nervous system, with a role in injury-induced sickness sleep. Sleep-promoting split-Gal4 lines label ascending neurons with cell bodies in the abdominal ganglia of the ventral nerve cord. Using synaptic tracing and labeling, we further show that abdominal multidendritic sensory neurons are presynaptic to these sleep-promoting ascending neurons. Activation of abdominal sensory neurons using the *ppk*-Gal4 driver decreases sleep and increases locomotion, whereas inactivation does not affect baseline sleep. Notably, abdominal injury reduces calcium activity in *ppk* neurons, and loss of *ppk* enhances injury-induced sickness sleep. Together, these results suggest an interaction between sleep and peripheral sensory neuron activity, in which sensory neurons inhibit sleep, while sleep, in turn, may suppress sensory neuron function.

544T From Mapping the Molecular Clock of Drosophila Neurogenesis to Understanding Human Brain Disease

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The Drosophila medulla, a complex visual center with 40,000 neurons, arises from around 2000 larval neuronal stem cells (NSCs). These NSCs generate neuronal diversity via a sequential cascade of temporal transcription factors (tTFs. However, the dynamics of this cascade—its precise timing, duration, and transition mechanisms—remain unknown. To define this molecular clock, we established a long-term primary NSC culture compatible with high-resolution live-imaging. Using CRISPR-Cas9, we endogenously tagged key tTFs with fluorescent proteins. This system revealed that the tTF progression is cell-intrinsic and that the NSC division rate is variable. Our platform allows for the precise quantification of temporal window durations, cell division numbers per window, and the timing of tTF transitions. This high-resolution temporal map provides a foundational blueprint for programming naive stem cells for cell replacement therapies. Furthermore, our innovative culture system offers a powerful new platform for deciphering human developmental brain diseases.

545T Glia are required for structural plasticity in mature adult neurons

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Neuronal plasticity is a fundamental property of neurons that underlies learning and memory and is dysregulated in neurological disorders such as autism spectrum disorder and PTSD. We use the *Drosophila* s-LNv principal circadian pacemaker neurons as “model neurons” to understand the molecular mechanisms of neuronal plasticity in adults. The projections of s-LNvs show dramatic structural plasticity every 24 hours: they expand at dawn to make new synaptic connections, and retract around dusk to break connections. Rhythmic s-LNv plasticity is controlled by the opposing actions of the small GTPases Rho1 and Rac1, which respectively drive retraction and expansion. Glia help shape the structure of neurons during development. However, the role of glia in plasticity in the adult brain is less clear. Our data show that glial phagocytosis is required for s-LNvs to retract their projections at dusk, but not for expansion of s-LNv projections at dawn. However, large parts of s-LNv projections remain in the retracted state. So how do glia know which parts of the neurons to eliminate? Glia recognize phosphatidylserine (PS) that has been externalized to the outer side of the plasma membrane using the glial PS receptor Draper. We found that Draper is also required for s-LNv projections to retract as is Orion, a chemokine-like receptor which also binds PS, typically on the neuronal side. We are currently testing the model that localized and/or timed activity of the Scramblase enzyme that externalizes PS is a key regulator of adult s-LNv plasticity. We are also investigating the interplay between neuronal activity, Rho GTPase activity and glial phagocytosis in the plasticity of adult s-LNv neurons.

546T Progenitor-Derived Regulatory Logic Generates Functionally Related Neuronal Subtypes

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The brain splits complex inputs into parallel, feature-tuned channels by deploying many neuronal subtypes, a landscape now richly characterized by single-cell atlases (e.g., 16 somatosensory subtypes in mouse dorsal root ganglia; 11 Purkinje subtypes in cerebellum). Despite this widespread pattern of subtype diversity within individual neuronal classes, how progenitors generate distinct yet functionally related subtypes remains unknown. Using the *Drosophila* lamina (five lamina neuron subtypes receiving photoreceptor input), we uncover the regulatory logic: a pan-class homeodomain transcription factor (HDTF), induced in progenitors and maintained in all lamina neurons, drives diversification within the lamina neuron class by orchestrating a four-step program across the progenitor-to-newborn neuron transition. Specifically, it (i) establishes progenitor identity, (ii) promotes cell-cycle exit, (iii) induces subtype-specific HDTFs, and (iv) acts as their obligate cofactor to specify distinct subtypes. Loss of subtype-specific HDTFs in newborn—but not older—neurons drives subtype-to-subtype fate conversions at molecular, morphological, and functional levels, including a contrast-to-luminance encoding switch. Extending beyond *Drosophila*, our analysis of single-cell atlases shows that in mouse dorsal root ganglia (somatosensory, 16 subtypes), cerebellum (Purkinje, 11 subtypes), and retina (retinal ganglion cell, 45 subtypes), each subtype expresses both pan-class and subtype-specific HDTFs, indicating evolutionary conservation of this regulatory logic. Together, these findings convert a longstanding mystery into a generalizable principle for within-class subtype diversification: pan-class HDTFs define neuron-class identity and partner with subtype-specific HDTFs to generate distinct subtypes. This framework lays the groundwork for subtype-precise reprogramming and cell replacement strategies.

547T Role of the Guanine Nucleotide Exchange Factor, *GEFmeso*, in the Drosophila Neuromuscular Junction

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Small Rho Family GTPases act as molecular switches that mediate signaling events at the cell surface with downstream cellular responses. These protein switches are activated by Guanine Nucleotide Exchange Factors (GEFs) and deactivated by GTPase Activating Proteins (GAPs). This study examines the role of the Drosophila gene, *GEFmeso* in the development of the neuromuscular junction. *GEFmeso* has been previously shown to regulate the Rho Family GTPases Rala and Cdc42 in specifying the Drosophila leg and wing morphology. *GEFmeso* is thought to function with a GAP partner, called *Crossveinless-C* (CV-C) in wing development. Recent work has described a role for CV-C in regulating the neuromuscular retrograde signal mediated Glass Bottom Boat (GBB). Loss of function *cv-c* mutations cause an increase in neuronal T-bars but have no effect on bouton numbers. Here, we extend this analysis to *GEFmeso* loss and gain of function mutations. We find that *GEFmeso* appears to be necessary and sufficient for proper neuromuscular junction formation in the developing larvae. We also characterized the subcellular localization of GEFmeso using an endogenously GFP tagged construct. We found that it localized to the neuronal cell body rather than the junction.

548T Discoidin Domain Receptors Inhibit Dendrite Branching of Nociceptive Neurons

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Nociception is the sensory process that detects noxious, or potentially tissue-damaging stimuli. We will describe our investigation of the functions of the *smoke alarm (smal)* and the *discoidin domain receptor (ddr)* genes. Our prior work found that RNAi targeting *smal* in nociceptors caused hypersensitive thermal nociception behavior as well as a hyperbranched morphology of nociceptive Class IV multi-dendritic neurons. However, a DNA deficiency for *smal* phenocopies the hypersensitive behavioral phenotype but has the opposite dendrite phenotype of reduced dendrite branching. Here, we show that while the behavioral phenotype for *smal* can be rescued by a duplication for *smal*, the reduced dendrite phenotype is actually worsened. This led us to hypothesize that the dendrite phenotype of the *smal* deficiency mutant is caused by something other than loss of *smal.* Both *smal* and *ddr* encode homologs for mammalian Discoidin Domain Receptors (DDRs). Mammalian DDRs are receptor tyrosine kinases that are activated by binding collagen. Analysis of *smal* and *ddr* coding sequences in flies suggest that only proteins encoded by the *ddr* locus contain an intracellular kinase. We find that *ddr* is dramatically overexpressed in the *smal* deficiency mutant, and this overexpression persists in the presence of the *smal* duplication. This suggested the possibility that *ddr* may function in this pathway to inhibit dendrite branching. Indeed, we find that overexpression of *ddr* on its own is sufficient to reduce dendrite branching. To determine if overexpression of *ddr* in the *smal* mutant causes the reduced dendrite phenotype, we have generated a *smal ddr* double mutant and find that *ddr* mutations suppress the reduced branching of the *smal* deficiency. Our evidence combined indicate that *ddr* and *smal* function together in a pathway that limits the extent of dendrite branching in larval nociceptors. Furthermore, behavioral hypersensitivity is not correlated with the degree of dendrite branching and may be independently regulated by this pathway.

549T Actin drives dendrite tip dynamics, while microtubules regulate diameter in Drosophila class IV da neurons

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Of all cells, neurons have the most complex structures, which are key to neuronal function and define neuronal identity. *Drosophila* larval class IV dendritic arborization (da) neurons are nociceptive neurons that sense noxious stimuli and form a highly branched dendritic arbor just under the cuticle. The arbor is shaped during development by the dynamic branching, growth, and retraction of dendrites: the stochastic transitions of dendrite tips between growing, shrinking, and paused states densely fill the subcuticular space. Our goal is to elucidate the cytoskeletal mechanisms underlying dendrite development. To investigate the roles of microtubules and actin in shaping dendrite morphology, we individually knocked down *α-tubulin84B* and *actin5C* using RNAi in class IV cells. Downregulation of *α-tubulin84B* and *actin5C* results in progressively attenuated dendritic branching, leading to reductions in branch number, arbor size, and total dendritic length as the larvae develop. Furthermore, we found that knocking down *α-tubulin84B* reduces the diameters of proximal dendrites, suggesting that microtubules may regulate dendritic width. Because the tip dynamics resemble the dynamic instability of microtubules, we hypothesized that microtubule growth and shrinkage drive the tip dynamics. However, existing microtubule markers lack the sensitivity to visualize individual microtubules in the fine terminal dendrites of these cells. We therefore developed new fly lines expressing mNeonGreen (mNG) fused to small domains of the tubulin-binding protein Jupiter. Under the spinning disk confocal microscope, mNG-Jupiter showed only weak fluorescence signals at the distal ends of dendrites near the tips. By contrast, the mNG signal underwent large discrete jumps several micrometers proximal to the tips. Our quantitative analysis suggests that the jumps correspond to single microtubules, and the weaker distal fluorescence corresponds to soluble Jupiter. We conclude from these observations that the terminal dendrite tips of class IV da neurons lack microtubules. In contrast to microtubules, actin filaments are distributed distally and extend all the way to the tip, where they grow, shrink, and pause in synchrony with dendrite growth, shrinkage, and pauses. Thus, actin filaments are in the right place to drive tip dynamics. Overall, our findings indicate that actin drives tip dynamics, while microtubules regulate the dendritic diameter of *Drosophila* larval class IV da neurons.

550T Regulation of neuronal development and function by the *Drosophila melanogaster* translation initiation factor *eIF4H1*

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Williams syndrome (WS) is a rare human multi-system disorder characterized in part by neurodevelopmental, behavioral, and cognitive phenotypes. WS is caused by a genomic microdeletion that results in hemizygosity of 25-27 genes. While the roles of some genes in this Williams syndrome critical region (WSCR) have been characterized, the full understanding of how this genomic region contributes to WS social and behavioral phenotypes is incomplete. To address this issue, we utilized *Drosophila melanogaster* to investigate potentially novel connections between WS genes and animal social behavior. Using a neuron-specific RNAi screen, we find that expression of the translation initiation factor *eIF4H1* in the fly nervous system is necessary for proper social behavior. Using a novel GFP-tagged allele, we find that eIF4H1 is expressed in a broad subset of neurons and glia both during development and at maturation. We also developed an *eIF4H1*^{null} allele and find that it shows severe social behavior defects, as well as variable effects on development and morphology across neuronal systems. These results indicate that eIF4H1 may have a nuanced role in the fly nervous system, with context-specific effects in limited numbers of cells that impact fly behavior. They also point to a underappreciated role for the fine-tuning of neuronal protein synthesis via translation factors in regulating the development and function of the nervous system.

551T Lethal phase analysis, NMJ phenotypes, and allelic comparisons for a new null allele of *wit*, encoding a Type II BMP receptor expressed in the nervous system

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The canonical Bone Morphogenic Protein (BMP) pathway is essential for the development of glutamatergic synapses in both vertebrates and invertebrates. In *Drosophila melanogaster,* the neuromuscular junction (NMJ) is used as a model for AMPA-type excitatory synapses of the mammalian central nervous system. Retrograde BMP signaling from postsynaptic muscle to presynaptic neuron is required for the scaling growth of synaptic termini proportional to larval muscle growth. Autocrine, activity-dependent BMP signaling is required for proper synapse density and organization of the presynaptic active zone. In the absence of any BMP signaling, NMJs severely undergrow and do not release neurotransmitter normally, leading to impaired locomotion. About twenty years have passed since the identification of the core BMP signaling components regulating synaptic growth and function and the community has long relied on transheterozygous combinations of alleles generated by EMS and generously shared through the Bloomington Drosophila Stock Center. Many labs have used these alleles over the past two decades to elaborate on BMP pathway regulation and NMJ development. However, a thorough description of the molecular nature of the two most commonly used alleles, *wit*^{A12} and *wit*^{B11} is lacking. Both are presumptive null alleles: *A12* is described as an early stop codon in the extracellular domain of the protein, however the *B11* allele was never defined molecularly.

We undertook an analysis of the publicly available alleles, examining lethal phase, bouton and active zone number, and sequencing to confirm *A12* and describe *B11*. We simultaneously made a new null allele of *wit* using CRISPR/Cas9, replacing the locus with an attP landing site, and compared the new allele with the *A12* and *B11* alleles for the above phenotypes. Our analysis to date will be presented comparing all three alleles, as well as a deficiency of the region. Lastly, we will share our attempts to visualize wild-type Wit at the NMJ using a V5-tagged version of the gene, generated by recombining it into the attP landing site.

552T Early-life infection and immune signaling perturb development

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Building a healthy adult brain is a complex process. Neural stem cell proliferation and differentiation to make neurons and glia, neuropil extension, and synaptogenesis followed by pruning depend on optimal nutrient, resource, environmental, and genetic conditions. These processes are tightly regulated because errors can have severe impacts on the developing brain and consequent adult behavior. For example, infection and aberrant immune activation, especially early in life, are associated with disrupted growth and cognitive development in children. The fruit fly *Drosophila melanogaster* has been used extensively to study both neural development and immune signaling. From these studies we know that *Drosophila* neural stem cells, or neuroblasts, grow and proliferate in a nutrient-dependent manner through growth signaling pathways and give rise to the neurons and glia. *Drosophila* has two humoral immune pathways, Toll and Imd, and data from our labs show that chronic Toll signaling results in a reduced larval growth and smaller flies. We hypothesized that an early-life infection activates immune signaling in larvae and leads to disruptions to brain development. Indeed, infecting freshly-hatched larvae with *Erwinia carotovora carotovora 15* (*Ecc15*) induces the antimicrobial peptide Drosomycin, a Toll pathway target. Feeding *Ecc15* to freshly-hatched larvae slows their growth compared to uninfected controls. In uninfected larvae, neuroblast reactivation from quiescence occurs by ~48 hours after hatching. However, our results show that *Ecc15* infection delays neuroblast reactivation by 24 hours. As a likely consequence of this delay, the infected larvae have smaller brains than controls. Activating Toll signaling in the absence of infection also results in smaller larvae and a slightly delayed neuroblast reactivation from quiescence. Overall development, such as time to pupation is delayed in the infected group but not in the Toll-active group compared to their respective controls. When the infected and/or immune-activated larvae grow to adult flies, the 1–2-week-old flies have a reduced daily baseline activity compared to their respective controls. So far, our results suggest that infection perturbs neural development, and the effects might be mediated by the immune system signaling. Future work will be conducting knock-down experiments to silence components of immune pathway and assess the effects of infection on the brain development. In addition, we aim to identify factors through which an enteric infection affects the brain - an immune-privileged organ. Our established larval infection model together with the expansive *Drosophila* genetic toolkit will enable us to unravel the biology of brain development and plasticity in response to early-life infection and immune signaling challenges.

553T Toll-7 acts via distinct signaling axes to promote midline crossing

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In addition to their well-defined roles in innate immunity, Toll-like receptors are emerging as regulators of key steps of nervous system development, from axon guidance and debris clearance to neuronal proliferation and survival. In *Drosophila*, Toll-7 promotes motor and olfactory axon targeting, though the mechanisms by which it acts are unknown. Toll-7 might also control other guidance decisions, including those required for the midline crossing of commissural axons, an essential step in establishing circuits coordinating the left and right sides of the body in all bilaterally symmetric animals. In flies, the guidance receptor Frazzled (Fra) promotes axon growth across the midline through both a Netrin-dependent pathway to reorganize the growth cone cytoskeleton and a Netrin-independent pathway to regulate gene expression. Importantly, many axons still cross the midline in *fra* mutants, indicating that additional pro-crossing pathways exist. We recently identified Toll-7 as a novel Fra interactor in a proteomic screen of embryonic fly neurons, suggesting that Toll-7 might work with Fra in midline circuit formation. Here we show that Toll-7 acts both with and in parallel to Fra to regulate commissural axon guidance. First, we find that *toll-7* is expressed in the central nervous system at midline crossing stages, and that *toll-7* mutant embryos display guidance defects that partly overlap with those of *fra* mutants, including failed midline crossing and breaks in axon tracks. Re-expression of *toll-7* in commissural neurons rescues these defects, indicating a cell-autonomous role. Co-immunoprecipitation assays show that Fra and Toll-7 physically associate via their cytoplasmic tails, suggesting that they may work together to guide commissural axons. Consistent with this, loss of a single copy of *toll-7* enhances the midline guidance defects of mutants with attenuated Fra signaling. Importantly, re-expression of Toll-7 in commissural neurons suppresses this enhancement, indicating that Toll-7 and Fra function together in these cells. Mechanistically, we find that Toll-7 contributes to the Fra canonical pathway, as the Netrin-dependent Fra gain-of-function phenotype is reduced in the absence of *toll-7* gene function. Notably, *fra, toll-7* double mutants show more severe midline defects than *fra* single mutants, indicating that Toll-7 also acts independently of Fra at the midline. Loss of the TIR domain protein *dSarm* also enhances the commissural and axon scaffold phenotypes of *fra* mutants, raising the possibility of a Toll-7-dSarm signaling axis acting in parallel to Fra-Toll-7. Our work identifies Toll-7 as a novel and essential regulator of commissural axon guidance and provides mechanistic insight into its roles, revealing that it can recruit at least two distinct signaling pathways involving central players of nervous system connectivity.

554T Dynamic role of cortex glia in controlling neuroblast behavior during *Drosophila* larval neurogenesis

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During *Drosophila* larval neurogenesis, neural stem cells, also called neuroblasts, reside in specialized honeycomb-like cell compartments classically referred to as niches. Niches are thought to provide an essential microenvironment for neuroblasts to produce the immense diversity of molecularly and functionally distinct neuron types. Cortex glia, a glial subtype, forms a major structural and signaling component of the neurogenic niche. Because genes and basic cellular processes are evolutionarily conserved, results from this work will provide insight into mammalian neural development. At the start of larval neurogenesis, cortex glia grow their cell membranes to establish an extensive interconnected glial network. While glial growth is required to reactivate neuroblasts from developmental quiescence, it is not completely understood whether glia control neuroblast behavior throughout larval neurogenesis. To study the ongoing interaction between neuroblast and cortex glia, I ablated cortex glia either constitutively or conditionally during late larval stages after neuroblast reactivation. I found that neuroblast survival and proliferation are unaffected following constitutive ablation of cortex glia. In contrast, conditional ablation of cortex glia impaired neuroblast survival and proliferation. I am currently investigating the differences in constitutive and conditional cortex glial ablation paradigms to define novel mechanisms underlying glial-neuroblast niches and interactions.

555T Larval Supplementation of PUFAs Modulates Adult Motor Function, Neural Circuit Excitability and the Bio-Active Lipid Profile in the Hyperexcitable Drosophila Na_V Channel Mutant *para*^{Shu}.

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Voltage-gated Na+ (Na_V) channels are critical for action potential generation and propagation. In humans, mutations in these channel-encoding genes are linked to epilepsy, autism, migraines and other neurological disorders. In flies, a single gene, *paralytic* (*para*), encodes all Na_V channels, with mutant alleles displaying diverse array of behavioral and neurophysiological phenotypes. One semi-dominant allele, *para*^{Shu}, displays spontaneous seizures that are exacerbated by high temperatures. Remarkably, feeding *para*^{Shu} mutants milk whey during the larval stage attenuates expression of adult excitability phenotypes, with intake of a component ω-3 poly-unsaturated fatty acid (PUFA), α-linolenic acid (ALA, C18-3ω3), implicated in the effect (Kasuya et al. 2019, 2023). To gain a broader perspective of dietary PUFAs' modulation of *para*^{Shu} phenotypes, we compared the effect of supplementing ALA with feeding the ω-3 PUFAs eicosapentanoic acid (EPA, C20-5ω3), docosahexaenoic acid (DHA, C22-6ω3), and the ω-6 PUFA linoleic acid (LA, C18-2ω6). Using IowaFLI Tracker and a machine-learning approach to identify seizure-related behavior, we found ALA, EPA, and DHA similarly improved behavioral phenotypes at both baseline and high temperatures, while LA was ineffective. To establish corresponding changes in neuronal excitability and motor circuit function, we monitored flight muscle motor unit spiking activity in a tethered fly preparation. *para*^{Shu}/*Y* flies display ongoing spiking (~8 Hz) punctuated by high frequency bursts (> 50 Hz) that are bilaterally synchronized. Feeding ω-3 PUFAs eliminated the burst discharges and reduced overall spiking. To isolate motor neuron-generated activity in *para*^{Shu} flies, we blocked excitatory cholinergic inputs. Compared to control-fed counterparts, motor neuron-driven spiking was markedly reduced in ω-3 PUFA-fed flies. Lastly, we employed a targeted lipidomic (LC-MS) approach to assess the composition of Bio-active PUFA metabolites in wild-type (WT) and *para*^{Shu}/+ adult flies fed either the control-, ALA- or LA-diets as larvae. Compared to WT flies on control diet, *para*^{Shu} flies displayed an increase in LA-derived oxylipins and a reduction in ALA-derived species. LA feeding exaggerated this imbalance, while ALA feeding reversed this effect. Together, our findings indicate ω-3 PUFA feeding ameliorates hyperexcitability in paraShu mutants at both circuit and neuronal levels and highlights long-lasting impacts of diet in modulating Drosophila brain function.

556T The role of glial ER membrane protein complex subunit 4 (EMC4) in larval development and behavior

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The endoplasmic reticulum (ER) is involved in the modification, packaging, and insertion of membrane proteins in the cell. The ER membrane protein complex (EMC) is a protein complex in the endoplasmic reticulum composed of 8-10 subunits that work together to facilitate protein biogenesis. Our lab observed that *Drosophila melanogaster* exhibited dramatically shortened lifespans of 5-6 days with an RNAi knockdown of EMC subunit 4 in glia. These flies also exhibited severe locomotion impairments (weakened climbing), an increase in protein aggregation, a decrease in developmental viability, and delayed development. Through a detailed developmental analysis, we observed approximately a one-day delay per stage: wandering 3rd instar larva, pupation, and eclosion, suggestive of a delay that begins in larval development. The larval stage is a critical developmental period to obtain energy necessary for pupation. Chemosensory perception guides larvae to food sources through neural protein signaling. Since the EMC is involved in membrane protein insertion and folding, we hypothesize that it plays a role in chemosensory processing. We examined olfactory-driven behavior in larvae with a glial-specific knockdown of EMC4 and observed no significant differences in olfactory preference index to ethyl acetate. These results suggest that the observed developmental delay may be due to other alterations in glial function besides olfaction. To investigate possible locomotive impairments due to the glial EMC4 knockdown, we used video tracking software to analyze larval locomotion parameters, such as speed and direction. Overall, our findings suggest that glial EMC4 plays an important role in the larval stage, but further research is needed to understand its contribution to developmental timing.

557T Variation in neural stem cell spatial patterning predicts distinct neuronal populations between two flies, *Drosophila melanogaster* and *Megaselia abdita*

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To survive their unique habitats, different species employ diverse behavioral strategies such as running, flying, and swimming. Behavioral diversity requires differences across neuronal populations. Yet, how this critical interspecies neuronal diversity arises during development remains unknown. Within an organism, neuronal diversity arises from two processes that pattern neural stem cells (NSCs): temporal and spatial patterning. Temporal patterning diversifies the progeny born from a NSC, known as a lineage, while spatial patterning differentiates a NSC lineage from all others. We propose interspecies diversity arises from changes to one or both patterning processes. To relate variation in NSC patterning and neural progeny requires directly linking a NSC to its lineage. Embryonic insects provide tractable system in which NSCs and their lineages can be identified. Across insects, the number and position of NSCs are conserved. Limited prior work compared NSC patterning between distantly-related beetles (*Tribolium castaneum*) and flies (*Drosophila melanogaster*). The authors found limited variation in temporal patterning but identified variation in spatial patterning for more than half of NSCs. However, comparisons of beetles and flies are confounded by significant morphological variation between legged-grubs and legless-maggots. It remains unclear how much NSC patterning varies between morphologically similar organisms.

Here, we compare spatial patterning between two flies species, the fruit fly *D. melanogaster* and the scuttle fly *Megaselia abdita*, which share the maggot body plan. Unpublished behavioral and gene expression data suggest interspecies differences in their neuronal populations. Therefore, we ask if *M. abdita* exhibits significant variability in NSC spatial patterning compared to *D. melanogaster* by visualizing expression of a suite of spatial patterning genes in abdominal segments. We find that the NSC number is conserved in *M. abdita*. However, for about half of NSCs, we find variation spatial gene expression between *M. abdita* and *D. melanogaster*. Our data suggests significant variation in NSC spatial patterning, even among species with a shared body plan. Furthermore, by considering pattering of specific NSCs, we will generate testable hypotheses for how neuronal populations vary between species.

558F TRMT1, a tRNA methyltransferase linked to intellectual disability, functions in glia to promote memory

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Nervous system development depends on regulated protein expression and its disruption has been linked to neurological disorders. Transfer RNAs decode mRNA and are extensively post-transcriptionally modified to maintain stable structure and high-fidelity protein synthesis. Pathological variants in human tRNA methyltransferase 1 (TRMT1) cause a rare form of intellectual disability. TRMT1 is responsible for the stabilizing dimethylation of guanosine 26 to N2,N2-dimethylguanosine (m2,2G), yet how the loss of this tRNA modification disrupts nervous system development and function remains unknown. We used CRISPR gene editing in Drosophila to investigate the in vivo role of TRMT1 in the nervous system. Nucleoside mass spectrometry analysis demonstrates that m2,2G methylation is lost in the absence of TRMT1 and fully restored upon re-expression of TRMT1. Loss of TRMT1 results in severe memory disruption. Surprisingly, TRMT1 is primarily expressed in glia in the nervous system and cell-specific expression of TRMT1 in glial fully restores memory. To determine the effect of loss of TRMT1-dependent tRNA methylation in the brain, we conducted whole-brain proteomics and RNA sequencing, revealing enhanced adenosine signaling. Through genetic interaction studies, we find that reducing adenosine levels suppresses disrupted memory in TRMT1 mutants. These findings reveal the importance of regulated adenosine signaling in learning and memory, and suggest a molecular basis for TRMT1-associated intellectual disability.

559F sens-2 is essential for proper 10B axonal projection patterns

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During nervous system development, individual stem cells divide and give rise to a population of neurons called a lineage. There are 34 lineages in the ventral nerve cord (VNC) of *Drosophila melanogaster* (fruit fly), which is analogous to the vertebrate spinal cord. The adult nervous system is built during metamorphosis from neurons of these lineages. Leveraging genetic tools available in the fruit fly, I aim to investigate the molecular mechanisms driving the development of lineage 10B neurons within the adult nervous system. Two recently published datasets have made the fly VNC a unique model system to study neural circuit development: single cell RNA sequencing (scRNAseq) provides transcriptome profiles for individual neuronal lineages and Electron Microscopy (EM) volume provides synaptic connectivity among neurons at a single synapse resolution. EM volume identifies a group of proprioceptive sensory neurons, called the femoral chordotonal (FeCo) neurons, that make strong synaptic connections with 10B neurons. scRNAseq analysis identified a set of key genes likely involved in cell-fate acquisition, axon guidance, and synapse formation of 10B neurons.

Using this information, I have been performing gain or loss of function experiments to elucidate which transcription factors are essential for 10B identity and which cell-to-cell communication molecules are needed for 10B neurons to integrate into neuronal circuits, particularly those with the FeCo neurons. The 10B lineage has approximately 72 neurons per neuromere. We have identified 3 distinct subsets of 10B neurons within the scRNAseq data set and are leveraging the sophisticated genetic tools available in the Drosophila system to target each of the three subsets using a triple genetic intersection strategy. We have also identified that the transcription factor *sens-2* is essential for proper 10B axonal projection patterns. Understanding the roles of different transcription factors in determining cell fate decisions within 10B neurons, may help us identify common patterns shared by other hemilineages which will deepen our understanding of neural circuit formation during neurodevelopment.

560F The role of Transglutaminase in *Drosophila melanogaster* Brain Development and Function: The Case of Dopaminergic Neurons

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Transglutaminases (TGs) are a family of enzymes characterized by their calcium-dependent transamidation activity. In higher mammals, among the eight catalytically active TGs, TG2 is the member with the highest expression in the brain, and current research suggests a role in neurodevelopment, neuronal viability, and differentiation. Despite this evidence, TG2 knock-out mice show no major neurodevelopmental abnormalities, which might be explained by compensation from the other five TG members also expressed in the mammalian CNS. Thus, simpler animal models are needed to study the contribution of TGs to brain formation and function. *Drosophila melanogaster* possesses only one TG-encoding gene (*dTg*), but little is known about the role of this protein in fly brain development or physiology.

Predicted models of different TGs indicate that *Drosophila melanogaster* TGs and their human orthologs exhibit high similarity in overall protein structure and catalytic site organization, suggesting that they may share similar functions. We observed that fly mutants for *dTg* showed marked morphological alterations in the mushroom body and a notable reduction in brain dopaminergic neurons. Cultured dopaminergic neurons from *dTg* knock-down larval brains exhibited reduced neurite branching compared to controls, supporting a contribution of dTG to the proper development of dopaminergic neurons. Furthermore, dTG-deficient animals displayed decreased locomotion and a pronounced asocial-like phenotype, two behaviors associated with the dopaminergic system. Interestingly, expressing human TG6 under the control of the endogenous *dTg* promoter in the mutant background rescued social behavior while failing to restore motor output. Lastly, transcriptomic analyses revealed changes in the expression of several genes associated with neurogenesis and neurodevelopment in the brains of dTG-deficient animals compared to controls. These alterations were partially rescued by the expression of human transglutaminases.

561F Notch-dependent gene regulatory networks drive neural specification in the *Drosophila* medulla

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The *Drosophila* medulla in the visual system contains around 150 neuronal types, making it an exceptional model for uncovering the principles that generate neural diversity. Previous studies have revealed that spatial and temporal patterning, together with Notch-dependent binary fate decisions, contribute to medulla neurogenesis. However, the gene regulatory networks that link Notch signaling to the activation of neuron-specific transcription factors (nTFs) remain poorly understood. Using 10x Genomics Multiome (ATAC + RNA), we profiled chromatin accessibility and gene expression simultaneously in single developing medulla cells. Comparative analysis of enriched motifs in the open chromatin showed that binding motifs of Notch pathway components are highly enriched in accessible regions of Notch-ON cells, suggesting that Notch signaling actively shapes chromatin accessibility during neuronal specification. As an example, we examined how Notch signaling regulates the expression of *bsh*, an nTF that marks the first-born Notch-ON progeny (Mi1 neuron). We identified that Hey, a canonical Notch effector, is necessary for *bsh* activation, as removal of *hey* results in complete loss of *bsh* expression. Multiome data identified several candidate cis-regulatory elements linked to *bsh*. One enhancer (peak #5) is both necessary and sufficient for *bsh* expression: reporter assays recapitulate endogenous *bsh* expression, and genomic deletion of this enhancer eliminates *bsh* expression in the medulla without affecting its expression in the lamina. Peak #5 contains two Hey-binding E-box motifs (CACGTG). Mutating these motifs abolishes enhancer activity, suggesting that Hey directly activates *bsh* through this enhancer, which is contrary to its canonical role as a repressor. Interestingly, Hey expression is transient, present only in immature Mi1, while *bsh* expression is maintained into adulthood, indicating that other factors stabilize *bsh* transcription after the initial Notch-dependent activation. Together, our results reveal a Notch-dependent gene regulatory mechanism in which a transient Notch/Hey input primes a stable enhancer state to specify neuronal fate. In future work, we will dissect the regulatory logic of Notch signaling in neural specification by extending our analysis to additional nTFs expressed in Notch-ON and Notch-OFF lineages.

562F The importance of pH dynamics in development

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Na^+/H^+ exchanger (Nhe) proteins are essential regulators of intracellular pH in the brain. The importance of these exchangers in brain development is evidenced by the fact that their dysfunction is associated with neural development disorders including autism spectrum disorders and epilepsy. Our lab studies the role of Nhe proteins in brain development using the model system *Drosophila melanogaster* (fruit fly). Flies have only 3 Nhe proteins (Nhe1-3) compared to 9 in humans, making them a more straightforward model to investigate Nhe functions. Previously, we investigated Nhe2, which maintains cytosolic pH. We found that *nhe2* knockout flies exhibited smaller brains with proliferation defects. We next wanted to determine whether the other two Nhe proteins influenced brain development. Nhe3 is predicted to maintain endolysosomal pH, while Nhe1 is predicted to maintain Golgi pH. This project sought to determine whether knocking out *nhe3* would affect brain size and neural progenitor proliferation. The results of this experiment proved to be similar to the *nhe2* knockout study. *nhe3* knockout animals displayed smaller brain sizes that were accompanied by proliferation defects which resulted in fewer brain cells. These results indicate that the regulation of pH within the endolysosomal pathway plays a crucial role in brain development by regulating the proliferation of neural progenitors. Understanding how subcellular pH regulation influences brain development will offer valuable insight into the underlying contribution of pH disruption in generating developmental disorders.

563F Control of neuron size and arborization growth through the Insulin/TOR signaling pathway

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Within the Metazoa, neurons show cell type specific differences in size across the nervous system and yet how neurons regulate their growth during development remains underexplored. The stereotyped development of complex nervous systems requires the precise orchestration of axonal and dendritic growth to ensure appropriate patterns of synaptic connectivity. In addition, these regulatory mechanisms must be plastic enough to allow the development of robust, functional networks under variable environmental conditions, such as fluctuations in nutrients and temperature.

We have found that the insulin/TOR signaling pathway plays an important role in regulating arbor size in *Drosophila* neurons during metamorphosis. Modulation of the insulin/TOR pathway in multiple types of neurons within the brain and ventral nerve cord (VNC) results in changes in the size of axonal and dendritic arbors. These arbor size changes result in corresponding changes in the number of functional synapses, suggesting that insulin/TOR signaling is an intrinsic mechanism for neuron scaling. These results lead us to hypothesize that the expression levels of Insulin-like receptor (InR) gate insulin/TOR signaling and the expression pattern of InR across neurons coordinates relative neuron size within the VNC.

Expression mapping of InR shows dynamic, patterned expression in the VNC throughout metamorphosis, with a general trend of larger neurons expressing higher levels of InR during metamorphosis. Consistent with the idea that InR levels are developmentally coordinated, relative InR expression levels and patterns appear unaffected in the smaller VNCs of nutrient restriction treated flies. Cell autonomous knockdown and over-expression in specific neuron populations show a dose-response relationship between relative neuron size and InR expression. Taken together, our findings suggest that InR expression modulates insulin/TOR signaling in developing neurons to regulate and coordinate neuron size across the developing pupa.

564F Investigating the Roles of Nmdar2 and M6 in Schizophrenia

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Schizophrenia is a highly heritable severe neuropsychiatric disorder that causes a great burden of disease for over 24 million people worldwide. Excessive synaptic pruning of glutamatergic synapses occurs during schizophrenia and is hypothesized to cause schizophrenia. Further investigation on the role of genes associated with schizophrenia on synaptic pruning is necessary for better understanding of this disease and improved treatments. Downregulation of synaptic gene *NMDAR2A* is associated with schizophrenia. Synaptic gene GP*M6A* is associated with schizophrenia and is downregulated in depressed suicide victims and chronically stressed animals. *NMDAR2A* encodes for a subunit of the NMDA receptor, which is involved in synaptic plasticity, memory, learning, and sleep. GPM6A encodes for a neuronal glycoprotein that is involved in synaptogenesis. Our work will investigate the role of *NMDAR2A* and *GPM6A* on synaptic pruning and schizophrenia symptoms using *Drosophila* as a model organism.

Using the glutamatergic *Drosophila* neuromuscular junction (NMJ), we are investigating how knocking down *Nmdar2*and *M6* in neurons affects synaptic pruning. Additionally, we are investigating how knocking down *Nmdar2* and *M6* affects *Drosophila* behaviors related to schizophrenia symptoms of social withdrawal and impaired memory. We have found that these genes affect the presynaptic boutons, post-synapses, active zones, glutamate receptors, phagocytic receptors, and microtubules in the NMJ. Additionally, knocking down these genes leads to the presence of ghost boutons, indicating impaired synaptic pruning. Overall, this research will provide insights into the role of schizophrenia associated genes on synaptic pruning and schizophrenia development.

565F How do astrocytes grow and function in the nervous system?

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Astrocytes are specialized glial cells with highly ramified fine processes that closely interact with surrounding neurons and other brain cell types. They perform a range of essential functions in neural circuits, which are thought to depend on their complex morphology and close association with neural circuits. By leveraging a combination of molecular-genetic tools and live-imaging techniques in *Drosophila* and zebrafish, the goal of our group is to understand how astrocytes develop and contribute to neural circuit formation and function. Our recent work identified an evolutionarily conserved phospholipid-binding GPCR signaling in astrocyte morphogenesis. Through genetic screening in *Drosophila*, we found that loss of Tre1 GPCR in astrocytes severely disrupts their morphological development and close association with synapses. In zebrafish, we demonstrated that S1pr1 (the functional vertebrate GPCR analog of *Drosophila* Tre1) is required to enable astrocyte fine process outgrowth upon receptor activation. In *Drosophila*, we now focus on defining the downstream signaling pathways of Tre1 GPCR in regulating astrocyte outgrowth. Additionally, we explore how neuron-astrocyte interactions influence astrocyte morphology and function using forward and reverse genetic screening approaches in flies. Lastly, we investigate how defects in astrocyte growth impact neurons and neuron-astrocyte interactions. Our preliminary data show striking motor behavioral defects and disrupted neurite patterning when astrocyte morphology is impaired. We will perform cell-specific genetic manipulations and advanced imaging to examine neuronal aspects of brain circuits and assay astrocytic and neuronal activities in vivo. Together, we expect our work to help deepen the understanding of the roles astrocytes play in the nervous system at the molecular, cellular, and circuit levels.

566F Dissecting synapse development and pruning in the *Drosophila* model of neurofibromatosis type 1

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Neurodevelopmental Disorders (NDD) often include changes in learning and behavior, which are driven in part by synaptic alterations. Neurofibromatosis type 1 (NF-1) is a monogenic multisystem disorder resulting from mutations in the Neurofibromin gene (*NF1*). NF-1 is an NDD that presents learning and behavior differences in patients along with higher rates of psychiatric comorbidities such as autism and intellectual disability, but it is unclear whether changes in synaptic development contribute to these differences.

Loss of Nf1 in *Drosophila* results in behavioral alterations, including increased grooming frequency. Further investigation of this phenotype revealed that Nf1 expression is required during a developmental critical period for normal grooming behavior. This suggests Nf1 as an essential developmental protein, therefore, it is necessary to identify the irregular neurodevelopmental mechanism during critical periods under Nf1 control responsible for this difference.

Because *Drosophila* Nf1 protein is conserved, as is function of its downstream pathways, it is an exceptional model for NF-1. *Drosophila* have also been used to understand neurodevelopmental mechanisms, further increasing their value to fill this knowledge gap. To investigate whether loss of Nf1 affects synapse development and pruning, we examined synapse formation and elimination in the Drosophila antennal lobe. By applying RNAi knock down of Nf1 in flies a well-established olfactory exposure paradigm, we present a powerful tool to illustrate neurodevelopmental irregularities brought about by Nf1 protein mutations. Preliminary results suggest that loss of Nf1 affects pruning in the ethyl butyrate-responsive VM7 glomerulus of the antennal lobe. By determining the neurodevelopmental mechanism underlying the learning and behavior differences in NF-1 models we can apply the same experimental design to investigate downstream pathways of Nf1 protein.

567F Posttranscriptional control of cytoskeletal regulators during neuronal remodeling

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A neuron's function relies on its form. In some neurons, morphogenesis continues after embryonic development through neuronal remodeling. Neuronal remodeling is a developmentally controlled process that refines morphology through pruning of exuberant neurites followed by regrowth of new neurites to establish a mature structure. A central question that still remains is: how is the cytoskeleton rapidly reorganized to support neurite regrowth at local sites during neuronal remodeling? We hypothesize that posttranscriptional regulation of mRNAs encoding cytoskeletal regulating proteins supports neurite regrowth in remodeling neurons because posttranscriptional regulation, such as RNA trafficking and local translation, allows for local regulation at sites distal to the cell body. We use Drosophila central nervous system mushroom body y neurons and peripheral sensory class IV dendritic arborization neurons as models to elucidate mechanisms underlying neuronal remodeling because they undergo stereotyped remodeling of their axons and dendritic arbors. Through genetic knockdowns of transcripts encoding actin binding proteins profilin and Ciboulot, we found that these proteins are required for successful remodeling of mushroom body axons and peripheral dendritic arbors and that they function synergistically during neuron morphogenesis. To study RNA trafficking dynamics during this process, we utilized live imaging of fluorescently labeled *profilin* RNA to characterize *profilin* RNA localization and motility

568F Hmx rewires Hippo pathway signaling to specify and maintain a photoreceptor subtype identity

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The Hippo signaling pathway is a highly conserved regulator of organ size, but more recent evidence suggests that it also plays key roles in neuronal differentiation. In the pupal *Drosophila* eye, the Hippo pathway is part of a feedback loop that mediates a binary photoreceptor fate decision between the blue-sensitive Rhodopsin Rh5 and the green-sensitive Rh6. During mitotic growth, the coactivator Yorkie (Yki) promotes the activity of the core Hippo pathway kinase Warts (Wts), which in turn phosphorylates Yki; this negative feedback loop limits growth in mitotic tissues. We discovered that in post-mitotic photoreceptors, the Hippo pathway is rewired to a positive feedback loop by the transcription factor H6-like homeobox (Hmx). Hmx is specifically expressed in Rh5 photoreceptors, where it represses Wts to maintain Yki activity and thereby establishes a positive feedback loop that maintains Rh5 photoreceptor fate.

To understand how Hmx controls Hippo signaling at the transcriptional level, we performed a modifier screen that identified the co-repressor Scribbler (Sbb; ZNF609 in humans) as a candidate Hmx cofactor. Knockdown of *sbb* phenocopied *hmx* loss, i.e., caused a loss of Rh5 and a gain in Rh6 photoreceptors. Moreover, a genetic interaction assay revealed that *sbb* suppresses Hmx overexpression phenotypes. These findings suggest that Sbb may cooperate with Hmx in Rh5 PRs. To validate whether Sbb and Hmx physically interact, we are currently performing co-immunoprecipitation *in vitro* and *in vivo*.

Taken together, we propose that Hmx and Sbb establish the positive feedback loop that underlies the mutually exclusive Rh5 vs. Rh6 subtype decision. Since Hmx, Sbb, and the core Hippo pathway components are conserved, these insights may facilitate the identification of similar mechanisms in the mammalian nervous system and mammalian disease contexts.

569F Role of Early Temporal Factors in Regulating Ensheathing Glia in Drosophila Brain

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The Drosophila melanogaster brain contains diverse type of neurons and glia, which arise from a small pool of neural stem cells, known as Type I & II. Type-II neural stem cells (T-II NSCs) generates tremendous diversity of neurons and glia that populates Drosophila adult brain Central Complex (CX), which is a higher order brain center that regulates sensory motor integration. Recent work has shown that these T-II NSCs undergo temporal patterning, changing their gene expression profile as they grow from young T-II NSCs to old. Early on, these T-II NSCs have been shown to express IGF-II-mRNA-binding protein (Imp), and the nuclear receptor Seven-up (Svp). Recent studies have shown the role of these early temporal factors in generating different neuron populations (Aisha et al 2024), but it is not known whether these early factors also generate glial diversity.

To answer this question, we are studying a special type of glia, called ensheathing glia that is found in Drosophila brain. This ensheathing glia is made by T-II NSCs, and it migrates from the CX to the Optic lobe (OL), where it ensheathes OL neurons. Knockdown of early temporal genes in T-II NSCs showed an increased ensheathing glial population suggesting early temporal genes have a role in regulating glial diversity.

Currently we are investigating what specific T-II NSCs make these ensheathing glia, and when are these glia made during development. Also, we will be doing lineage specific knockdown of guidance cues to find how migration of ensheathing glia happens to the OL, and what important function they perform there. This study will determine whether glia respond to these temporal switches, similar to neurons, which may reveal a previously unrecognized mechanism of neural diversity.

570F Deep learning-enabled prediction and interpretation of cis-regulatory sequences specific for Drosophila neuronal subtypes

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Alterations in neural circuit development have been linked to various neurological disorders. The Drosophila visual system is an excellent model for understanding how diverse neural cell types are generated and form complex circuits. Distinct combinations of transcription factors, known as terminal selectors (tsTFs), establish and maintain cell-type identity in postmitotic neurons, but how cis-regulatory regions read out the various tsTFs combinations in a cell-type-specific manner remains largely unknown. To address this, we generated a single-cell multiomics atlas of developing optic lobes at four key stages, providing a high-resolution characterization of gene expression and chromatin accessibility for all its cell types. We then trained deep learning models on cell-type-specific accessibility profiles and inferred the underlying grammar of cis-regulatory sequences by interpreting these models. As a proof of concept, we present BPNet models of pseudobulk ATAC-seq profiles at various developmental time points for two cell types, Tm1 and Tm4. Interpretation of these models revealed canonical pioneering motifs like GAGA, architectural TF motifs such as CTCF, and promoter elements known to be associated with accessible regions. Interestingly, the models also effectively capture the binding motifs of key tsTFs in each cell type. To evaluate the utility of these models, we tested model-derived mutations on a Tm1-specific enhancer of the tsTF Drgx, which we had previously shown to be critical for specifying Tm1 fate over Tm4. These mutations resulted in increased or decreased expression of the reporter construct, as predicted by the models. Our results demonstrate that accurate interpretations of BPNet models reveal cis-regulatory rules of tsTFs and provide a way to design targeted mutations to disrupt the regulatory circuitry.

571F Alterations in GABAergic Signaling in the Hyperexcitable *Drosophila* Na_V Channel Mutant *para*^{Shu}.

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Neural circuit function often requires a balance between excitatory and inhibitory neurotransmission. In both vertebrates and flies, GABA is the primary inhibitory neurotransmitter. Disruption of GABA synthesis or release machinery can contribute to the emergence of seizure activity, and several anti-seizure drugs target GABA receptor function. In this study we compare inhibitory GABAergic signaling in wild-type (+/+) flies to seizure-prone flies carrying a gain-of-function allele in the voltage-gated Na^+ channel gene *paralytic* (*para*^{Shu}/+). Measurements of whole-fly GABA levels (via GC-MS) reveal significant increases in *para*^{Shu}/+ compared to +/+ counterparts. Potentially, elevated GABA levels could compensate for *para*^{Shu}-related neuronal hyperexcitability. To examine this possibility, we created double-mutants carrying *para*^{Shu} allele and a loss-of-function mutation in the vesicular GABA transporter gene (*VGAT*$^{T2A\text{-}Gal4}$). Using a tethered fly preparation to monitor changes in flight muscle motor unit activity, we noted clear non-additive interactions between the mutations. While *para*^{Shu}/+ and *VGAT*$^{T2A\text{-}Gal4}$/+ displayed occasional spikes related to grooming activity (<0.1 Hz), the *para*^{Shu}/+;*VGAT*$^{T2A\text{-}Gal4}$/+ double-mutant showed ongoing spontaneous spiking (~1.5 Hz). This activity persisted following pharmacological blockade of excitatory inputs to the motor neuron. Potentially, the *VGAT* mutation serves to disrupt an increase in GABAergic tone that normally counters *para*^{Shu} hyperexcitability. Combining the *VGAT*$^{T2A\text{-}Gal4}$ with a fluorophore reporter (CD4-GFP, nls.redStinger) enabled visualization of endogenous *VGAT* expression. Consistent with elevated GABA levels in *para*^{Shu} flies, whole-brain imaging of *para*^{Shu}*/GFP-redStinger;VGAT*$^{T2A\text{-}Gal4}$/+ flies revealed a significant increase in *VGAT* expressing cells in the brain, most prominently in the mushroom body and fan-shaped body neurons. Lastly, we examined the effect of feeding α-linolenic acid (ALA, an ω-3 poly-unsaturated fatty acid) on the phenotypes above. Previously, we have found feeding ALA during the larval stage attenuates hyperexcitability in adult *para*^{Shu} flies. In ALA-fed *para*^{Shu}/+;*VGAT*$^{T2A\text{-}Gal4}$/+, the spontaneous firing phenotype significantly reduced compared to control diet-fed counterparts, while the number of *VGAT*-expressing cells in the brain increased further. Overall, our findings suggest that plasticity within GABAergic systems contributes to the modulation of hyperexcitability in *Drosophila.*

572S Temporal Regulation of Gad1 Translation and Activity During GABAergic Neuron Maturation in *Drosophila melanogaster*

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GABAergic neurotransmission underlies the balance of excitation and inhibition in the nervous system, so understanding how GABA synthesis is temporally controlled during development is essential for elucidating the mechanisms that ensure circuit stability. To investigate how GABA production is regulated during neuronal maturation, we examined the developmental dynamics of Glutamic Acid Decarboxylase 1 (Gad1), the enzyme responsible for GABA synthesis, across pupal stages in *Drosophila melanogaster*. We generated and validated a Gad1 antibody, confirming consistent labeling with a Gad1 reporter and reproducing previously reported neuromuscular junction staining, despite Gad1 not being expressed in motor neurons. Using this antibody, we found that nearly all GABAergic neurons in the thorax clear and downregulate GABA production by 24 hours after pupa formation while retaining high levels of Gad1 protein throughout pupal development, revealing an uncoupling between enzyme presence and neurotransmitter availability.

To determine whether this uncoupling reflects differences between neuronal birth orders, we distinguished primary and secondary neurons using immortalization and EdU labeling. Primary neurons retained robust Gad1 protein, whereas secondary neurons expressed low levels during early metamorphosis, suggesting that temporal regulation of Gad1 may gate the onset of GABA synthesis in newly generated neurons. Surprisingly, fluorescent in situ hybridization showed comparable *gad1* mRNA levels between both populations, raising the possibility that translational control or differential protein stability underlies these patterns.

To test whether post-transcriptional regulation contributes to these differences, we misexpressed the Gad1 coding region and observed high mRNA and protein levels confined to neuroblasts and newborn neurons, with rapid loss in mature neurons. In non-neural tissue, Gad1 protein accumulated but failed to generate GABA, indicating that additional neuronal factors are required for enzymatic activity. Ongoing work examines whether Gad1 untranslated regions stabilize its transcript and whether predicted microRNA-190 binding within the 3′UTR contributes to translational regulation. Together, these findings indicate that Gad1 expression is tightly controlled at multiple post-transcriptional levels to ensure the correct temporal onset of GABA synthesis during neuronal maturation.

573S SPARC is a transcriptional target of BMP signaling that promotes synaptic density and apposition

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The *Drosophila* neuromuscular junction (NMJ) is an important model for understanding glutamatergic synapse assembly and function. The Bone Morphogenetic Protein (BMP) pathway from the TGF-β superfamily is required in neurons for NMJ synaptogenesis and synaptic function. Loss of BMP pathway function results in smaller synaptic terminals and fewer active zones. In addition, the organization of the active zone cytomatrix is disrupted in BMP pathway mutants, preventing proper neurotransmitter release. Given the importance of BMP signaling in NMJ development, we screened predicted transcriptional targets of neuronal BMP signaling by RNAi. Since forward genetic screens were previously conducted looking for changes in synaptic terminal size (and these identified components of the BMP pathway itself), we focused on identifying disruptions in active zone density or apposition of pre-and post-synaptic compartments. We screened 155 RNAi lines representing 87 genes, and identified 12 genes with appreciable differences in active zone size, density, or apposition to the post-synaptic density. One particularly interesting gene identified in this screen is *SPARC* (Secreted Protein Acidic and Rich in Cysteines). *SPARC* is a transcriptional target of the BMP pathway which encodes an ECM protein that has been shown to bind to growth factors including those from the BMP/TGF-β families. In mouse, SPARC and its paralog Hevin are required for synapse elimination and synapse formation, respectively. In *Drosophila*, there appears to be only one homolog, so it is possible that *Drosophila* SPARC mediates one or both of these roles. Our preliminary results show that neuronal loss of *SPARC* presents with an apposition defect as well as defects in synapse density and active zone size. We are continuing to study whether *SPARC* interacts with the BMP pathway to affect synapse formation and/or elimination by closely examining the composition and organization of active zones and post-synaptic densities of NMJs.

574S Investigating the Role of *SATB1* in Eye Development and Craniofacial Disorders

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Special AT-rich sequence binding protein 1 (SATB1) is a well-known global chromatin organiser. Initially, SATB1 was known to be majorly involved in T-cell development. Recent studies suggest that SATB1 also plays a major role in neurodevelopment. Mutations in *SATB1* are implicated in a craniofacial developmental disorder in humans called Den Hoed-de Boer-Voisin (DHDBV) syndrome. In *Drosophila*, the K-50 homeodomain transcription factor *defective proventriculus* (*dve*), an ortholog of human SATB1, acts as a critical dorsal fate selector gene essential for Dorso-Ventral (DV) patterning. Loss of function of Dve leads to dorsal eye enlargement, whereas its gain of function results in complete eye suppression. Previous structure-function analyses have identified specific domains of Dve responsible for these effects. To investigate the functional conservation between *dve* and *SATB1*, this study employs genetically engineered *Drosophila* models with targeted mutations in specific domains of SATB1 to highlight their roles in eye and brain development. The findings of the eye and brain development will be presented. These findings aim to enhance our understanding of developmental mechanisms, growth, and patterning, and provide insights into the etiology of early developmental birth disorders.

575S Structural insights into the Beat-Side interactions reveal roles in Drosophila neuromuscular circuit wiring

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The precise control of animal behaviors depends on the appropriate assembly of neural circuits. The Drosophila larval neuromuscular system consists of 33 motor neurons and 30 muscles with invariant connectivity patterns in each body wall hemisegment. Cell surface proteins play critical roles in nervous system assembly. In Drosophila, Beaten Path (Beat) and Sidestep (Side) are two interacting cell surface protein families whose members have been described as axon guidance receptor-ligand pairs, in addition to roles in specifying synaptic connectivity. To understand the molecular basis and specificity of Beat-Side interactions, we report here the first Beat-Side structure, Beat-Vc bound to Side-VI. The structure showed a binding topology similar to other neuronal immunoglobulin superfamily receptors, including Nectins, SynCAMs, Dprs and DIPs, despite lack of established evolutionary relationships. Using a structure-based rational approach, we generated Drosophila larvae carrying mutations in the endogenous side locus that abrogate Beat-Ia binding: these mutants show a loss of ventral muscle innervation. Additionally, unlike overexpression of wild-type Side, overexpression of mutant Side in muscles does not alter motor neuron innervation of dorsal muscles. This gain-of-function phenotype can be rescued by co-overexpression of wild-type Beat but not Beat carrying mutations in the interaction interface. These results suggest that the Beat-Ia–Side interaction is required for appropriate assembly of the Drosophila neuromuscular system.

576S Gene Regulatory Mechanisms Involved in *Drosophila* Optic Lobe Neuron Specification

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The *Drosophila* optic lobe comprises a diverse array of neuronal types that arise through distinct patterning mechanisms, including temporal, spatial, and Notch-dependent patterning. The temporal patterning in *Drosophila* neural progenitors, called neuroblasts, is attained through sequential expression of specific Temporal Transcription Factors (TTFs) in a predetermined order. Expression of specific TTFs in the neuroblasts leads to specific neuronal fates in the progenies, even in cases where the expression of the TTF is not maintained in the progenies. Even though the different temporal stages and the respective TTFs are known, it is not clear how the expression of these TTFs in the neuroblasts translates to specific neuronal fates in the progenies. We hypothesize that the TTFs, along with Notch-dependent binary fate choice, regulate specific neural transcription factors (nTFs) in the progenies, and combinations of these nTFs regulate neuron-specific genes. We performed single-cell multiomic sequencing of medulla cells to draw correlative linkages between gene expression and chromatin accessibility. Our analysis identified the cis-regulatory elements (CRE) associated with many prominent nTFs in different temporal stages. These CRE elements were then validated through reporter expression in appropriate neural types. Interestingly, some of these CREs are made accessible in corresponding temporal stages in the neuroblasts themselves, although nTF expression is seen only in the neurons. To further test our hypothesis that the basis of temporal patterning of progeny neurons is through chromatin accessibility changes guided by TTFs, we did a series of scATAC experiments in TTF-RNAi backgrounds, and analyzed how the chromatin accessibility changes. Our data suggests that the chromatin accessibility of medulla cells changes in the absence of these TTFs, and respective TTFs are required to regulate the CRE accessibility, thereby guiding the expression of downstream nTFs. For example, CREs of nTFs expressed when the TTFs D and Scro are expressed, like Ets65A, have D and scro binding sites. Mutation of D or Scro binding sites in the CRE abolished the reporter expression. Following CUT&RUN experiments showed that these TTFs bind convergently to these regulatory regions in the neurons. These results delineate how TTFs regulate nTFs in *Drosophila* optic lobe medulla, and potentially in other similar systems of cell fate determination.

577S Role of a novel transmembrane protein in localizing a visual signaling complex to the light-sensing compartments

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Drosophila photoreceptor neurons contain specialized light-sensing compartments (rhabdomeres) that house light-sensing pigments and phototransduction proteins. The phototransduction proteins are assembled into a signaling complex by the scaffolding protein INAD (Inactivation No Afterpotential D). INAD is a five PDZ domain-containing protein, and each PDZ domain interacts with a specific phototransduction protein. Previous studies have shown that the INAD complex is preassembled in the cytoplasm before it is targeted to the rhabdomeres. However, the mechanism that targets the scaffolding protein and its interactors to the rhabdomere remains poorly understood.

We showed that the scaffolding protein INAD and its interactors INAC, TRP and NINAC, are already expressed during early rhabdomere development. In addition, we discovered a novel INAD interactor, Mps (Major photoreceptor stabilizer), which is a four transmembrane domain protein. Mps colocalizes with INAD and the light-sensitive cation channel TRP in the developing rhabdomeres. Strikingly, both INAD and TRP are mislocalized to the cell body in *mps* null mutants, indicating that Mps is required for the rhabdomeric localization of INAD and TRP. Since INAD is required for localizing the eye-specific kinase INAC to the rhabdomeres, we found that INAC is also mislocalized to the cell body in *mps* mutants.

Taken together, we identified a novel interactor of the scaffolding protein INAD, the transmembrane protein Mps, which is required to localize the INAD complex to the rhabdomeres. These results enhance our understanding of the mechanisms that target scaffolding proteins and their interactors to specialized neuronal compartments.

578S IgSF cell adhesion molecules restrict synaptic growth at fly larval and adult NMJs

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Proper synaptic connectivity and maintenance are essential for neural circuits to properly function. Interactions between complementary cell surface proteins (CSPs) have been implicated in synaptic connectivity, but how these synapses are maintained is less well understood. The *Drosophila* DIPs and Dprs are immunoglobulin superfamily (IgSF) CSPs mediate several steps of circuit assembly, including axon pathfinding and synaptic connectivity. For example, DIP-α and Dpr10 interact to regulate synaptic partner matching at the embryonic/larval neuromuscular junction (NMJ). Notably, DIP-α and Dpr10 continue to be expressed into adulthood, intimating potential roles in synaptic maintenance or growth. Here, we characterize their roles in synaptic growth at larval and adult NMJs and elucidate cell type-specific requirements.

We find that DIP-α and Dpr10 are required for restricting synaptic growth at the larval NMJ, as loss of either protein leads to NMJ expansion at ventral longitudinal muscle 12 (m12). Since *dpr10* is expressed by both m12 and an innervating Is motor neuron (MN)—the ventral common exciter (vCE)—we investigated cell type-specific requirements for *dpr10*. Muscle-specific, but not Is-MN-specific, *dpr10* knockdown recapitulated the overgrowth phenotype observed in $dpr10^{-/-}$ mutants, indicating a muscle-autonomous role. To establish whether this function is conserved in adults, we examined the adult dorsal longitudinal muscle (DLM) NMJ. Indeed, loss of either *dpr10* or *DIP-α* produced similar overgrowth phenotypes. Expression analysis at the adult DLM NMJ revealed that *DIP-α* is expressed by dorsal longitudinal motor neurons (DLMns). While *dpr10* expression in muscles and MNs has been characterized at various stages, in adults, we observed expression in DLMns and peripheral glia, suggesting potential glia–MN crosstalk. Notably, *dpr10* expression in glia has not been previously reported. Ongoing experiments are confirming cell type-specific requirements for *dpr10* and *DIP-α* and examining the roles of other Dprs and DIPs in synaptic growth and maintenance. This study highlights the multifaceted roles of Dprs and DIPs and provides insight into how the same CSPs can be repurposed for unique functions in different developmental stages.

579S Astrocytic DYRK1A overexpression induces neurodegenerative phenotypes in *Drosophila melanogaster* and mammalian astrocytes *in vitro*

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Background: DYRK1A is a multifunctional kinase that regulates diverse neuronal and astrocytic processes. Its overexpression is known to trigger several pathological cascades in neurons, contributing to neurodegeneration and Alzheimer's disease (AD) progression. However, the consequences of DYRK1A overexpression in astrocytes—cells essential for neuronal support and homeostasis—remain poorly understood.

Methods: To investiate astrocytic DYRK1A function and pathology, we employed an astrocyte-specific DYRK1A overexpression *Drosophila melanogaster* model, assessing developmental and neurolocomotor phenotypes. Additionally, *in vitro* assays of Aβ or LPS stimulated mammalian astrocytes exposed to a pharmacological DYRK1A inhibitor were used to evaluate DYRK1A's contribution to astrocyte dysfunction by regulating astrocyte reactivity—a neurotoxic state driven by Ca^{2+} upregulation—and excitotoxicity—the astrocytic failure to clear synaptic glutamate, a process modulated by CaMKII.

Results: Astrocytic DYRK1A overexpression disrupted normal thorax bristle development and impaired fly locomotion. In cultured mammalian astrocytes, Aβ/LPS stimulation increased DYRK1A and GFAP levels, dysregulated Ca^{2+} dynamics and enhanced DYRK1A–CaMKII interaction. Pharmacological DYRK1A inhibition mitigated both these *in vivo* phenotypes and *in vitro* molecular alterations.

Conclusions: Our findings suggest that DYRK1A overexpression in astrocytes disrupts neuron–astrocyte communication, enhances astrocyte reactivity, and compromises glutamate uptake mechanisms. Collectively, these impairments could promote the loss of astrocyte-mediated neuroprotection. Thus, targeting astrocytic DYRK1A offers a novel therapeutic avenue to promote neuronal homeostasis and survival in AD.

580S Investigating a Role for Ecdysteroid Reception in Glia of Peripheral Nerves During *Drosophila* Metamorphosis

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Metamorphosis in Drosophila melanogaster is associated with significant restructuring of form and function. An example is the process of reorganization of abdominal peripheral nerves where 5-pairs of posterior nerves fuse to form the Terminal Nerve Trunk (TNT). The fusion of nerves is initiated at 28h APF and completed by 36h APF, during the 4 day period of metamorphosis (Subramanian et al 2017). Each larval nerve is ensheathed by an extracellular Neural Lamella (NL), followed by three layers of glia: Perineurial Glial (PG), Sub-Pernineurial Glial (SPG) layer, the blood brain barrier and Wrapping Glial (WG) layer, the inner most layer wrapping individual axons, (Stork et al 2008). Remodelling of each ensheathing layer occurs in a characteristic spatial and temporal manner (Subramaiam et al., 2017). In examining the expression of ecdysteroid receptors (EcRs) in peripheral nerve associated glia, our pilot data have demonstrated expression in the most external PG layer (n= 50 animals, A1-A8 nerves). Studies are underway to test the hypothesis that EcR expressed in this layer of glial cells (PG) influences the nerve fusion that generates the TNT. A PG specific driver line is being used to express EcR-RNAi and EcR-DN to test the impacts on TNT formation, and the adult specific behavior of eclosion. A third set of experiments is underway to eliminate individual glial layers using Diphtheria Toxins (DT-A) to determine the role of these glial layers during reorganization of larval abdominal nerves during metamorphosis.

581S Two cell surface protein subfamilies modulate axon terminal morphology of Drosophila motor neurons

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The *Drosophila* Dpr and DIP immunoglobulin superfamily (IgSF) proteins form an extensive network of cell-surface interactions that shape circuit assembly throughout the nervous system. Although several Dpr-DIP pairs have been characterized in various circuits, the functions of most family members remain unknown. We showed that Dprs/DIPs are expressed in the fly larval neuromuscular system, suggesting roles for Dpr-DIP interactions. For example, DIP-epsilon (DIP-ε) and DIP-zeta (DIP-ζ) and their Dpr partners are expressed in specific motor neurons and/or muscles. DIP-ε and DIP-ζ are sparsely expressed in subsets of Type I motor neurons, while their Dpr partners are broadly expressed in nearly all motor neurons. Additionally, one of these Dprs, Dpr19, is also expressed in muscles. Sequence analyses indicate that DIP-ε and DIP-ζ show high sequence identity within their first Ig domain, which is required for interactions with Dprs. The sequence similarity is even stronger at the Dpr binding interface. This high sequence conservation suggests functional redundancy or shared signaling capacity between the two DIPs. However, the roles of DIP-ε, DIP-ζ, and their interactions with specific Dpr partners at the neuromuscular junction (NMJ) remain unresolved. This study leverages the stereotypy and accessibility of the fly NMJ to decouple the roles of specific Dpr-DIP interactions in synaptic development.

To investigate these interactions, we performed loss-of-function analyses of DIP-ε, DIP-ζ, and their Dpr partners. Analysis of the number of synaptic boutons, at Type Ib and Is motor neuron terminals revealed that some of these CSPs inhibit growth, while others promote it. These data suggest unique functions for specific Dpr/DIP combinations. Further, bouton size was also altered in a subset of these mutants, indicating multifaceted functions at the same NMJ. Rescue experiments will test where each Dpr and DIP is required to assess if cis and/or trans Dpr-DIP interactions are driving these functions. We are also performing genetic interaction experiments to examine redundancy and potentially independent roles of each Dpr and DIP. These findings position DIP-ε and DIP-ζ as key regulators of NMJ growth, with distinct but overlapping roles from their Dpr partners. This work extends the known roles of the DIP-Dpr network in development and highlights a conserved molecular strategy by which cell-surface recognition molecules guide synapse development in the motor system.

582S An ecdysone-independent role for the EcR-B1 receptor in neural stem cell temporal patterning

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The medulla is the largest structure of the *Drosophila* optic lobe, comprising ~150 neuronal cell types, making it a powerful model to study how neural stem cells generate neuronal diversity. At the onset of the 3rd instar larval stage, medulla neuroblasts integrate spatial and temporal cues to generate diverse neuronal types. We have recently shown that medulla neuroblasts are additionally patterned by opposing gradients of the early Imp and late Syp RNA-binding proteins. Imp/Syp and their downstream transcription factors (Chinmo, Mamo and E93) confer unique identities to neurons based on the time they are specified during development. In central brain neuroblasts, the ecdysone hormone extrinsically regulates the early Imp/Chinmo to late Syp/E93 transition. However, whether ecdysone signalling regulates Imp/Syp patterning in the developing medulla remains unclear. Here, we show that the ecdysone receptor, EcR-B1, regulates Imp/Syp patterning in the developing medulla in an ecdysone-independent manner. As a first step, EcR-B1 expression initiates at 48 hours after-larval-hatching (ALH), which coincides with the onset of medulla neurogenesis. Expression levels peak at 60 hours ALH and steadily decline until the end of larval neurogenesis. Next, we show that EcR is necessary for the expression of early factors and is both necessary and sufficient to repress late factors; *UAS-EcR-RNAi* clones fail to express the early factors Imp/Chinmo and precociously express the late factor Syp, whereas *UAS-EcR-B1* gain-of-function (GOF) clones show decreased Syp expression. Surprisingly, expressing the dominant-negative (DN) form of EcR-B1, which cannot bind ecdysone, fails to activate Syp/E93 and prolongs the expression of Imp/Chinmo/Mamo. This shared phenotype of reduced Syp expression between the DN and GOF clones suggests that EcR-B1 regulates gene expression in the absence of ecdysone, consistent with the repressive function of unliganded EcR-B1. Indeed, using an ecdysone sensor system, we find that there is no ecdysone signalling activity in medulla progenitors. Our data supports a model in which EcR-B1 acts in an ecdysone-independent manner to activate Imp and suppress Syp expression in the developing medulla. We anticipate that the mechanisms uncovered here will play a conserved role in the temporal patterning of neural progenitors.

583S Concurrent temporal patterning of neural progenitors in the developing *Drosophila* optic lobe

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The spatial and temporal patterning of neural stem cells is a powerful mechanism to generate the neuronal diversity required for complex neural circuitry. In recent years, the medulla of the *Drosophila* visual system has emerged as a model system to study how neural stem cells integrate spatial and temporal patterning inputs. Medulla neurons are generated during larval stages from neuroblasts (NBs) patterned by independent spatial and temporal inputs: spatially the NBs express different transcription factors based on their position, while temporal patterning arises through the expression of a series of transcription factors as NBs age. Recently, our lab has uncovered a third patterning axis where the neuroepithelial (NE) cells which give rise to the NBs are patterned by opposing temporal gradients of the Imp and Syp RNA-binding proteins. While it has been shown that Imp and Syp are necessary for the generation of neuronal diversity within one spatial-temporal address, Vsx1-Hth, it is unclear if Imp and Syp are also necessary for neuron type diversification in other spatial-temporal windows. To determine whether Imp/Syp act as a global patterning mechanism in the developing medulla, we have characterized the neuronal outputs generated at the intersection of the Vsx1 spatial and Ey temporal windows. As a first step, we generated a *vsx1-ey* split-Gal4 line that only produces a functional Gal4 protein in cells expressing both the *vsx1* and *ey* genes. We next used this line to drive a MultiColor FlpOut (MCFO) genetic construct which stochastically labels single-cell clones in adults. By comparing the morphology of the neurons labeled by MCFO to the *Drosophila* optic lobe connectome, we identified eight unique neuronal types that are born at the Vsx1-Ey address: LPi3-4, Pm4, Cm6, Mi17, Tm29, Tm31, Tm32, and Tm37. The observation of unexpected diversity at the Vsx1-Ey intersection suggests that Imp/Syp patterning acts to further diversify neuronal fates at this spatial-temporal address. Since Imp/Syp temporally pattern medulla progenitors across neurogenesis (from 48 h after larval hatching (ALH) to P15), it is expected that unique Vsx1-Ey neuronal types will be generated in distinct developmental windows. Indeed, using an EdU-based birthdating technique, we found that LPi3-4 neurons are born only during the later stages of medulla neurogenesis (post 92 h ALH), when Syp levels are high. We are currently performing loss-of-function experiments for Imp and Syp to determine if these genes are required for the specification of the eight Vsx1-Ey cell types. Our finding that Imp/Syp diversify neuronal fates beyond the Vsx1-Hth spatial-temporal window, to include Vsx1-Ey, suggests that Imp/Syp act as a global neural stem cell patterning mechanism in the developing medulla.

584S Metabolic stress activates neurodegenerative and neuroprotective signaling in the motor system

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Altered metabolism is a prominent feature of many neurodegenerative disorders. It is often unclear how these metabolic shifts fit causally in disease progression, as well as mechanistically in the loss of axons and synapses. Uncovering signaling pathways that respond to metabolic stressors offers a route for understanding nervous system metabolic homeostasis and identifying potential therapeutic targets. This project examines loss of pyruvate kinase (PyK, 10th glycolysis enzyme) on the integrity of axons and synapses in the Drosophila motor system. PyK loss causes progressive neurodegeneration, which is promoted by the dual leucine zipper kinase (DLK) and the enzyme SARM1. This metabolic defect also reveals a protective mechanism that is uncovered when PyK-deficient axons are physically injured prior to degeneration onset, resulting in delayed axon breakdown that coincides with reduction of SARM1 axonal localization. Together, these data support a rheostat mechanism capable of switching metabolically-stressed neurons between a protective state and a degenerative state.

585S Mechanisms of Glial Response to Neural Injury and Facilitation of Neural Repair

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Annually, ~300,000 people suffer peripheral nerve injuries, resulting in pain and movement difficulties. Achieving full axon regeneration in the human central and peripheral nervous systems (CNS, PNS) remains elusive. CNS and PNS glia are distinct, generating inhibitory and permissive environments for axon regeneration respectively. Despite advances, our understanding of mechanisms of glial injury responses, generating permissive environments for axon regeneration, remains incomplete. Larval fly PNS sensory glia resemble Schwann cells as they ensheathe axons and are essential for axon regeneration. Using axotomy and long-term imaging, I identified a novel glial phenomenon: the release of large mitochondria-containing vesicles (~3 mm) near the axon injury site. These vesicles morphologically resemble exophers, large (~3 um) vesicles, documented in *C. elegans*, mouse, and human neurons, which sequester and remove damaged mitochondria to decrease cellular stress. I hypothesize that glial exophers alleviate glial stress following axon injury and may have roles in promoting axon clearance and regeneration, mediated by different factors. I found that following axon injury, glia experience stress, which is resolved by the time axon regeneration takes place. Glial-specific knock-down of *Rab11* and *Atg9,* members of the cell's membrane recycling pathway, perturbs glial exopher morphology and results in disrupted axon clearance. Additionally, glial-specific knock-down of *Atg7*, an autophagy regulator required for normal exopher formation in mice and *C. elegans* respectively, also perturbed fly glial exopher morphology, but instead disrupted axon regeneration. Understanding the functions of these novel fly glial exophers may uncover a new mode of exopher action in glial recovery from the stresses of axon injury to clear axon debris and permit further axon regeneration.

586T A quantitative model of phenotypic variability

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Phenotypic variability is a hallmark of many Mendelian disorders, which are disorders that are caused by mutations in single genes and that follow predictable inheritance patterns. Given that the presence of a pathogenic allele alone cannot predict an associated phenotype, it is important to understand the contributions to phenotypic variability in order to determine the best course of treatment for patients. The underlying causes of phenotypic variability are thought to be a combination of modifier genes, environment, and stochasticity, but disentangling these contributions remains challenging. Here, we establish the *Drosophila* larval tracheal system as a model for studying phenotypic variability. We focus on the specification of terminal cells (TCs), which are extensively branched cells that supply oxygen to surrounding tissues during larval stages. We study the dorsal TCs, which are present in 8 pairs along the anteroposterior axis of the larva in segments referred to as tracheal metameres. It is known that both gain- and loss-of-function mutations in FGF-ERK signaling result in gain and loss of TCs, respectively. In flies, the FGF ligand Branchless (Bnl) is haploinsufficient; removing one copy of Bnl results in a severe reduction in the number of dorsal TCs per larva. We show that this effect can be partially rescued by introducing an additional gain-of-function mutation in MEK, highlighting the contribution of modifier genes to phenotypic variability. Additionally, we find that in both the half-dose Bnl and rescue conditions, the tracheal metameres respond differently, with some metameres being more strongly affected than others. Thus, we identify cryptic variation across metameres that is hidden in wild type conditions but is revealed upon introducing perturbations to the system. To determine the origins of this variation, we used an FGFR pYtag, a receptor tyrosine kinase biosensor, to identify any differences in receptor activation across metameres that may exist prior to TC specification. We also performed quantitative live imaging of TC specification in both wild type and Bnl half-dose embryos to determine when this structural defect arises. We find that in wild type embryos, TC specification follows a stereotypic trajectory both in terms of gene expression and morphological changes. In mutant embryos, these trajectories become more variable.

587T The Role of Akirin and Its Conserved SYLS Motif in *Drosophila* Muscle Development

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Akirin is a small adaptor protein that binds to various protein complexes and participates in diverse processes ranging from transcriptional activation to nuclear import. This versatility is intriguing, and its underlying mechanisms remain unclear. The last four amino acid residues at the Akirin's C-terminus form the SYLS motif, conserved across distant species from flies to humans. Recently, the C-terminal region of the closest human homolog was shown to bind the 20S proteasome to facilitate its nuclear import in mitotically active cells. In this study, we analyzed Akirin's contribution to adult myogenesis, with a particular focus on the role of the SYLS motif. RNAi-mediated knockdown (KD) of *akirin* using the muscle-specific *Mef2*-Gal4 driver resulted in complete lethality at the pharate adult stage, with flies failing to eclose. Muscle specification and differentiation in KD pharates appeared normal, but their muscles were significantly smaller. Inducing *akirin* KD with late muscle drivers (*fln*-Gal4, *Act88F*-Gal4) had no effect, whereas the early, myoblast-specific driver (1151-Gal4) reproduced the *Mef2*-Gal4 phenotype, indicating that *akirin* functions during early myogenesis. Overexpression (OE) of *akirin* had no detectable effect on muscle morphology or function (as assessed by flight and jump tests). In contrast, OE of *akirin* lacking the SYLS motif (ΔSYLS) caused partial lethality and muscle dysfunction, suggesting a dominant-negative effect. Collectively, our findings indicate that in myogenesis *akirin* acts in myoblasts and that its C-terminal SYLS motif is essential, possibly by enabling proteasomal nuclear translocation. Additional experiments are underway to test this hypothesis.

588T Broad expression regulation in egg chamber cells by the ecdysone and JAK/STAT signaling pathway in *Drosophila*

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During oogenesis, epithelial cells in the anterior follicular epithelium of *Drosophila* egg chamber undergo changes from cuboidal to the flattened and stretched squamous shape. Following the transition, our lab previously reported that a few key components of the ecdysone and JAK/STAT pathways regulate the transcription factor Broad (Br) to control the morphological changes of squamous cells (SC). However, it was still unclear whether all or partial components of the pathway regulate Br to impact SCs. To further understand how the two pathways affect Br expression, we utilized anterior follicle cell specific A90-Gal4, UAS RFP flies to knock down all known components of ecdysone and JAK/STAT pathways one by one to visualize their effects on Br expression and SCs accumulation in stage 10 anterior follicle cells. For instance, ecdysone pathway component UAS- EcR-B1 DN had a strong Br regulation in all SCs with strong accumulation at the anterior terminal. Downstream gene UAS-Eip75B also showed a strong Br upregulation in anterior SC. Contrarily, pathway component UAS-EcR-B2 DN showed no Br upregulation in SCs but had slight SC accumulation at the anterior. JAK/STAT pathway components UAS-dome RNAi and UAS-Stat92E RNAi presented weak Br upregulation in some SCs with cluster accumulation, whereas pathway component UAS-hop RNAi and positive regulator UAS-Cnot4 RNAi presented no Br upregulation with clustered SC accumulation. While many genes regulate both Br regulation and SC accumulation, some genes only impact one, suggesting Br regulation and SC accumulation are not interdependent.

589T Screening Essential Genes for *Drosophila* Squamous Cell Development

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Epithelial cells have three basic shapes: cuboidal, columnar, and squamous. *Drosophila* egg chambers have all three types of epithelial cells and can be used to study morphological changes within their epithelium. Cell morphological transitions are a highly interesting topic of study. In a single cell RNA (scRNA) sequencing analysis of *Drosophilia* egg chambers from our lab, over 300 genes were found to be highly expressed within squamous cells at stage 9 during the morphological change. We hypothesize that these highly expressed genes play a critical role in egg development, shape change, and migration. Reducing their expression may cause adverse morphological effects, which can be studied to understand signaling pathways regulating morphological changes. Using UAS-RNAi stocks ordered from Bloomington Drosophila Stock center, the top 100 genes are sequentially reduced using the established A90 Gal4, UAS RFP, Gal80ts system. A90 Gal4 is used for tissue specific expression in the anterior squamous cells. Use of Gal80ts will allow for control over specific time of expression by shifting flies to 29°C to activate Gal4, UAS expression system. Ovaries are subsequently immunostained and examined with confocal microscopy. Of the so far examined ovaries, we have seen 75% with upregulation of Br, which was identified by our lab to be involved in the morphological changes. Most of this upregulation was seen in the anterior follicle cells of egg chamber. Some examined egg chambers also showed squamous cell accumulation. Some egg chambers had border cell (BC) defects including arrival delay and break down of the BC clusters.

590T Splicing mediated by U2-associated Scaf6/CHERP is necessary for myogenesis in *Drosophila* and vertebrates

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RNA regulation and alternative splicing play an important role in myogenesis. Mis-regulation of splicing alters the balance of gene isoform expression, impacting steps in muscle development from myoblast division and differentiation to sarcomere assembly and contractile function. Altered isoform expression dynamics further contribute to muscle aging, atrophy and disease. RNA binding proteins regulate RNA processing, but the majority of these proteins have never been studied in muscle. Here we identify spliceosome associated factor Scaf6/CHERP to play an essential and conserved role in muscle development. Whole animal Scaf6 mutant flies display myofibril defects and are flightless due to detachment and atrophy of the flight muscles. 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 select sarcomere proteins, notably Tm1, Sallimus, Unc-89, Zasp-52 and Zasp-66. The altered balance in sarcomere protein expression induces aberrant myosin contractility and myofiber loss. Interestingly, Scaf6 is required cell-intrinsically for splicing in both muscle and neurons, and neuronal-specific loss of Scaf6 results in behavioral defects and decreased motor neuron axon branching. We further demonstrate that Scaf6 has a conserved function in regulating myoblast proliferation, and knockdown of the vertebrate homologue CHERP in mouse C2C12 cells results in decreased proliferation and premature differentiation. From pull-down mass-spectrometry experiments in mouse and human muscle cells, we demonstrate that CHERP is tightly associated with the Sf3b1 complex, which is a core unit of the U2 spliceosomal complex. We also find that CHERP is misexpressed in myotonic dystrophy patient cells. Our results thus establish a novel, disease-relevant function for Scaf6/CHERP in myogenesis in both flies and vertebrates, and provide insight into how the physiological roles of splicing factors are defined by temporal and tissue-specific requirements.

591T microRNA-274 is essential for early head and eye formation

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MicroRNAs (miRNAs) are small non-coding RNAs that finetune gene expression at the post-transcriptional level, playing critical roles in various developmental and metabolic processes. Through gain of function and loss of function approaches, we identified 46 miCats (**mi**croRNAs **C**ontrolling **A**dipose **T**issue) in *Drosophila melanogaster*. Lipids are essential for cell membrane structure, signaling molecules, and energy storage and thus, play a central role in supporting cellular activities and biosynthesis. Given that nervous tissue exhibits high levels of microRNA expression, we hypothesize that miCATs also regulate nervous system development. Notably, brain-specific expression of *miR-274*, one of the identified miCATs, significantly impairs eye development and head symmetry. Approximately ten percent of the flies survive with microcephaly and reduced or absent eyes. To further dissect eye-specific effects, we expressed *miR-274* exclusively in the eye using the GMR promoter, which results in glossy eyes, *GMR-Gal4; UAS-miR-274* (*GMR>miR-274*). To further confirm that the eye phenotype results from *miR-274* overexpression, we knocked down its expression using a *miR-274* sponge line. When *GMR>miR-274* was combined with the *miR-274* sponge line, the eye phenotype was rescued. In a striking difference, early eye-specific expression of *miR-274* under the *eyeless* promoter, produced headless flies, *eyeless -Gal4; UAS-miR-274* (*eyeless>miR-274*), indicating that *miR-274* plays a critical role in early head and eye formation. Remarkably, incubation at 18^{o}C completely suppressed the eye phenotype in *GMR>miR-274* flies, but not in *eyeless>miR-274* flies.

592T *kayak* is required for photoreceptor development and corneal patterning in the fly eye

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The *Drosophila* gene *kayak* (*kay*), the sole fly homolog of the evolutionarily conserved *fos* class of genes coding for basic region-leucine zipper transcription factors, is primarily known for its essential embryogenesis role during dorsal closure, yet its functions in imaginal tissues have been described much less. Here, by making use of homozygous *kay* mutant clones, we characterized phenotypes affecting photoreceptor organization, external eye morphology and mechanosensory bristles. Loss of *kay* reduces photoreceptor number and alters planar cell polarity, while also disrupting ommatidial patterning, bristle morphology, and corneal ultrastructure. These morphological defects correlate with impaired phototactic behavior, suggesting a functional consequence of *kay* loss in visual performance. Our findings establish *kayak* as a key factor in eye morphogenesis and cellular differentiation. Ongoing work aims to determine whether *kay* loss affects photoreceptor differentiation through altered cell survival or fate specification. Together, these studies uncover novel, JNK-independent roles for Kay/Fos in shaping the architecture and function of the fly visual system. Funding: UR307 UNAM

593T Downstream transcriptional targets of *Drosophila* Egf receptor signaling play roles in eggshell morphogenesis

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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 previously 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. This screen identified multiple genes activated by Egfr signaling that appear to have roles in the morphogenesis, rather than the patterning, of eggshell features, with loss of function phenotypes including severely decreased chorionic integrity, shortened eggs, and various dorsal appendage malformations, as well as decreased fertility. We have also used the CRISPR-Cas9 system to create mutations in several of these genes, with a goal of generating null mutant lines. Characterization of these mutants has so far revealed expected (previously observed) phenotypes, and in some cases additional eggshell phenotypes not seen with the original knockdown or P-element flies. We are continuing to utilize these CRISPR lines for further study and characterization of our genes of interest, for example by investigating possible underlying ovarian defects via fluorescence microscopy to look at ovary structure. Preliminary results suggest possible roles in nurse cell dumping and in oocyte positioning. Future work will continue to investigate the roles of these Egfr target genes in *Drosophila* development.

594T Investigating the function of Ecdysone during dorsal closure in Drosophila embryogenesis

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20-hydroxyecdysone (20E) is a steroid hormone required for major developmental changes in Drosophila. 20E surges during mid-embryogenesis, before each larval molt, before pupariation, and during terminal differentiation of the adult structures. During metamorphosis, 20E signaling regulates tissue-specific gene expression that results in cell death of the larval tissues and morphogenesis of the imaginal discs. Previous studies showed lack of 20E signaling in the embryo results in germband retraction, head involution, dorsal closure, and cuticle synthesis defects. However, the detailed function of 20E during embryogenesis is not well understood. Here we are focusing on Dorsal closure (DC) that is a process where two sheets of epidermis cover an open gap through interactions between multiple tissues. To gain a mechanistic understanding of the function of the 20E during dorsal closure, we are characterizing phenotypes associated with loss of *disembodied* (*dib*) and *shroud* (*sro*), two “Halloween” genes that encode p450 cytochrome enzymes required for 20E synthesis. Immunostaining of *dib* and *sro* mutant embryos during dorsal closure shows that JNK signaling, an initiating pathway of DC, appears normal. Likewise, the formation of the actomyosin cable on the leading edge is normal in mutant embryos. However, time-lapse imaging of Ecad and Sqh labeled *dib* and *sro* mutant embryos reveals slower germ band retraction, abnormal shape, and pulsatory behavior of amnioserosa cells during DC, and poor formation of the canthus, reduced filopodia number and activity at the leading edge, and defective zippering of the contralateral epidermal sheets. To better characterize the tissue requirements for 20E signaling, we expressed a dominant negative 20E receptor (EcR-DN) using tissue-specific GAL4 lines. Each of the ubiquitous, epidermis and, amnioserosa specific EcR-DN expressions showed distinct DC defects. As we noted that many of the Dpp pathway defects in DC were similar to what we observe in Halloween mutants, we wondered if we could suppress the DC phenotypes of sro mutant embryos by more strongly activating Dpp signing using an activated form of the Dpp receptor (TkvQD). To better understand the tissue-specific response of 20E signaling in DC, we plan to express GFP is the amnioserosa or the dorsal epidermis, disrupt the embryos and sort the cells, and conduct bulk RNA seq on these tissues.

595T Developmental ionome of a *Drosophila* multispecific transporter mutant

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Malvolio (Mvl) is the Drosophila ortholog of the mammalian Solute Carrier Protein Slc11a2, which transports divalent metals. As a multispecific transporter, Mvl mediates the cellular transport of iron, copper, and manganese. In this study, we determine the Drosophila developmental ionome by quantifying the ontogenic elemental abundances in the embryo, larval, and adult stages of the Oregon R strain (control) for comparison with the *Mvl* loss-of-function mutant. We highlight the relative abundance of the following twelve elements in the control and mutant strains: Iron, Copper, Manganese, Zinc, Bromine, Strontium, Phosphorus, Sulfur, Magnesium, Sodium, Potassium, and Calcium. We also detect abnormal developmental morphology in the *Mvl* mutant. Ongoing studies are expected to reveal tissue-specific ionomes from the larval and adult stages with a focus on elemental abundances in select organs during development. Our results spotlight a potential correlation between developmental ionome imbalance and organ morphogenesis.

596T Arginine kinase1 controls flight muscles development by regulating energy homeostasis

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To better understand the molecular mechanisms regulating muscle development, we built a high resolution reference cell atlas of the adult muscle precursors (AMPs) associated with the third instar larval wing disc giving rise to the flight muscles. The cell atlas comprises ~30,000 cells and identifies AMPs giving rise to direct and indirect flight muscles, as well as AMPs at different stages of cell differentiation. We identified Arginine kinase 1 (Argk1), as a one of the highly expressed genes in a sub-type of AMPs. Interestingly, Argk1-depletion specifically in the muscles causes animal lethality at pharate stage, thus suggesting that Argk1 may have an important role in muscles. Both Argk1 and its homologue in mammals, creatine kinase, belong to the phosphagen kinases family. The phosphagen kinases play an important role in energy homeostasis in cells with high and variable rates of energy turnover. Also, they are important in maintaining ATP homeostasis during short bursts of contractile activity in muscles. While dysfunctional creatine kinase has been associated with myopathies in mammals, the role of these kinases during muscle development has not been studied yet.

Here, we aimed to investigate how Argk1 regulates flight muscle development. First, we examined the overall structure of Argk1-depleted muscle using the pan-muscle Mef2-Gal4 driver. Both transverse and sagittal cross-sections of the Argk1-depleted flight muscles showed a significant reduction in muscle size and abnormal myofibrillogenesis. Additionally, depleting Argk1 using a late muscle driver, Act88F-GAL4, revealed defects in myofibril assembly and muscle growth, thus indicating that Argk1 is required during late stage of flight muscle development. To examine the role of Argk1 in early muscle development, we profiled Argk1-depleted AMPs associated with the larval wing disc by scRNAseq, and analyzed changes in their transcriptomes compared to the wild type cell atlas that we established. We found that the loss of Argk1 does not affect the overall composition of the AMPs clusters, or their cell states. However, differential gene expression and pathway enrichment analyses revealed that several metabolic pathways were broadly altered in Argk1-depleted AMPs, which is an indication of defects in energy homeostasis. Accordingly, Argk1-depleted muscles showed a severe reduction in ATP content and a more reductive cellular state, as determined by the NAD/NADH ratio. Furthermore, mitochondrial morphology was highly abnormal in these muscles. Thus, our data established an essential role for Argk1 as ATP buffers and regulators of energy metabolism, which are important during flight muscles development.

597T An *ex vivo* embryonic midgut model reveals a calcium-driven mechanism for tissue folding

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During organ morphogenesis, single-cell molecular programs collectively generate reproducible, tissue-scale mechanics that sculpt stereotyped 3D shapes. Tubular organs, such the gut, follow a well-conserved developmental motif in which cellular tubes constrict and regionalize into chambers. It is now known that calcium pulses in the gut visceral muscle are required for tissue folding, but how these morphogenetic calcium pulses are regulated within individual cells, and coordinated in space and time, remains unclear. To decouple organ-intrinsic mechanisms from interactions with adjacent tissues, we developed an *ex vivo* culture system for the embryonic midgut, eDiGESt (embryonic Drosophila midGut Ex vivo System), which provides us with unprecedented optical, pharmacologic, and mechanical accessibility. Using this assay, we uncover an organ-intrinsic, intracellular calcium cycle in which electrically excitable visceral muscle cells flux calcium through voltage-gated calcium channels. This, in turn, induces calcium-induced calcium release (CICR) from internal stores to generate whole-cell calcium pulses. Given that constriction-stage muscle cells are very thin, but nearly 150 µm long, this mechanism enables signal amplification of local stimuli–ensuring coordinated calcium-driven actomyosin contractility across the cell. Furthermore, pharmacologic activation of the mechanosensitive ion channel, Piezo, induces calcium pulses, but pharmacologic blockade of Piezo has no effect. This suggests that mechanosensitive mechanisms may fine tune, but not direct, the gut's morphogenetic calcium pulses. Finally, high frequency imaging of our eDiGeSt system via oblique plane microscopy provides the spatiotemporal resolution needed to dynamically track the movement of individual nuclei in the deforming midgut while simultaneously recording calcium reporter activity. By relating muscle cell contractility to calcium pulses, we propose a mechanical model of gut morphogenesis in which calcium pulses elicit a ratcheting-contraction-like response in single cells, which collectively confer viscoelastic tissue contractions. Together, these results implicate an organ-intrinsic calcium program as the key driver of *Drosophila* midgut morphogenesis and advance our understanding the mechanochemical underpinnings of organ dysfunction in congenital diseases like intestinal malrotation and volvulus.

598T Understanding the Role of HOX genes in Early development of the *Drosophila* Ovary

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Throughout the animal kingdom, male and female gonads develop differently in a process that is regulated by the Doublesex and mab-3 related (DMRT) family of transcription factors. Our lab studies how *Drosophila* Doublesex (Dsx) controls the development of the somatic gonad of the ovary and testis. Much is known about testis development, while much less is known about the development of the ovary. Therefore, to understand how Dsx controls sexual dimorphism in the somatic gonad, we first need to understand how the somatic stem cells and support cells of the ovary form and attain their distinct identities. In embryogenesis, Somatic Gonadal Precursors (SGPs) coalesce with germ cells to form the initial gonad. SGPs have clear anterior and posterior identities dependent on the Hox genes, *abdominal-A (abd-A)* and *Abdominal-B (Abd-B)*. Anterior SGPs express only *abd-A*, while posterior SGPs express *abd-A* and *Abd-B*. *Abd-A* is essential to proper SGP formation, while changing *Abd-B* expression alters SGP cell fate. In testis, loss of *Abd-B* results in loss of male-specific SGPs, but *Abd-B's* role in other SGPs has not been studied. In addition, *Abd-B's* role in the ovary has not been well characterized, as ovary development is halted until later larval stages. To decipher the role *Abd-B* plays in ovarian cell identity, it is essential to know which cells express *Abd-B*. Using lineage tracing and *in situ* hybridization, I have preliminarily determined that *Abd-B* is expressed in a subset of intermingled cells during larval stages. Additionally, I am utilizing tissue-specific CRISPR to target the *Abd-B* locus and characterize which cells require *Abd-B* expression. Together, this work will uncover how the HOX code of the SGPs influences the development of different cell types in the gonads.

599T Eye or Head? The Antagonistic interaction between Dpp–Dve in Fate Specification.

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Fate determination during organogenesis involves interactions among multiple signaling pathways and regulatory genes. One of the most important aspects of craniofacial development is the delineation of the eye field from the surrounding head primordium, a process that requires spatio-temporal coordination between morphogens, transcription factors and signaling pathways. In Drosophila eye, Decapentaplegic (Dpp), a member of the evolutionarily conserved Dpp/Bone Morphogenetic Protein (BMP) pathway, is crucial for initiating morphogenetic furrow progression at the posterior margin, regulating retinal differentiation. Dpp interacts antagonistically with wingless (wg) to modulate retinal differentiation. Recently, we identified defective proventriculus (dve), a K-50 transcription factor, the ortholog of human SATB1, as a dorsal fate selector gene in eye development, which induces wg to promote head-specific fate. However, eye development is a highly coordinated process, and how dve integrates into this broader regulatory network remains unknown. In this study, we explore the interaction between the dve and the Dpp signaling pathway, highlighting how this interplay determines the fate of the Drosophila eye versus the head cuticle. Our findings indicate that Dpp and dve are mutually antagonistic in defining eye versus head identity. Misexpression of dpp in the head vertex region shifted the fate from head to eye, overriding the wg influence. In contrast Misexpression of dve in the eye domains represses dpp expression and photoreceptors and this repression can be rescued by co-expression of dpp, confirming that dpp acts downstream to dve. Finally, human SATB1, the vertebrate ortholog of Dve, phenocopies its function, highlighting evolutionary conservation, given that SATB1 mutations, including SATB1 E413K , within the CUT1 domain, are linked to craniofacial developmental defects such as hypertelorism. These variants similarly repress Dpp signaling and dpp co-expression restores retinal fate. Together, these results establish a conserved dve/SATB1–Dpp regulatory axis whose balance defines eye–head identity and provides mechanistic insight into how its disruption contributes to craniofacial malformations.

601T The first cell fate decision is made in the plasma membrane

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Embryogenesis in higher animals begins with cleavage divisions that generate numerous naïve daughter cells. These naïve cells must then be assigned a lineage through the asymmetric activity of molecular determinants. Embryonic regions often contain the activities of multiple determinants that need to be resolved to singular lineage outcomes. In this work, we investigate the overlap between the determinants of the primordial germline and the posterior endoderm of the Drosophila embryo. The primordial germline determinants are RNP (germ) granules that actively promote production of the phospholipid PIP2 in the posterior plasma membrane, which enables formation of primordial germ cells (PGCs). We find that the neighboring posterior endoderm determinant, signaling by the RTK Torso, antagonizes germ granule activity by recruiting and activating PI3K which converts PIP2 to PIP3. PIP3 prevents PGC formation, ensuring endoderm specification. Reciprocally, germ granules produce Germ cell-less, a ubiquitin ligase component that targets Torso for destruction, protecting PIP2. The net outcome of this antagonism is a PIP3/PIP2 boundary that informs the endoderm/PGC split. This work determines both the mechanism by which a binary fate outcome is achieved from overlapping determinants and demonstrates that the phospholipid composition of the plasma membrane can inform lineage specification.

602T The GPCR Pathway Mediating Apical Constriction in the *Drosophila* Salivary Gland Requires the Transcription Factor Huckebein for Appropriate Trafficking of its Ligand, Folded gastrulation (Fog)

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The analysis of complex signal transduction pathways is essential for our understanding of how animals achieve appropriate organ morphology during development. *Drosophila melanogaster* is a valuable model organism for revealing the cellular and molecular events that control terminal organ shape, size, and location. Our laboratory utilizes the embryonic *Drosophila* salivary gland (SG) as a simple model of epithelial tube formation analogous to the more intricate mammalian lungs and vascular system. While the cellular mechanisms contributing to SG invagination have been described, the signaling pathways coordinating the initial process of apical constriction remain under investigation. We hypothesize that the SP1-like transcription factor Huckebein (Hkb) mediates the localized apical secretion of the signaling molecule Folded gastrulation (Fog), thus activating the downstream G-protein-coupled-receptor(s) (GPCRs) critical for SG invagination. This highly regulated and time-sensitive event requires appropriate trafficking of Fog and/or its GPCR to the apical surface by vesicles whose apical trafficking requires the kinesin-binding protein Klarsicht (Klar) of which transcription in the SG is *hkb*-dependent. Utilizing new null alleles of *hkb* created by homologous recombination, in combination with tissue-specific overexpression of Hkb, we test the model that Hkb regulates apical secretion of Fog. Our work reveals that high level Fog secretion is normally limited to the SG cells about to invaginate, and that ectopic expression of Hkb affects the levels and distribution of secreted Fog, resulting in aberrant invagination events. Preliminary data also suggests that loss of Klar affects the size, density, and distribution of Fog-containing vesicles. In comparison, loss of *hkb* often results in very small SGs with altered morphologies. These *hkb* null SGs frequently fail to invaginate and/or elongate and are thus found in the head region at late stages. We observe these SG phenotypes even when the *hkb* mutants are deficient in apoptotic capacity, suggesting that the small SG size is not due to loss of SG cells. Our findings suggest that Hkb facilitates the localized secretion of Fog from SG cells, which then activates the downstream GPCR-mediated pathway required for the cell shape changes that underlie invagination.

603T Non-Canonical Role of Septate Junctional Proteins in Border Cell Migration of Drosophila

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Border cell migration (BCM) is a useful model to study collective cell migration, a process important in tissue morphogenesis, wound healing, and cancer metastasis. Within the egg chamber, polar cells secrete signaling molecules that recruit nearby follicular somatic cells to surround them, forming a cluster that delaminates and then migrates between nurse cells to the anterior side of the oocyte. BCM begins in early Stage 9 and is completed by Stage 10A of oogenesis. Throughout this process, the cluster exhibits anterior-posterior, apical-basal, and rotational polarity.

Our main interest is understanding the roles of septate junction (SJ) proteins in BCM. As intercellular junctions in invertebrate epithelia, SJs connect cells and restrict paracellular flow, analogous to tight junctions in vertebrate tissues. During BCM, mature septate junctions have not yet formed between the follicle cells; however, previous studies of *Drosophila* SJ proteins suggest they have additional developmental roles independent of their occluding function, including in BCM.

To quantify the effect of SJ protein knockdown on BCM, we employed the GAL4/UAS system using the slbo-Gal4 line with RNAi to target *Kune-kune* (Kune), *Macroglobulin complement-related* (Mcr), and *Coracle* (Cora). Using this approach in combination with antibody staining, we found that the knockdown of SJ proteins caused incomplete migration, failed delamination, or dissociation of the cell cluster during migration. Potential hypotheses are that septate junction proteins may contribute to border cell polarity and orientation during migration, maintain adhesion within the cluster, drive movement through actin–myosin contractions, or act through a combination of these mechanisms.

To test these hypotheses, we are using Gal4 drivers that allow cell-type–specific knockdown: slbo-Gal4 (border cells), unpaired-Gal4 (polar cells), and C306-Gal4 (both). Comparing phenotypes across these conditions will help distinguish whether SJ proteins act within border cells, polar cells, or at their interface. To quantify cluster polarity, we will analyze not only migration completion but also cluster morphology and organization during migration, including shape, filopodia number, length, and direction, as well as the subcellular localization of proteins such as Mcr, which we previously found to localize asymmetrically toward the leading edge. Understanding how SJ proteins coordinate adhesion and polarity in this system may reveal conserved mechanisms underlying collective cell migration across species.

604T How Do We Build a Muscle? Let Me Count the Genes...

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Akirin is a nuclear cofactor that links transcription factor activity with chromatin remodeling complexes to regulate gene expression during embryonic heart development and skeletal myogenesis. In Drosophila, Akirin interacts with chromatin remodelers such as CHD4 and the NuRD complex to control cardiac gene pathways, a mechanism conserved across metazoans. To identify additional factors that cooperate with Akirin in myogenesis, we are investigating potential genetic interactions with Ppn (pupation prominent) and hyd (hyperplastic discs).

We have begun a series of genetic interactions studies with akirin and either hyd or Ppn, focusing on early skeletal and cardiac morphogenesis. Our preliminary results have indeed demonstrated an interaction between akirin and these loci during these processes. Our results have expanded the known Akirin interaction network during skeletal myogenesis and heart morphogenesis. We anticipate that these findings will contribute to understanding conserved mechanisms underlying similar congenital skeletal and cardiac musculature defects in other organisms, particularly mammals.

605T Deciphering EGFR Signaling in *Drosophila* renal development

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Molecular signaling is a crucial factor influencing development of tissue shape. The EGF Receptor (EGFR) signaling cascade plays a critical role in many cellular processes that drive development in different organisms, such as proliferation, migration, and cell shape change. To understand the role of EGFR in organogenesis, we study the developing *Drosophila* renal system, the Malpighian tubules. In this system, a proximal-distal EGFR signaling gradient coordinates myosin contractions to drive tubule elongation. Furthermore, when EGFR signaling is decreased or increased, the resulting tissue phenotype is the same. These results indicate that there is a precise level of EGFR signaling that is necessary for tubule elongation and suggest that there is feedback from myosin contractility on EGFR signaling. However, it is unknown precisely how EGFR signaling instructs myosin dynamics, nor is it known how specific levels of EGFR signaling are maintained to drive proper organ formation. The EGFR pathway is multifaceted and which branch is active in tubule cells is unknown; thus, to identify the factors that drive tubule extension I am taking an unbiased proteomics approach. To assess the validity of potential EGFR signaling intermediates, I will quantify myosin dynamics during tubule extension in the absence of these potential intermediates. By comparing myosin dynamics in control tubules as well as tubules with increased and decreased EGFR signaling I will determine how potential intermediates act to modulate myosin dynamics and tubule extension. My preliminary data of control tubules recapitulate polarized basal myosin pulses and identify apical and cortical myosin dynamics. To determine if myosin contributes to feedback regulation of EGFR signaling regulation, I will first characterize the signature of EGFR signaling during tubule extension using EGFR signaling biosensors. Using the *in vivo* ERK biosensor (ERK-KTR) during tubule extension, I recapitulated the observed proximal-distal gradient in EGFR signaling. Next, I will increase or decrease myosin activity and observe how these EGFR signaling signatures change to identify if there is negative or positive feedback from myosin to EGFR signaling. Together, these approaches will determine how the EGFR signaling cascade interacts with myosin to sculpt tubular organs.

606F Arc1 and Acetobacter *aceti* collaborate to regulate early brain growth in Drosophila larvae

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Growth is one of the few universal experiences among metazoans. Likewise, the mechanisms that regulate this growth are well conserved across taxa. Growth is influenced through the individual and synergistic contributions of one's genetics, environment, diet, and more recently recognized, microbiota. Bacteria have been shown to promote growth through supplementation to the diet and by promoting host signaling pathways including immune and insulin/insulin-like signaling. In Drosophila, germ-free larvae have an extended growth period and are smaller compared to those hosting a microbiota. We have shown that the capsid forming neuronal protein Arc1 collaborates with Acetobacter aceti to promote larval growth. Arc1 null germ-free larvae take significantly longer to reach pupariation and are even smaller than wild-type germ-free larvae. This is accompanied by dysregulated insulin signaling. These defects can be rescued through monoassociation with A. aceti. However, these markers only assess organismal growth and do not reveal potential developmental or organ-specific effects of Arc1 and the microbiota. Here, we explore the independent and synergistic contributions of Arc1 and A. aceti to early larval brain development. Preliminarily, we have found that despite having smaller bodies, Arc1 null germ-free larvae have disproportionately large brains compared to wild type germ-free larvae. We are investigating whether Arc1 or A. aceti regulate neuroblast proliferation and how they may contribute to brain sparing mechanisms that maintain brain growth when systemic growth is stunted. In addition, we are investigating whether the growth promoting effects of Arc1's collaboration with bacteria are dependent on Arc1's capsid forming abilities. These results will contribute to our understanding of how bacteria affect neural development and identify potential new roles for Arc1 in the brain.

607F Trade-offs between Dorsal gradient robustness and Cactus lifetime

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The NF-κB/IκBα pathway regulates key biological processes, such as development, immunity, and inflammation. In *Drosophila*, the homologous Dorsal (Dl) system patterns the dorsoventral axis of the blastoderm embryo. Dl is retained in the cytoplasm through binding to the inhibitor Cactus (Cact). Signaling through the Toll receptor results in the dissociation of the Dl/Cact complex, freeing Dl to enter the nuclei and form a spatial gradient. Recently, endogenous tagging of Cact (Schloop et al. 2025) has confirmed that Cact is present in the nucleus. This finding supports earlier studies which suggested that *in vivo* measurements of the Dl gradient are composed of both free Dl and the Dl/Cact complex and that nearly all Dl in the dorsal-most nuclei must be bound to Cact to maximize the robustness of the Dl gradient.

By itself, Cact is an unstable protein with a high turnover rate. When bound to Dl, Cact is protected from degradation. It is believed that most of the Cact present in the embryo is bound to Dl because, in embryos with classical Dl knockout alleles, Cact levels become undetectable. Yet, the recent finding that Cact is present uniformly in the nucleus presents a paradox between this stable nuclear Cact pool and Cact's intrinsic instability.

Given these conflicting observations, we propose a trade-off between the average lifetime of Cact and the proportion of Dorsal (Dl) that remains bound in complex with Cact. To investigate this, we developed a one-cell model of Dl/Cact interactions, constrained by recent quantitative data on Dl and Cact concentrations. We performed a random parameter search over six orders of magnitude, filtering for physiologically plausible sets. Our analysis found clear trade-offs between our two objectives: Cact lifetime and the percentage of Dl bound to Cact. Our model reveals that at above ≈ 50% Dorsal-Cactus binding, the Cact lifetime becomes longer than expected for a high-turnover protein. This finding places an upper limit on the possible robustness of the Dl gradient. We further validated this finding by expanding the analysis to model the Dl/Cact system over the entire embryo, which yielded results consistent with the one-cell model.

In particular, our model predicts that direct experimental measurements of (1) the *in vivo* binding dynamics of the Dl/Cact complex and (2) the lifetime of Cact are required to determine the robustness of the Dl gradient. Our results highlight the need for a quantitative framework to understand the emergent properties of morphogen gradients.

608F The Drop Out Kinase is Essential for Proper Hedgehog Signaling

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In the Drosophila melanogaster wing imaginal disc, Hh signaling across the Anterior/Posterior (A/P) compartment boundary organizes the patterning of the wing. Previous work has shown the importance of Hh in short and long range signaling. To investigate this process, we examined the role of Microtubule-Associated Serine/Threonine (MAST) kinases. We generated genetic mosaics with mutant clones for drop out (dop), the Drosophila homolog of MAST kinases. As dop is essential, we generated clones homozygous for the null allele, dop[10], by the Minute technique. We investigated the effects of these clones in both compartments on Hh target genes and cytoskeletal organization, particularly basal actin projections known as cytonemes, which are believed to facilitate Hh trafficking.

In the anterior compartment, dop[10] clones that ran along the anterior side of the boundary had decreased Ptc and Dpp, two target genes of the pathway. However, when these clones ran along the posterior side of the boundary, an increase was observed in the expression of target genes directly on the other side of the boundary. These clones also showed increased en expression in both the posterior and anterior compartments. The anterior en expression causes repression of cubitus interruptus (ci), the main transcription factor of the pathway, potentially explaining the decrease in Ptc and Dpp.

When using a hh::GFP construct, in posterior dop[10] clones, Hh was notably diminished at the basal surface, suggesting a trafficking defect rather than impaired transcription. In the anterior compartment, Hh appeared on the anterior side of anterior dop[10] clones, indicating that intercellular transport is not completely blocked. CD8RFP was used to mark clones and observe the effects of dop[10] on cytonemes. In the anterior compartment, Hh puncta appeared to colocalize with CD8 and were seen along basal projections. Additionally, in both compartments, staining actin using fluorescent phalloidin showed that mutant clones had irregular actin distribution and elevated actin expression. These clones appear to send long projections across the boundary and even to cross the boundary.

When using a construct of lacZ downstream from the hh promoter as an assay for the transcription levels of hh, no difference was observed in lacZ fluorescence between dop[10] and wt cells. Together, these findings suggest that MAST kinase activity is required for proper Hh trafficking.

609F Golgi-localized PI 4-kinases drive microvilli biogenesis and membrane compartmentalization in cellularization

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Microvilli are like waves at sea; from a distance, they appear unassuming, but a closer look reveals the dynamic and complex remodeling of these intricate membrane protrusions. These actin-supported protrusions are essential for epithelial function, and defects in their morphogenesis can cause severe diseases such as Microvillus Inclusion Disease. Drosophila cellularization, the embryo's first complete cytokinesis, provides a valuable model for studying the dynamics and function of microvilli. During cellularization, cleavage furrow ingression is strongly coupled to the consumption of apical microvilli, which act as a membrane reservoir for furrow extension.

Our previous work showed that two conserved Golgi-localized phosphatidylinositol 4-kinases, PI4KIIα and PI4KIIIβ/Four wheel drive (Fwd), function redundantly to promote furrow ingression during cellularization. Using spinning-disk confocal and scanning electron microscopy, we found that PI4KIIα PI4KIIIβ double knockdown embryos display a marked reduction in apical microvilli during cellularization. Live imaging revealed that most double knockdown embryos exhibit defects before or at the onset of cellularization, often arresting prior to the formation of furrow canals – membrane compartments at the basal ends of the ingressing furrow. In the embryos that completed cellularization, furrow canals formed incompletely, showing reduced myosin II recruitment and abnormal overlap between myosin and basal adherens junctions – two structures that are normally well segregated in wild-type embryos. These embryos also showed defects in apical morphology, which initially resembled wild type but failed to develop dense, irregular microvilli arrays. To explore the underlying cause, we examined Rab11 compartments, a key mediator of endocytic recycling and exocytosis. In wild-type embryos, Rab11 rapidly clustered around centrosomes after the syncytial-to-cellular transition, whereas in double RNAi embryos, these clusters dispersed prematurely despite normal microtubule organization.

Together, our results show that depletion of PI4KIIα and PI4KIIIβ disrupts both apical microvilli biogenesis and basal membrane compartmentalization, likely through misregulation of Rab11-mediated vesicle trafficking. These findings uncover a critical role for Golgi PI4-kinases in coordinating plasma membrane organization during epithelial morphogenesis.

610F Investigating Membrane Trafficking of Glypicans

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Morphogen signaling is important for tissue patterning and organ formation during development. Its regulation depends on how signaling molecules such as Wingless (Wg/Wnt) and their modulators are trafficked within cells. In *Drosophila*, glypican Dally-like protein (Dlp) is a lipid-anchored heparan sulfate proteoglycan that modulates extracellular Wingless (Wg/Wnt) distribution and signaling at the cell surface. Dlp can both enhance or restrict Wg signaling by interacting with the ligand on the cell surface. Therefore, the surface levels of Dlp affect Wg availability for signaling. The mechanisms governing Dlp's internalization and trafficking remain unknown. Because Dlp lacks a transmembrane and cytoplasmic domain, it cannot directly recruit classical endocytic machinery for its internalization, raising the question of how cells control its cell-surface levels. Elucidating how Dlp interacts with the cell membrane during internalization is critical to understanding how Wg gradients are established and maintained across tissues. We hypothesize that Dlp interacts with transmembrane domain containing proteins to facilitate its internalization. To test this, we will employ a SNAP-tagged *dlp* construct expressed in the *Drosophila* wing disc using *ptc*-GAL4 driver. Live imaging of surface-labeled Dlp will allow us to monitor internalization dynamics *in vivo*. Potential regulators of Dlp internalization will be identified using In-silico Alpha Fold screen, which uses known and predicted protein structures to identify direct protein-protein interactions. The predicted proteins associated with Dlp on the membrane will be tested for their role in Dlp's internalization using RNAi knockdown and mutant alleles. Preliminary data suggest that Dlp surface dynamics are tightly coupled to Wg distribution, and perturbation of its internalization alters both the range and intensity of Wg signaling. These studies will establish the molecular pathway by which a lipid-anchored glypican is internalized *in vivo* and reveal how Dlp regulates Wg ligand availability and signaling. Defining this mechanism will provide fundamental insight into glypican-mediated regulation of developmental signaling and may inform understanding of conserved processes in vertebrate development and diseases.

611F No Sexual Dimorphisms on Drosophila Heart Tube Formation

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Heart development starts with the formation of a tube. During heart tube formation, cardiac progenitors migrate from opposite sides of the embryo, meet medially and fuse, in a process conserved in both vertebrates and invertebrates. Defects in heart tube formation can cause congenital heart defects. Thus, understanding the mechanisms of heart tube formation may contribute to the development of therapeutic interventions to treat or prevent congenital heart disease. There are clear differences in the incidence of congenital heart disease between males and females. For instance, severe congenital heart anomalies are 25% more common in males than females. However, the underlying causes of sexual dimorphism during heart development are not understood. We are using *Drosophila* embryos and quantitative live microscopy to investigate potential morphogenetic differences in heart development in males and females. In *Drosophila*, two highly-ordered contralateral rows of cardiac progenitors (cardioblasts) migrate from opposite sides of the embryo to form the heart tube. Previously, we showed that cardioblasts move directionally, but not monotonically, taking forward (longer) and backward (shorter) steps as they migrate, resulting in a net-forward movement. Cardioblast oscillations are associated with periodic cell shape changes and cytoskeletal waves "rectified" by a supracellular actin cable at the trailing edge of the cells. We imaged and tracked cardioblast migration in embryos expressing a fluorescent reporter of cardioblast nuclei. Embryonic sex was determined in one of two ways: 1) by allowing embryos to develop into adults after imaging; or 2) using Sxl-Pe-EGFP to identify female embryos prior to imaging. Using PyJAMAS (pyjamas.readthedocs.io), an open-source tool for quantitative image analysis that we develop, we measured features including cardioblast velocity, oscillation period and dynamics, cell shape changes or rate of heart tube closure. Strikingly, we found no significant differences between male and female embryos, suggesting that there are no sexual dimorphisms in the dynamics or morphogenesis of the heart tube during *Drosophila* embryonic development.

612F Functional evolution of anterior determinants in dipteran embryos: Target genes of maternal Odd-paired in the moth fly Clogmia albipunctata

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We are interested in how embryos evolve and have established experimental tools and genomic resources for a set of non-traditional dipteran model organisms to study how complex developmental mechanisms can change and diversify in the course of evolution. Previously, we discovered that different fly species use unrelated anterior determinants to establish the anterior-posterior (AP) axis of the embryo. While Drosophila's anterior determinant (Bicoid) is a widely studied morphogen model, lower dipterans use a range of unrelated transcription factors encoding anterior determinant genes to establish the primary body axis. Bicoid initiates symmetry-breaking during nuclear cleavage cycles (NCs) by engaging in a concentration-dependent competition with nucleosomes at the loci of a small set of transcription factor (TF) genes that are expressed in the anterior embryo. We are examining the symmetry-breaking mechanism of a moth fly, Clogmia albipunctata, in which a maternally expressed transcript isoform of the pair-rule segmentation gene odd-paired (Cal-opaMat) serves as anterior determinant (cf. Yoon et al., 2019, eLife). Recently, we found that maternal Cal-opa is required for chromatin accessibility and anterior expression at Clogmia's homeobrain and sloppy-paired loci during nuclear cleavage cycles (cf. Amiri et al., 2025, bioRxiv). Homologs of homeobrain and sloppy-paired (of which most insect genomes contain multiple copies) could potentially serve a more widely conserved role in the initiation of anterior pattern formation given their early anterior expression and function in head development across holometabolous insects. We therefore seek to answer the following two questions: First, does Cal-OpaMat target fewer genes than Bicoid, potentially to avoid interference with the conserved pair-rule gene function of zygotic odd-paired activity during NC14, when Bicoid is still active? Second, are homeobrain and sloppy-paired sufficient to establish embryo polarity in Clogmia? To answer these questions, we have started to conduct ChiP-seq (Cut&Run) experiments in Clogmia at relevant blastoderm stages using newly raised Cal-Opa antiserum and antibodies against active promoter and enhancer marks (H3K4me3 and H3K27ac). We are also analyzing the function of homeobrain and sloppy-paired homologs in Clogmia. In the future, we plan to extend this work to better understand how the segmentation GRNs of dipterans diverged during their radiation over ~250 million years.

613F The Role of GPCR Signalling and Mechanobiology in Tubular Organ Morphogenesis

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During development, cells, tissues, and organs reliably adopt specific morphologies that are essential for their optimal function. Many organs in the body, such as blood vessels, the heart, kidneys, urinary tract, gut, and neural tube, share a tubular architecture. Even minor errors in the development of tubular organs can result in severe congenital defects such as anorectal malformations or spina bifida. While the focus of developmental studies is frequently on the role of genetics in generating these morphologies, mechanical forces play an essential role in morphogenesis by generating the physical shape changes. Indeed, there is a constant dynamic interplay between biochemical and mechanical signalling during organ formation.

The Drosophila embryonic salivary gland (SG) provides an excellent model to probe the role of mechanical forces in morphogenesis since its development consists of transforming a 2-dimensional sheet of cells into a 3-dimensional tubular organ. An essential part of early salivary gland development is the coordinated apical cell constriction that drives formation of the invagination pit. Folded gastrulation (Fog), through binding to a G-protein coupled receptor (GPCR) and activating downstream non-muscle myosin II (MyoII), the force-generating motor protein, has been shown to ensure the correct apical cell area within invaginating SG cells. By combining genetically encoded tools, including nanobodies and RNA interference to disrupt gene expression and protein localization, with quantitative approaches, including fluorescent probes and advanced microscopy to measure mechanical forces, we are investigating the role of Fog in inducing downstream mechanical changes to determine tissue geometry. Our aim is to unravel the coordination between biochemical signals and mechanical processes that sculpts tubular organs.

614F The Hippo pathway and matrix metalloproteinases regulate coordinated growth between two epithelial layers in the *Drosophila* wing disc

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Epithelia in living organisms seldom develop in isolation and are typically tethered to other tissues. How growth is coordinated between tissue layers that are mechanically coupled is poorly understood. The Hippo pathway, originally discovered in *Drosophila*, regulates the nuclear localization of a transcriptional co-activator Yorkie (Yki, YAP/TAZ in mammals) which binds to a sequence-specific DNA-binding protein Scalloped (Sd, TEAD proteins in mammals) to promote expression of genes that promote growth and inhibit apoptosis. When nuclear Yki levels are low, repressor proteins bind to Sd and inhibit expression of those same genes. The Hippo pathway was thought to regulate organ size during development. However, this role has been questioned because eliminating both activator and repressor functions had little effect on growth within individual layers in imaginal discs. Our work has demonstrated a clear role for the Hippo pathway in regulating coordinated growth between two tissue layers under physiological conditions. The wing imaginal disc of Drosophila has a layer of columnar epithelium (the disc proper, DP) and a squamous layer (the peripodial epithelium, PE) which are tethered to each other. We have previously shown that growth of the PE, unlike that of the DP, is relatively insensitive to morphogens, and that its growth is driven by that of the DP (a leader and follower mechanism) likely by mechanical stretching (Friesen and Hariharan, BioRxiv, 2023). We now show that inactivation of Ajuba, a protein that mediates mechanosensation by the Hippo pathway, compromises PE growth, as does combined elimination of both activator and repressor functions of the Hippo pathway. We show that these effects are mediated in part by the microRNA bantam and two matrix metalloproteinase genes, Mmp1 and Mmp2. Taken together, our work demonstrates a key physiological role for the Hippo pathway in regulating coordinated growth between linked epithelia via mechanical coupling.

615F Developmental Mechanisms of Wing Hinge Morphogenesis

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The wing hinge plays a crucial role in insect flight by converting small oscillations of the flight muscles into the sweeping motions of the wing. Despite its importance, the morphogenesis of the wing hinge remains poorly understood due to its small size and structural complexity. To elucidate the cellular and molecular mechanisms underlying the hinge development, we used *Drosophila melanogaster* to investigate hinge morphogenesis during pupal development.

Using the hinge driver *zfh2*-Gal4, we performed both static and live *in vivo* time lapse imaging from 14h to 30h after puparium formation (APF) to visualize the dynamics of hinge contraction. Quantitative 3D analysis revealed minimal changes in hinge area between 14h and 18h APF, followed by a gradual decrease in hinge area from 18h to 30h APF, with the most pronounced contraction occurring between 18h and 24h APF. 2D surface analysis revealed that cell area is visually larger in 16h APF compared to 24h APF pupal wing hinge. In the 16h APF pupal wing hinge, we identified a region where neighboring cells appear stretched along the proximal-distal (P-D) axis and narrow along the anterior-posterior (A-P) axis. We hypothesize that this region is the center for hinge contraction and termed it 'the hinge center'. The stretching pattern of cells initiates around the hinge center and expands over time, indicating spatially coordinated cell deformation during hinge contraction.

Analysis of the dorsal hinge layer, labelled by ap-Gal4, revealed a ~2fold increase in cell layer thickness at 24h APF compared to 16h APF, suggesting a cuboidal to columnar cell shape transition associated with tissue contraction. Moreover, heatmap analysis showed strong accumulation of non-muscle myosin II (*sqh)* in the hinge center before 24h APF, suggesting that myosin II drives tension for hinge contraction. Future studies on tracking cell shape change, quantifying cell areas over time and assessing myosin II functionality through RNAi experiments, along with defining the spatial distribution of hinge associated gene expression relative to the hinge center are being undertaken to provide novel insights into the cellular and molecular mechanisms of wing hinge morphogenesis.

616F PI4-Kinase Fwd Regulates Cell Surface Expansion and Epithelial Morphogenesis in *Drosophila* Embryos

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Embryonic lethality and congenital birth deformities stem from defects during morphogenesis, which frequently involve the remodeling of epithelial tissues. It is therefore vital to understand the physical and molecular mechanisms that dictate cell shape changes underlying epithelial morphogenesis. The formation of the ventral furrow (VF) during *Drosophila* gastrulation serves as an example of epithelial folding, an important mechanism that creates 3D tissue forms. During VF formation, apical constriction powered by actomyosin contractility drives cell shape changes that facilitate furrow invagination. Our lab previously identified Four wheel drive (Fwd), a phosphatidylinositol 4-kinase IIIβ ortholog, as an important regulator of these shape changes. Depletion of Fwd impairs apical-basal elongation of constricting cells and apical stretching of the neighboring flanking cells, causing aberrant VF morphology. These observations suggest that Fwd promotes force-mediated cell surface expansion, but the underlying mechanism is unclear. We found that prior to gastrulation, Fwd-marked Golgi apparatus became enriched at the periphery of the perinuclear cluster of Rab11-marked compartments and colocalized with discrete, bright Rab11 puncta. Interestingly, the perinuclear cluster of Rab11 compartments became disintegrated in *fwd* RNAi embryos, suggesting that Fwd regulates the formation and spatial organization of Rab11 compartments. Furthermore, we observed that Fwd knockdown induced marked remodeling of the apical cell surface before gastrulation, including a reduction in apical membrane protrusions and the appearance of a more uniform hexagonal organization throughout the tissue. Interestingly, despite altered cell shapes, apical myosin activation and the mechanism of actomyosin-driven constriction remain unchanged in *fwd* RNAi embryos, suggesting that the shape defects arise not from impaired force generation. To test whether defects in intracellular trafficking underlie the phenotype observed in Fwd-deficient embryos, we are adapting the Retention Using Selective Hooks (RUSH) technique in early *Drosophila* embryos to visualize how loss of Fwd affects surface delivery of secretory cargoes. In future experiments, we aim to investigate not only the molecular pathways that regulate cell surface expansion, but also the physical properties of the tissue and how constraints on membrane expandability influence passive tissue mechanics.

617F Quantitative Proteomics of Cell Fate Specification in the Early *Drosophila* Embryo

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The transition from a single fertilized zygote to diverse tissues in a multicellular organism requires tightly regulated protein expression across space and time. Yet, global proteomic dynamics during embryonic cell fate specification remain poorly characterized. Although bulk proteomic studies in frog, fish, and fly embryos have profiled thousands of proteins during early development, these approaches average across heterogeneous tissues. In vitro differentiation systems may not faithfully recapitulate developmental contexts, and single-cell proteomics, while promising, remains insufficiently reliable for comprehensive quantification of the thousands of proteins that define cell fate.

To overcome these challenges, we developed *Drosophila* morphogen-flattened (MF) embryos, in which endogenous patterning cues are genetically fixed to uniform levels across the embryo. This strategy produces multicellular embryos composed of a single cell type prior to gastrulation, providing sufficient and homogeneous material for quantitative proteomic analysis of cell fate–specific differentiation.

Using MF embryos representing distinct cell fates, we quantified ~6,500 proteins using accurate multiplexed proteomics across four developmental time points. At the blastoderm stage, when embryos are composed of a single cell type, proteomes already diverged by ~10% between neurogenic and lateral hindgut MF embryos and ~25% between the neurogenic and dorsal ectoderm MF embryos. These differences intensified during subsequent development, reaching up to 40% within two hours, underscoring how modest early patterning cues are amplified at the protein level in ways not always predictable from transcript abundance.

Our results demonstrate that early developmental patterning programs rapidly establish distinct, fate-specific proteomes, providing a framework for understanding how small differences in embryonic inputs are amplified into the tissue diversity of multicellular organisms.

618F Investigating the roles of sphingolipid glycosylation enzymes in *Drosophila* border cell migration

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Collective cell migration is a highly conserved and critical process that drives tissue formation during development, supports wound healing, and promotes cancer invasion and metastasis. Understanding how cells move together as cohesive groups is essential for deciphering the mechanisms that govern multicellular organization and dynamic tissue behavior. In the *Drosophila* melanogaster ovary, this process is exemplified during stage 9 of oogenesis, when a cluster of border cells detaches from the anterior follicular epithelium, invades the germline cells, and moves toward the oocyte. This migration requires the precise integration of signaling pathways to coordinate cell polarity and cytoskeletal dynamics. Our previous work showed that the small GTPase Rap1 is critical for maintaining group polarity during border cell migration. To identify additional factors involved, we performed a genetic modifier screen for genes that influence Rap1-induced migration defects. In a pilot screen, several deficiency lines were found to modify the migration delay caused by Rap1 hyperactivation. Secondary screening of candidate genes revealed that a transposon insertion in α4GT1 (α1,4-N-acetylgalactosaminyltransferase 1) significantly suppressed the $Rap1^{V12}$-induced migration delay, a result that was recapitulated by α4GT1 overexpression. Because α4GT1 functions in glycosphingolipid biosynthesis, we further examined upstream glycosylation enzymes (egghead, brainiac, β4GalNAcTA/B, and α4GT2) and found that their dysregulation also altered Rap1-mediated migration phenotypes. Future studies will analyze the mutant clones of these genes to elucidate their role in collective cell migration. Given the links between glycosphingolipid modification and cancer metastasis, our findings may uncover conserved mechanisms that regulate coordinated cell movement in development and disease.

619F Genetic screen for nuclear membrane protein that interacts with *dysfusion* in *Drosophila* oogenesis

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During embryonic development, collective cell migration plays a crucial role in morphogenesis, yet its molecular mechanisms remain incompletely understood. To investigate how coordinated cell movement is regulated, we used the border cell cluster in *Drosophila melanogaster* oogenesis as an in vivo model system. The cluster consists of six to eight cells that migrate together toward the oocyte during mid-oogenesis. Previous studies have shown that the JAK/STAT signaling pathway is essential for determining the number of border cells and maintaining their persistent migration. Reduced pathway activity results in fewer migrating cells and migration defects, whereas excessive activation induces additional migratory cells. Therefore, proper modulation of JAK/STAT signaling is critical for ensuring the correct size and coordinated movement of the migrating cluster. Our previous work revealed that the nuclear membrane protein *dysfusion* (*dysf*) inhibits STAT nuclear import, leading to border cell migration defects. Immunostaining indicated that Dysf may localize to the inner nuclear membrane, suggesting that it could interact with nuclear pore complex (NPC) components to regulate nucleocytoplasmic transport and affect STAT localization. To test this hypothesis, we conducted a systematic genetic screen targeting multiple nucleoporins, nuclear transport factors, and Ran-binding proteins. In total, dozens of mutant lines were analyzed to assess their effects on border cell migration defects induced by dysf overexpression. Several genes were found to modify the dysf-dependent phenotype, suggesting that Dysf may cooperate with specific nucleoporins or transport proteins to regulate STAT nuclear import and JAK/STAT signaling during collective cell migration.

620F The *Drosophila* micropyle is shaped through reciprocal interactions between follicle cells and the chorionic extracellular matrix they secrete

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Collective movements of epithelial cells not only shapes tissues and organs but can also create lasting extracellular structures when coupled with new protein secretion. How cells influence and are influenced by the extracellular matrix (ECM) they secrete remains poorly understood. An ideal place to study this is the *Drosophila* micropyle, the conical tube within the eggshell ECM through which a sperm will enter to fertilize the egg. ~36 cells build this complex, multilayered tube through sequential secretion of distinct eggshell layers, while also undergoing drastic changes in cell shape. The mechanisms of lengthening this tube are not known, nor whether reciprocal interactions between the cells and secreted ECM inform its morphogenesis. We employed 3D super-resolution confocal microscopy to quantify changes in cell apical size and shape that occur during lengthening of the micropyle. We found lengthening to be associated with large changes in cell apical area and the direction in which individual cells are elongated. Starting from pre-stressed circumferentially elongate shapes, a two-step process of apical shrinkage is followed by apical elongation along the orthogonal (micropyle) axis. Additionally, we imaged micropyles in egg chambers of controls and those in which the follicle cells were depleted of an early endochorion protein, s36, to determine the effects of reduced chorionic ECM on micropyle formation. Live imaging revealed that micropyles continued to narrow even after cells had deposited a substantial amount of endochorion, implying a circumferentially constrictive force that the chorion only partly counteracts. This idea is further supported by the observation that micropyle-forming cells depleted of s36 produce highly deformed micropyles that are excessively narrow along part or all of their entire length. These findings suggest that reciprocal mechanical interactions between the follicular epithelial cells lengthening the tube, and the chorionic ECM they are secreting inform the shape of the micropyle.

621F Systematic Characterization of Phosphatases Implicated in BMP-Dependent Wing Patterning and Growth in Drosophila

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The highly conserved BMP pathway plays important roles in cell differentiation, tissue development, and homeostasis, and exhibits diverse behavior in response to input signals. Gain-of-function or loss-of-function mutations in the Bone Morphogenetic Protein (BMP) pathway are associated with disorders affecting multiple organ systems, including the musculoskeletal, gastrointestinal, urinary, cardiovascular, pulmonary, neurological, and ophthalmic systems (Wang et al., 2014). Clinical treatment remains challenging due to the complexity, extensive crosstalk, and context-dependent roles of the pathway, and a better understanding of the quantitative regulation of this pathway may lead to improved targeting of this pathway for BMP-related diseases. In particular, response speed, noise filtering, and output sensitivity are competing performance objectives that vary for a particular developmental context based on non-conserved parameters across various organisms, where cellular phosphatase concentrations are predicted to have the most significant impact on pathway tunability (Shaikh et al., 2024). Consequently, we characterized ten phosphatases implicated in the BMP pathway by expressing RNAi lines with the nub-Gal4 and ap-Gal4 drivers and evaluated the phenotypes of adult wings. We found that several phosphatases, including Dullard and Pp1c isoforms, demonstrated the most consistent and severe phenotypic abnormalities, including ectopic veins, blistered/crumpled wings, or were lethal. The other phosphatases demonstrated driver-specific phenotypes or were less severe, suggesting potential indirect regulation of BMP signaling as additional components that tune performance objectives governing cell and tissue fate. Overall, these results provide a phenotypic signature of phosphatases in wing development and suggest that phosphatases are key functional regulators of pathway dynamics.

622F Genetic regions influencing dorsal closure in *Drosophila*: Using a 3R deficiency screen to investigate cell sheet morphogenesis

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Cell sheet morphogenesis is fundamental to metazoan development and underlies key events in vertebrate development such as gastrulation, neural tube closure, palate formation, and wound healing. Dorsal closure (DC) in *Drosophila melanogaster* provides a key model system for studying the genetic regions that are required for normal cell sheet morphogenesis. Such genetic regions are efficiently identified by analyzing the effects of homozygosing genomic deficiencies (*Df*s) available in the Bloomington *Df* kit that covers the right arm of chromosome 3 (3R). The effect of homozygosing a *Df* on DC is tested by live confocal imaging of fluorescent proteins. These are found on the second chromosome and are either incorporated into cell junctions (cadherin encoded by ubicadherin) or the cytoskeleton (nonmuscle myosin 2 encoded by sqhGFP-RLC or F-actin using GFP fused with the actin binding domain of moesin, encoded by sGMCA). Time-lapse images of homozygous *Df* embryos determine whether or not the *Df* includes genes whose zygotic expression is required for DC. When *Df*s display a DC phenotype, the *Df* is further imaged in trans to available sub-*Df*s that overlap the parent *Df* in order to hone in on the DC gene(s) responsible for the *Df* phenotype. Both *Df(3R)ED5780* and *Df(3R)ED7665* have moderate to strong DC phenotypes. *Df(3R)ED5780* is characterized by abnormal head involution, malformed canthi, tears in the amnioserosa and the head, and puckering. Homozygous *Df(3R)ED7665* embryos exhibited cigar shaped dorsal openings, lagging canthi, puckering, and tearing, most frequently at the interface of the lateral epidermis and amnioserosa, adjacent to the supra-cellular actomyosin rich cables found near the leading edge of the dorsal most epidermal cells of the lateral epidermis. Ultimately, we seek to find the DC gene(s) responsible for the DC deficiency phenotypes. Further imaging of *subDfs* has narrowed down the region of interest for the phenotype seen in *Df(3R)ED5780* embryos, while imaging *Df(3R)ED7665* embryos with several subdeficiencies has revealed that each partially phenocopy some aspect of *Df(3R)ED7665* embryos. Imaging has also revealed minor DC phenotypes in *Df(3R)Exel7326*, including head involution defects and an abnormal amnioserosa. Candidate DC genes are being investigated, including *stranded at second* (*sas*) for *Df(3R)ED7665*. Grant support: R35GM127059 and a grant for undergraduate students from Duke University.

623F 3D Chiral Morphogenesis of the Embryonic Midgut, Part I: Dynamics and Mechanics

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Organ morphogenesis reflects a dynamic interplay between chemical signaling and mechanical forces to form functional organs. A key feature of many organs is that they display highly stereotyped, chiral morphologies. While left-right asymmetry can arise from asymmetric gene expression, it also emerges from cell-intrinsic chirality driven by cytoskeletal components, including unconventional myosin motors Myo1C and Myo1D. The embryonic Drosophila midgut begins as a symmetric tube and coils into a stereotyped, chiral loop–with no known left-right asymmetric genetic patterning. Tuning the expression of Myo1C and Myo1D can invert its chirality, but the mechanical forces that govern this process are currently unknown. We show that chiral coiling follows a two-step symmetry-breaking process. First, the tissue undergoes left-right asymmetric bending, with a secondary contribution from tissue twist. Elongation under confinement then amplifies these initial asymmetries into a stereotyped coil. We demonstrate that longitudinal visceral muscle cells control coiling direction and propose a cellular relay mechanism dependent on the activity of Myo1C and Myo1D. Altogether, these results point to a bistable “mechanical Waddington landscape”, wherein molecular inputs bias organ shape toward one of two chiral outcomes that are canalized by mechanical and geometric constraints. Our results highlight how cell and tissue-scale mechanisms collectively generate large-scale organ asymmetry, offering insight into the physical basis of 3D chiral morphogenesis

624F Genetic Control of Dorsal Closure: Morphological Mutations in Embryogenesis Identified in Chromosome 3R Deficiency Screen

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Dorsal closure (DC) is a process during *Drosophila melanogaster* embryogenesis that serves as a model for cell sheet morphogenesis across chordates. DC involves the coordinated movement of lateral epidermal cells to close a gap filled by a sheet of thin, squamous amnioserosa cells on the dorsal side of the embryo. This model serves as a powerful tool for studying the genetics and genomics of cell sheet migrations including those that impact human pathogenesis, including anencephaly, spina bifida, and cancer. Genetic contributions to DC were investigated with forward genetic deficiency (Df) screens on the second chromosome. Here we extend such screens to the right arm of chromosome 3 (3R). *Df* chromosomes, available in Bloomington's *Df* kit for 3R are crossed into an "imaging line" with fluorescent markers for cell junctions or the cytoskeleton and a fluorescently marked balancer. Embryos homozygous for the *Df* are imaged live with confocal microscopy and analyzed for DC phenotypes. Embryos homozygous *Df(3R)BSC47* and homozygous *Df(3R)BSC464* each have strong DC phenotypes characterized by jagged outlines of the dorsal opening and the actomyosin rich cable included in the leading edge of the lateral epidermis, tearing of cell junctions between the amnioserosa and the lateral epidermis, amnioserosa degradation and abnormal rolling of the embryos during time-lapsed recording. Embryos trans-heterozygous for *Df(3R)BSC47* and *Df(3R)BSC464* phenocopy the homozygotes, indicating that the genes that are lost and responsible for the phenotype reside within the overlapping region, thereby reducing genetic candidates from 89 to 36. Embryos homozygous *Df(3R)BSC819* or *Df(3R)ED6280* show strong deformations in cell shape, and have an abnormally wide cigar shaped dorsal opening. To quantify the severity of the cigar-shape, we measured changes in height:width ratios of the dorsal opening over time. *Df(3R) BSC819* was further investigated through trans-heterozygous crosses with sub-*Df(3R)ED10820*, sub-*Df(3R)ED10811* and adjacent kit *Df (3R)BSC43* with negative results. Trans-heterozygous *Df(3R)ED6280* / sub-*Df(3R)ED6277* embryos phenocopied DC mutants, reducing the candidate genes from 8 to 4. Additionally, it suggests Gp93, a gene that encodes an endoplasmic reticulum heat shock protein and is involved in epithelial function, as a possible candidate DC gene. We continue to hone in on the DC genes responsible for these interesting DC phenotypes with appropriate genetic and molecular analyses. Grant Support: R35GM127059 and Duke University for undergraduate research.

625F Control of cell division orientation by patterned cell-surface receptors during axis elongation

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Directionally oriented cell division is a conserved phenomenon of epithelial remodeling that elongates tissues and separates functionally distinct cell populations. An excellent model for studying oriented cell divisions is germband extension of the early *Drosophila* embryo, in which hundreds of epithelial cells divide in a coordinated manner along the anterior-posterior (AP) axis. The elongating germband comprises two morphologically distinct tissues: the lateral neuroectoderm (LNE) and the ventral mesectoderm (VME). Tissue-level mechanical forces generated by contractile actomyosin can influence the direction of cell division during germband extension. Previous studies have demonstrated that tension along the AP axis orients cell divisions in the VME, gap gene mutants with disrupted AP-patterning show defective cell division orientation in the NE[1]. **However, the nature of the molecular cues that cells directly use to orient cell division remains unclear.** The AP-patterning system culminates in the striped expression of four leucine-rich repeat receptor proteins (Toll-2, Toll-6, Toll-8 and Tartan) in the germband, which are required for planar polarized actomyosin and directional cell intercalation. Therefore, we tested whether these same striped cues are also responsible for directing cell division angles.

626S Assembly and Age-Related Degeneration of the *Nasonia vitripennis* Oosome

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Germline specification in many insects relies on maternally provided ribonucleoprotein (RNP) granules. A unique system for studying this process is offered by the parasitoid wasp *Nasonia vitripennis*, as its germ plasm is organized into a single, massive RNP body called the oosome. The oosome is evolutionarily divergent from the well-studied polar granules of *Drosophila*, differing in its enormous size, dynamic migration, and unique protein architecture. However, fundamental questions remain about how this complex structure is assembled and how it is affected by external factors such as maternal age. To understand its formation, the spatio-temporal hierarchy of oosome assembly during oogenesis is being investigated. The precise order in which core proteins and RNAs are integrated is being mapped using super-resolution microscopy combined with fluorescence in situ hybridization and immunofluorescence. Furthermore, parental RNA interference is used to functionally dissect the roles of core components in orchestrating this process. To determine the impact of maternal age on the fidelity of oosome assembly, oosomes from young and old mothers are compared, and age-related changes in volume, internal architecture, and composition are quantified. The functional consequence of these changes is assessed by quantifying the number of pole cells specified in the resulting embryos. We show *Nasonia* tudor is critical for oosome integrity, as its knockdown results in smaller oosomes and causes the mislocalization of barkbeetle RNA into an ectopic shell-like structure. Despite these defects, the core oskar mRNA meshwork is still able to form. Strikingly, oosomes from aged mothers are found to be significantly smaller and compositionally deficient in key RNAS compared to those from young mothers. This research provides a high-resolution analysis of the assembly of this unique RNP granule and establishes a direct link between maternal age and the structural integrity of the germ plasm.

627S Dissecting Cis- and Trans-Regulation of Notch during Drosophila Wing Vein Refinement

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The Notch pathway is vital for various developmental processes across metazoans. In *D. melanogaster*, for example, Notch signaling is necessary for pupal wing vein refinement, during which the initially 6-8 cell-wide provein stripes narrow to 1-2 cells over roughly 16 hours. Proveins exhibit elevated expression of the Notch ligand Delta (Dl) compared to the surrounding tissue. Dl in provein cells activates Notch in adjacent non-vein cells, generating stripes of Notch activity flanking each provein region. The increased Notch activity in these flanking cells represses vein fate, ensuring that the adult veins are the appropriate size. Beyond the flanking regions, non-vein tissue exhibits both low Notch activity and low Dl expression, which defines intervein areas. In addition to trans-activating signaling in neighboring cells, Notch ligands can block pathway activity within the same cell through cis-interactions with the receptor. Dl has been proposed to mediate such “cis-inhibition” in proveins to prevent inappropriate Notch activation, though this has not been formally demonstrated. Our understanding of how cis-inhibition may influence vein patterning is further complicated by the overlap of high Dl expression and strong Notch activity during the refinement process. It remains unclear whether cis-inhibition operates in specific cell types, at defined stages of refinement, or both. Using somatic mosaics, we found that Notch ligand knockout within the provein regions leads to ectopic Notch signaling, nominally consistent with loss of cis-inhibition. However, our interpretation of these results is limited as large clones severely disrupted concurrent Notch-dependent events in wing development. To address this concern and more rigorously characterize the importance of cis-inhibition in vein development, I am generating smaller clones to knock out Notch ligands within specific regions of the wing. Additionally, I am using genetic drivers specific to the provein, flanking, and intervein regions to perturb ligand expression and/or function within these areas. By analyzing whether these manipulations result in wildtype vein patterns or Notch loss-of-function/gain-of-function venation phenotypes, we aim to determine whether cis-inhibition occurs in these regions, the contexts in which it acts, and how its regulation differs from that of trans-activation. Overall, these studies will provide insight into the similarities and distinctions in Notch regulation across wing regions, contributing to a more comprehensive understanding of Notch-mediated vein refinement.

628S Identifying key genetic regions for cell sheet morphogenesis on chromosome 3R using a *Drosophila* deficiency screen in dorsal closure

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Morphogenesis is an essential component of metazoan development and involves highly conserved movements of cell sheets. In vertebrates, cell sheet morphogenesis is required for heart and palate formation, wound healing, gastrulation, and neural tube development. The process of dorsal closure (DC) in *Drosophila melanogaster* embryos mimics cell sheet movements in vertebrates and serves as a genetically tractable model system. As the dorsal hole closes, amnioserosa cells ingress and apoptose. Simultaneously, contractile forces in the amnioserosa and actomyosin-rich cables contribute to the spreading of lateral epidermal cell sheets to effect DC. We explore the genes that are required for DC through a forward genetic deficiency (*Df*) screen. We use the Bloomington deficiency kit, which is a set of genomic deletions that remove 98.5% of the right arm of chromosome 3. Two crosses successfully isolate homozygous *Df* embryos carrying a fluorescent protein for live imaging. Here, our focus is on kit *Df(3R)s BSC43, BSC141*, *BSC467*, and *BSC567*. *Df(3R)BSC141* embryos are characterized by strong tears between the amnioserosa and the lateral epidermis, unstable lateral and dorsal epidermis, moderate puckering, and failure of the amnioserosa integrity. *Df(3R)BSC43* has a “cigar-shape” phenotype in which the canthus-to-canthus length of the opening decreases at a slower rate than wildtype. In contrast, *Df(3R)BSC567* embryos are wild-type. Ultimately, we seek to identify the specific gene(s) responsible for the DC *Df* phenotype.

Of particular interest is *zen* (removed by *Df(3R)467*), a gene well-known for its contribution to early dorsal-ventral patterning (Rushlow and Levine 1990 Adv. Genet. 27:277). *Zen* embryos show an instructive “pre-DC” phenotype. Null alleles lack amnioserosa, but *zen* hypomorphs show varying numbers of amnioserosa cells. Preliminary analysis suggests that a minimum threshold of 20 amnioserosa cells is required for proper canthi formation – smaller islands of amnioserosa close but without canthi. Why canthi fail to form when only small numbers of amnioserosa cells are present is an open research question. We expect that understanding a parts list of genes required for proper DC will translate from this model to others, allowing for greater insight into the robust process of morphogenesis. Grant support: R35GM127059 and a grant for undergraduates from Duke University.

629S Molecular Sponge Reveals the Importance of Steroid Hormone Ecdysone in Regulating Salivary Gland Morphology in *Drosophila melanogaster*

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Hormonal signaling controls essential physiological processes and is vital during metazoan development. In , the steroid hormone ecdysone (20E) coordinates development by activating the nuclear hormone receptor Ecdysone Receptor (EcR). EcR binds DNA and recruits coregulators to either repress or activate gene expression. Traditionally, tools lacked the precision to determine how specific coregulators influence EcR’s transcriptional function at individual promoters or developmental stages. To address this, we created the transgene *UAS-EcR*^{LBD}, which allows tissue- and time-specific expression of EcR’s ligand-binding domain (LBD). EcR^{LBD} functions as a molecular sponge, competing for cofactor binding, including 20E, without disrupting EcR’s DNA binding. EcR^{LBD} interferes with EcR-driven regulation of promoters, tissues, and developmental processes. Notably, *UAS-EcR*^{LBD} also serves as a platform to study the regulatory roles of individual coregulators in EcR-driven biology. As a proof of concept, we introduced the A483T mutation into our LBD tool, *UAS-EcR*$^{LBD\text{-}A483T}$, which disrupts its interaction with the corepressor Smrter. In salivary glands, EcR^{LBD} expression blocks secretion and expectoration of glue protein without affecting its production, whereas EcR$^{LBD\text{-}A483T}$ does not. EcR^{LBD} expression also results in morphological changes in the salivary gland, including disorganization of secretory cells, a decrease in cell number and nuclear size, and lumen reduction. Surprisingly, EcR^{LBD} does not alter EcR’s transcriptional activity, suggesting these phenotypes are independent of EcR›s transcriptional function. These phenotypes are rescued by expressing EcR$^{LBD\text{-}A483T}$, indicating that Smrter-mediated repression of EcR is vital for proper salivary gland development and function. Overall, the data highlight that Smrter-mediated repression is crucial for salivary gland biology, and that 20E and Smrter are essential for maintaining tissue structure and lumen integrity. Additionally, EcR activity in salivary glands may occur independently of the LBD. We plan to expand our observations by introducing other mutations into the LBD platform to determine whether other EcR domains are important for salivary gland biology. These new transgenes will complement our existing toolkit and enable us to dissect the role of EcR-driven transcriptional activation and repression at the promoter, cellular, tissue, and behavioral levels during *Drosophila* development.

630S The role of Slit-Robo signaling in Caudal Visceral Mesoderm (CVM) migration during embryogenesis

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Caudal visceral mesoderm (CVM) cells migrate across the developing embryo during Drosophila development, guided by a network of signaling molecules and cell–cell interactions. Although these cells make the longest migration of any cells in the Drosophila embryo, we still do not know much about the signaling pathways that influence their migration. Previous studies suggest that the Slit–Robo signaling pathway, known for its role in axon guidance, may also regulate other types of cell migration, including muscle cell migration. The Robo1 receptor is expressed in migrating CVM cells, while Slit is expressed in the trunk visceral mesoderm (TVM) cells, the substrate on which the CVM cells migrate. Preliminary data shows that embryos completely missing *slit* display mis-migration of CVM cells. We are currently investigating whether Robo2 is also expressed in the CVM and whether absence of *robo1* or *robo2* also result in CVM mis-migration. These findings will help clarify whether Slit–Robo has a conserved function outside axon guidance, particularly in regulating mesodermal cell migration.

631S The serine-like protease *masquerade* (*mas*) plays an important role in tracheal tube formation

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The embryonic trachea is a convoluted series of epithelial tubes that provide oxygen to all cells and tissues in the embryo. Proper formation of these tubes involves several distinct cellular processes, including invagination, collective migration, and cell intercalation. *Masquerade* (*mas*) is a serine-like protease that is expressed in the embryonic trachea. In the absence of *mas*, we found individual tracheal metameres had missing dorsal trunk or dorsal branches while the rest of the trachea formed normally. Moreover, we found that, when the lumen was missing, tracheal nuclei were not present. Using btl-GAL4::UAS-myrGFP, which marks the entire cell membranes of tracheal cells, we found misshapen tracheal cells and uneven dorsal trunks. Currently, we are looking at cell-cell junction markers. We are also in the process of generating over-expression constructs of *mas* to see what happens in the trachea with too much Mas present. We are currently in the process of looking at whether there is a link between mas and FGF signaling in the trachea. Overall, this work will be instrumental to our understanding of tracheal tube formation.

632S FGF signaling regulates epithelial stability during *Drosophila* dorsal closure.

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Dorsal closure (DC) is an excellent system for the study of epithelial mechanics, yet how underlying mesodermal tissues contribute to ectodermal function is not understood. A forward genetic, deficiency (*Df*) screen showed that embryos homozygous for *Df(2R)ED2247* have a distinct, epithelial instability phenotype — seams formed during DC are kinematically active, distorted and have zigzag trajectory. In time-lapse, seams repeatedly displace away from, then recoil back to, the dorsal midline. This dynamic instability differs from previously described DC defects and reveals a unique failure mode of epithelial biomechanics.

With sub-*Dfs* and other mutants, we mapped this phenotype to the FGF ligands Pyramus and Thisbe. Loss of either ligand, the FGF receptor Heartless (Htl), or the FGFR scaffold Stumps cause highly penetrant, zigzag seams. Tissue-specific mesodermal, but not ectodermal expression of Htl^{WT} rescues seam morphology, demonstrating that FGF signaling acts in the mesoderm to regulate the ectodermal function.

UV laser ablations reveal reduced recoils in *FGF* signaling mutants, consistent with defects in tension generation rather than changes in viscoelastic relaxation, further indicating altered epithelial mechanics.

Embryos homozygous for *Df(3R)BSC141* phenocopy the *FGF* zigzag seam phenotype. This *Df* removes genes that encode Phosphatidylinositol 3-kinase 92E (Pi3K92E), which contributes to cell and tissue growth and Leucine Rich Repeat Kinase (Lrrk), which contributes to mRNA translational control, cytoskeleton regulation and vesicle trafficking among other functions. Mutations in either *Pi3K92E* or *Lrrk* do not cause the phenotype, suggesting that the two kinases function synergistically to drive DC. Preliminary data indicate that normal mesodermal morphology is preserved in *Df(3R)BSC141* embryos, suggesting that epithelial distortion occurs despite a structurally intact mesoderm.

Mesodermal expression of Dpp partially rescues the mesodermal phenotypes of *htl* null embryos (specific cardiac features are restored) and reduces zigzag severity, though overall mesodermal organization remains abnormal. We speculate that Dpp may be modifying contractility in the perturbed ectoderm.

Together, we identify an FGF, mesodermal to ectodermal signaling axis that is required for the mechanical stability of the ectoderm. We find that zigzag seams emerge as a sensitive readout of this inter-germ-layer communication. Grant support: R35GM127059 to DPK.

633S Roles for *jumeau* and *Checkpoint suppressor 1-like* in alary muscle morphogenesis: expanding the cardiogenic functions of Fox genes

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The heart requires specialized support structures, such as the pericardial ligaments and the diaphragm in mammals, to allow it to withstand mechanical stress and maintain its stability and function. In *Drosophila*, the Alary Muscles (AMs), the evolutionarily conserved analogs of these supporting tissues, anchor the Seven up-expressing pericardial cells (Svp-PCs) of the heart to the embryonic/larval body, thereby ensuring its mechanical stability, lumen integrity, and proper rhythmic function. Forkhead box (Fox) transcription factors are master regulators of heart development, with mutations in four Fox genes associated with human congenital heart disease. Previous studies from our laboratory had revealed the conserved functions of two *Drosophila* Fox genes, *jumeau* (*jumu*) and *Checkpoint suppressor 1-like* (*CHES-1-like*), in regulating cardiac progenitor cell specification, division, differentiation, and spatial organization. Building on that prior work, we now find that these same two *Drosophila* Fox genes are also essential for AM morphogenesis. Loss-of-function mutations in either *jumu* or *CHES-1-like* lead to truncated, incorrectly attached, or abnormally patterned AMs, suggesting defects in alary muscle founder cell specification, myoblast fusion and elongation, or attachment site selection. Similar AM phenotypes in embryos mutant for the *jumu*-activated kinase-encoding gene *back seat driver* (*bsd*) suggest further that these three genes may function in a shared regulatory pathway essential for AM development. To elucidate the mechanisms underlying these morphogenetic defects, we propose three hypotheses: (1) incorrect specification of AM founder cells, (2) impaired myoblast fusion and elongation processes, and (3) defective positioning of AM attachment sites (Svp-PCs) due to errors in Fox transcription factor-mediated cardiac progenitor cell division. Using phenotypic analyses, genetic interaction assays, and fixed and live imaging with transgenic reporter lines, we aim to identify which of these hypotheses is/are correct. Understanding how Fox genes coordinate the development of cardiac support tissue development in *Drosophila* will provide insight into conserved pathways that maintain heart integrity in mammals and reveal how subtle defects in pericardial ligaments, the pericardium, or the diaphragm may contribute to sudden cardiac failure.

634S Defining supporting structures: the Back seat driver kinase orchestrates the development of heart-anchoring alary muscles

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The *back seat driver* (*bsd*) gene encodes a serine/threonine kinase that regulates gene expression epigenetically and is essential for larval somatic muscle development in *Drosophila*. Previous studies indicate that Bsd preserves muscle identity by preventing the fusion of somatic myotubes derived from distinct lineages. Its human ortholog, *VRK1* (*vaccinia-related kinase 1*), is a proto-oncogene whose expression increases under pathological conditions such as vascular injury and cancer, hinting at a conserved role in regulating cellular organization and proliferation. In our study of *bsd* null mutants, we observed a striking defect in alary muscle formation. Alary muscles are a subset of somatic muscles that anchor the *Drosophila* heart tube to the body wall, providing structural support analogous to human pericardial ligaments. Disruption of these muscles often leads to heart tube collapse and impaired cardiac stability. In *bsd* deficient mutants, over 70% of the embryos exhibited severe disruption of alary muscles, and approximately 60% lacked at least one alary muscle, indicating that Bsd is essential for their proper development and organization. Based on the known role of Bsd in restricting cross-lineage myotube fusion, we hypothesize that the missing or disrupted alary muscle phenotypes arise from inappropriate fusion events between developing alary muscle myotubes and adjacent somatic muscle myotubes, resulting in hybrid or incorrectly patterned muscle fibers. To test this, we plan to employ MHC-tau-GFP in combination with an alary muscle-specific marker to visualize all somatic and alary muscles myotubes simultaneously. This strategy will reveal if other muscle types occupy the positions of missing alary muscles in *bsd* mutants and will determine if lineage mixing causes this phenotype. Collectively, our results establish Bsd as a key factor in maintaining the architecture and function of alary muscles that are critical for heart support.

635S Reconstructing the modular effector subnetworks that enable a Fox transcription factor to generate diverse cardiac progenitor cell divisions

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Forkhead box (Fox) transcription factors orchestrate multiple conserved cardiogenic processes in both mammals and *Drosophila*. In *Drosophila*, the Fox genes *jumeau* (*jumu*) and *Checkpoint suppressor 1-like*(*CHES-1-like*) coordinate three distinct categories of cardiac progenitor cell division: asymmetric, symmetric, and cell division at an earlier developmental stage. Our prior work demonstrated that *jumu* regulates a pathway comprising Polo kinase and the kinesin Nebbish (*Neb*) to mediate symmetric and earlier stage cardiac progenitor cell divisions independently of *CHES-1-like*. These findings prompted two questions: (1) whether *jumu* exclusively regulates additional effector genes that mediate cardiac progenitor cell divisions in a manner similar to *polo* and *neb,* and (2) whether such effectors mediate all three categories of cardiac progenitor cell divisions or only specific subsets thereof. To address these questions, we conductedcomparative transcriptomic profiling of wild-type, *jumu* loss-of-function, and *CHES-1-like* loss-of-function mesodermal cells, identifying multiple genes activated exclusively by *jumu*. Phenotypic analysis of mutations of these exclusively *jumu*-regulated genes revealed that the anilin-encoding gene *scraps* (*scra*), the citron kinase-encoding gene *sticky* (*sti*), the kinesin-encoding gene *pavarotti* (*pav*), and the Rho GTPase-encoding gene *tumbleweed* (*tum*) are required for all three categories of cardiac progenitor cell divisions, though only a minimal threshold level of Scra is needed for asymmetric cardiac progenitor cell divisions. Synergistic genetic interactions between *scra*, *neb*, *jumu*, and *polo*, and the absence of such synergistic interactions between either *scra* and *CHES-1-like* or between *neb* and *CHES-1-like*, demonstrate that *scra* and *neb* are integral components of a *jumu-polo* regulatory subnetwork mediating a specific subset of cardiac progenitor divisions. Ongoing pairwise genetic interaction assays aim to determine whether *sti*, *pav*, and *tum* are additional components of this subnetwork. Future work employing qPCR and rescue assays will elucidate the topological hierarchy of these subnetwork components in relation to one another. Collectively, our findings illustrate how a single regulator can generate diverse developmental outcomes by selectively engaging distinct effector modules to regulate discrete cellular processes.

636S Dissecting the individual and combined roles of *Drosophila spalt* paralogs in heart tube morphogenesis to model SALL1/4-linked congenital heart defects

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Mutations in the human *SALL1* and *SALL4* genes, which encode zinc-finger transcription factors, are associated with Townes–Brocks syndrome and Duane radial ray syndrome, respectively. Both disorders are characterized by congenital heart defects (CHDs), underscoring the essential roles of these transcription factors in cardiogenesis. To investigate the genetic basis of such CHDs, we are studying the roles of *spalt major* (*salm*) and *spalt-related* (*salr*), the *Drosophila*orthologs of *SALL1/4,* leveraging the conserved genetic pathways governing heart development in fruit flies and mammals. However, dissecting the individual roles of *salm* and *salr* poses a challenge due to their status as adjacent paralogous genes with potential functional redundancy. To disentangle their individual and combined contributions, we generated null mutations in each gene using CRISPR/Cas9 and combined these with the *Df(2L)Exel6029* deficiency, which removes both loci, enabling a comprehensive analysis of their roles during heart morphogenesis. In wild-type *Drosophila* embryos, the heart is a tubular structure that is closed at the posterior end and consists of two bilaterally symmetric rows of myocardial cells, with contralateral hemisegments of these cells from the left and right sides of the embryo pairing and aligning precisely at the dorsal midline to produce a straight dorsal vessel. In contrast, embryos lacking both *spalt* gene activities display severe cardiac abnormalities: misalignment of myocardial hemisegments, aberrant curvature of the heart tube, atypical clustering of Tinman-expressing myocardial cells in abdominal segment seven, and incomplete posterior closure of dorsal vessel. These phenotypes indicate that the *spalt* genes are essential for proper heart development. To define the origins of these defects, we intend to employ real-time live imaging to track the spatiotemporal dynamics of heart development and determine where and when these anomalies first emerge. By elucidating the cardiogenic roles of *salm* and *salr* in dorsal vessel assembly, this work aims to uncover the evolutionarily conserved genetic mechanisms that may underlie CHDs associated with *SALL1* and *SALL4* dysfunction in Townes-Brocks and Duane-radial ray Syndromes, offering potential insights into the molecular etiology of these human disorders.

637S Dissecting Fox transcription factor-mediated regulation of Polo kinase activity essential for cardiac progenitor cell divisions

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Forkhead box (Fox) transcription factors are essential for heart development in both mammals and Drosophila. Our laboratory previously demonstrated that the DrosophilaFox gene jumeau (jumu) mediates three distinct categories of cardiac progenitor cell division—asymmetric, symmetric, and cell division at an earlier developmental stage—by upregulating Polo kinase activity. However, when we compared the transcriptional expression profiles of flow cytometry-sorted mesodermal cells from wild-type embryos and jumu null mutants, we found that jumu does not transcriptionally regulate polo. This result suggests that that the Fox-mediated activation of polo in cardiac progenitor cell division may occur posttranscriptionally. A vital step in the posttranscriptional activation of polo is the posttranslational phosphorylation of the Polo protein. Two serine-threonine kinases that activate Polo via phosphorylation are Aurora B (AurB) and Back Seat Driver (BSD). Intriguingly, both aurB and bsd, the genes encoding these kinases, are transcriptionally activated by jumu, suggesting that they may be promising candidates through which the Fox gene may activate polo. While aurB is known to have multiple roles in mitosis, bsd has previously been characterized only as mediating the postmitotic activation of Polo necessary for somatic muscle morphogenesis. However, this did not rule out the possibility that bsd is also utilized for mitosis in cardiac progenitor cells. If jumu uses aurB and bsd to activate Polo for cardiac progenitor cell division, then we would expect aurB and bsd mutants to exhibit cardiac defects similar to those seen in jumu and polo mutants. In this study, we show that loss-of-function mutations in aurB and bsd do, in fact, reproduce all three categories of cardiac progenitor cell division defects observed in jumu and polo mutants, consistent with our hypothesis.

To definitively determine whether aurB and bsd mediate Polo activation through jumu, we are conducting genetic interaction and rescue assays. If jumu utilizes one of these genes, it should exhibit synergistic genetic interactions with both jumu and polo. Additionally, cardiac-specific ectopic expression of the candidate gene should partially rescue defects in jumu mutants but not in polo mutants. These experiments will define the mechanism by which jumu governs cardiac progenitor cell division and advance our understanding of Fox-dependent regulation of Polo kinase activity.

638S Drosophila *no ocelli* as a model for human *ZNF503* function: regulation of cardiac progenitor cell divisions in heart development

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The human transcription factor zinc-finger transcription protein 503 (ZNF5030 was first identified as a putative cardiogenic protein through predictive epigenetic and gene expression signatures observed in mouse and human embryonic stem cells differentiating along the cardiac lineage. Functional validation using shRNA-mediated knockdown in human induced pluripotent stem cells further established its essential role in cardiomyocyte differentiation, as loss of *ZNF503* led to a marked disruption of normal cardiac developmental trajectories. Due to the conserved roles of cardiac transcription factors across species, we investigated the *Drosophila* ortholog, *no ocelli* (*noc*), to define its function in vivo during heart development. In wild-type embryos, the dorsal vessel forms as two bilaterally symmetrical rows of myocardial cells whose precise pattern arises from three tightly controlled categories of cardiac progenitor divisions: symmetric divisions that give rise to Tinman-expressing cardial cells; asymmetric divisions that produce paired Seven up–positive cardial and pericardial fates; and an earlier progenitor division that establishes the Seven up lineage. We found that embryos lacking *noc* exhibit an irregular distribution of Tinman-expressing cardial cells—a defect indicative of disrupted symmetric cell divisions—and abnormal numbers of Seven up–expressing cells, reflecting errors in both asymmetric and earlier progenitor divisions. Collectively, these findings demonstrate that *noc* is essential for coordinating cardiac progenitor cell divisions, suggesting a conserved role for *ZNF503* in human cardiogenesis. Now we are focusing on exploring the downstream transcriptional network through which *noc*acts. We will combine expression profiling with targeted phenotypic analyses to identify direct effectors and genetic interactors mediating each division category, thereby resolving whether *noc* engages distinct effector modules for symmetric versus asymmetric/early divisions. By unraveling the role of *noc* in *Drosophila*cardiogenesis, we aim to illuminate conserved mechanisms underlying cardiac defects, offering insights that may connect developmental genetics to human cardiovascular disorders. More broadly, our results underscore the power of *Drosophila* genetics to dissect complex, evolutionarily conserved pathways governing heart development and to generate testable hypotheses for human cardiovascular biology.

639S Dual mechanisms of cardiac patterning: Fox genes ensure proper cardiac cell positioning by regulating progenitor cell divisions and repressing 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 Fox transcription factors (TFs) Checkpoint suppressor 1-like (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 cells within individual hemisegments. Since defective divisions of cardiac progenitor cells in CHES-1-like and jumu loss-of-function mutants often lead to individual hemisegments having different numbers of cardial cells compared to their counterparts across the dorsal midline, we first examined whether the steric constraints caused by this asymmetry were a potential cause of incorrect positioning. Indeed, in Fox mutants, consistent with our hypothesis, contralateral hemisegments with unequal numbers of cardial cells showed significantly more positioning defects than contralateral hemisegments with the same number of cardial cells. However, our statistical analysis also showed that steric constraints could not explain all of the positioning defects: contralateral hemisegments with equal number of cardial cells in Fox mutants showed significantly more positioning defects than those with equal numbers from wild-type embryos. 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 ECM proteins Viking, Collagen type IV alpha 1, and Terribly reduced optic lobes were all overexpressed in Fox mutants. Our preliminary phenotypic analysis 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 ECM genes in the mesoderm phenocopies the cardiac cell positioning defects observed in CHES-1-like and jumu loss-of-function mutants.

640S Twin roles of the zinc finger transcription factor Castor: specification of cardiac cell subtypes and regulation of cardiac progenitor cell division

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Mutations in the zinc-finger transcription factor-encoding gene *CASZ1* lead to aberrant heart development in humans, *Xenopus*, and mice, underscoring its evolutionarily conserved role in cardiogenesis. Our analysis of null mutations of *castor*(*cas*), the *Drosophila* ortholog of *CASZ1*, reveals that *cas* plays two distinct roles in heart development. First, *cas* is required for mediating all three types of cardiac progenitor cell division: asymmetric, symmetric, and cell divisions at an earlier stage of development. Second, *cas* prevents subsets of cells in the most anterior region of the heart, the anterior aorta, from being specified as *seven up*-expressing cardial cells (Svp-CCs). In wild-type embryos, Svp-CCs are found exclusively in the posterior aorta and the even more posterior heart proper, regions of the heart patterned by the expression of the Hox genes *Ultrabithorax (Ubx)* and *abdominal A (abd-A)*. Intriguingly, both *Ubx* and *abd-A* repress *cas*,and ectopic expression of either of these two Hox genes in the anterior aorta leads to the ectopic specification of Svp-CCs there—a result which phenocopies *cas* loss-of-function mutants. Collectively, these data raise the possibility that that *Ubx* and *abd-A* specify Svp-CCs in the posterior aorta and the heart proper by repressing *cas* in those regions. In contrast, in the anterior aorta, in the absence of both *Ubx* and *abd-A*, *cas* levels may be sufficiently high to repress the Svp-CC fate. We are presently testing this hypothesis for *cas*-mediated Svp-CC specification and attempting to delineate the molecular pathways through which *cas* regulates cardiac progenitor cell divisions

641S Role of PAPS synthetase in regulating apical extracellular matrix during salivary gland morphogenesis

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Epithelial tubes are fundamental for the structure and function of many organs. Their morphology and luminal integrity are shaped by the apical extracellular matrix (aECM), a specialized layer of proteoglycans and fibrous proteins at the apical surface of epithelial cells. Sulfation, a conserved post-translational modification, is critical for modifying the ECM. The universal sulfate donor, 3′-phosphoadenosine-5′-phosphosulfate (PAPS), is synthezised by PAPS synthetase (Papss), a key enzyme required for sulfation.

Using the Drosophila salivary gland (SG), we investigate how sulfation influences tubular morphogenesis. Our previous work in the embryonic SG has shown that loss of Papss disrupts lumen expansion, causing apical disorganization, condensed aECM, and defects in the Golgi. In this study, we extend our analysis to the larval SG, which offers a larger surface area and is well suited for live imaging of vesicle dynamics and secretory processes. Knockdown of Papss using the SG-specific driver fkh-Gal4 resulted in a thin, irregular lumen with apical membrane blebs, reduced Sec15 signals (exocyst marker), and disrupted Golgi organization, indicated by altered ManII signals. Time-course analysis across larval stages (96–100, 100–110, 110–120, and >120 hours after egg laying) revealed persistent failure of luminal expansion in Papss knockdown SG at all stages. Ongoing live imaging of ex vivo cultured larval SGs is aimed at elucidating how Papss influences vesicular trafficking, secretory granule biogenesis, and aECM organization. In parallel, we are investigating the roles of key components of the aECM, zona pellucida (ZP) domain proteins, in lumen organization in the embryonic SG. These studies will elucidate how sulfation coordinates intracellular trafficking and extracellular matrix organization to maintain epithelial tube architecture and integrity. These findings may be generally applicable to the development and diseases of tubular organs.

642S On organ form and function: quantifying shape variation in the Drosophila renal system

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The development of an organ requires coordination of cells to form the correct 3D tissue structures for its proper physiological functioning, and yet some structural variability is tolerated. The degree of variability can reveal how strongly linked form and function are for a given organ structure. Here I propose to determine the variation tolerance in organ shape of the embryonic Drosophila melanogaster renal system, the Malpighian tubules. This organ is responsible for osmoregulation and waste excretion, and the tubules become physiologically active at the end of embryogenesis. Tubule development involves the specification of four cylindrical buds of cells attached to the gut, these buds proliferate, and then each of these buds elongate into four functional epithelial tubules, with two tubules extending towards the anterior and two tubules extending towards the posterior. The tubules each have a characteristic fold about two-thirds along their length towards the distal end. The final organ form is described as “stereotypic”, but this organ’s shape variability has yet to be quantified. To address this challenge, I am capturing high resolution images in 3D of the organ shape using confocal microscopy of embryos expressing a tubule-specific membrane marker and a whole-embryo apical protein marker (ctB>palm::mkate, crb::GFP). I will quantify morphometric features of the organ to identify variable and stereotyped regions. To test the impact of organ form on its function, I will leverage several known manipulations that impact tubule shape development and positioning. I will determine which features of Malpighian tubule shape are altered in these genetically perturbed embryos and quantify uric acid secretion as a readout of physiological function. Taken together, these approaches will identify the metrics and features of organ shape critical for physiological function and lay the groundwork to identify the molecular players that are essential for sculpting organs.

643S A new mutation in *zipper* exhibits dominant negative character

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Cell sheet morphogenesis is a highly conserved process across metazoans and contributes to wound healing, neural tube closure, and palate formation. During embryogenesis, dorsal closure (DC) in *Drosophila melanogaster* provides an excellent, genetically tractable model system to study epithelial sheet morphogenesis. During DC, lateral epidermal sheets migrate dorsally to seal an opening covered by a squamous epithelium called the amnioserosa. Closure is driven by multiple coordinated forces: amnioserosa cells undergo apical constriction, and an actomyosin cable at the leading edge of the lateral epidermis generates contractile force. Together, these forces collaborate with zipping to close the dorsal hole. In an F2, fail to complement screen for a deficiency that removes nonmuscle myosin 2 heavy chain, we generated mutations in three lethal complementation groups. One complementation group included an allele that encoded an SDS-PAGE electrophoretic variant of Myosin 2 heavy chain. That complementation group in turn failed to complement *zipper (zip)* which had been recovered in the Nüsslein-Volhard/Wieschaus screen for pattern formation mutations, but which had not been identified molecularly. A new allele of interest is *zip*D38, which exhibited higher penetrance for severe tearing at the interface between the lateral epidermis and the amnioserosa and for incomplete closure than *zip* nulls. To investigate the mechanism, we examined *zip*D38 in trans to a deficiency removing *zip* or the null allele *zip*2. These transheterozygotes showed reduced tearing and improved closure compared to *zip*D38 homozygotes, consistent with a dominant-negative mechanism. We introduced an extra copy of *lethal(2) giant larvae (lgl)* via a transgene into the *zip*D38 background. Extra *Lgl* increases the penetrance of dorsal closure defects, apparently by titrating the maternal load of Zip protein (see Franke et al. 2005 Curr. Biol. 15:2208). As expected, extra *Lgl* did not significantly enhance the *zip*D38 tearing phenotype. These results are consistent with *zip*D38 disrupting DC primarily by interfering with Myosin 2 function via a dominant negative mechanism and confirms that successful epithelial morphogenesis depends critically on the nonmuscle Myosin 2 activity.

644S Genetic Control of Dorsal Closure: Morphological Mutations in Embryogenesis Identified in Chromosome 3R Deficiency Screen

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Dorsal closure (DC) is a process during Drosophila melanogaster embryogenesis that serves as a model for cell sheet morphogenesis across chordates. DC involves the coordinated movement of lateral epidermal cells to close a gap filled by a sheet of thin, squamous amnioserosa cells on the dorsal side of the embryo. This model serves as a powerful tool for studying the genetics and genomics of cell sheet migrations including those that impact human pathogenesis, including anencephaly, spina bifida, and cancer. Genetic contributions to DC were investigated with forward genetic deficiency (Df) screens on the second chromosome. Here we extend such screens to the right arm of chromosome 3 (3R). Df chromosomes, available in Bloomington’s Df kit for 3R are crossed into an “imaging line” with fluorescent markers for cell junctions or the cytoskeleton and a fluorescently marked balancer. Embryos homozygous for the Df are imaged live with confocal microscopy and analyzed for DC phenotypes. Embryos homozygous Df(3R)BSC47 and homozygous Df(3R)BSC464 each have strong DC phenotypes characterized by jagged outlines of the dorsal opening and the actomyosin rich cable included in the leading edge of the lateral epidermis, tearing of cell junctions between the amnioserosa and the lateral epidermis , amnioserosa degradation and abnormal rolling of the embryos during time-lapsed recording. Embryos trans-heterozygous for Df(3R)BSC47 and Df(3R)BSC464 phenocopy the homozygotes, indicating that the genes that are lost and responsible for the phenotype reside within the overlapping region, thereby reducing genetic candidates from 89 to 36. Embryos homozygous Df(3R)BSC819 or Df(3R)ED6280 show strong deformations in cell shape, and have an abnormally wide cigar shaped dorsal opening. To quantify the severity of the cigar-shape, we measure changes in height:width ratios of the dorsal opening over time. Df(3R)BSC819 was further investigated through trans-heterozygous crosses with sub- Df(3R)ED10820, sub-Df(3R)ED10811 and adjacent kit Df (3R)BSC43 with negative results. Trans-heterozygous Df(3R)ED6280 / sub-Df(3R)ED6277 embryos phenocopied DC mutants, reducing the candidate genes from 8 to 4. Additionally, it suggests Gp93, a gene that encodes an endoplasmic reticulum heat shock protein and is involved in epithelial function as a possible candidate DC gene. We continue to hone in on the DC genes responsible for these interesting DC Df phenotypes with appropriate genetic and molecular analyses. Grant Support: R35GM127059 and Duke University for undergraduate research.

645S *Drosophila* Trus, the orthologue of mammalian PDCD2L, is required for proper cell proliferation, larval developmental timing, and oogenesis

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Toys are us (Trus) is the *Drosophila melanogaster* ortholog of mammalian Programmed Cell Death 2-Like (PDCD2L), a protein that has been implicated in ribosome biogenesis, cell cycle regulation, and oncogenesis. In this study, we examined the function of Trus during *Drosophila* development. CRISPR/Cas9 generated null mutations in *trus* lead to partial embryonic lethality, significant larval developmental delay, and complete pre-pupal lethality. In mutant larvae, we found decreased cell proliferation and growth defects in the brain and imaginal discs. We also found that *trus* is required for proper oogenesis. Mapping relevant tissues for Trus function using *trus RNAi* and *trus* mutant rescue experiments revealed that imaginal disc defects are primarily responsible for the developmental delay, while the pre-pupal lethality is likely associated with faulty central nervous system (CNS) development. Examination of the molecular mechanism behind the developmental delay phenotype revealed that *trus* mutations induce the Xrp1-Dilp8 ribosomal stress-response in growth-impaired imaginal discs, and this signaling pathway attenuates production of the hormone ecdysone in the prothoracic gland. Additional Tap-tagging and mass spectrometry of components in Trus complexes isolated from *Drosophila* Kc cells identified Ribosomal protein subunit 2 (RpS2), which is coded by *string of pearls (sop)* in *Drosophila,* and Eukaryotic translation elongation factor 1 alpha 1 (eEF1a1) as interacting factors. These findings and similarities of *trus* mutant phenotypes with the haplo-insufficiency phenotypes produced by heterozygosity for mutants in Minute genes support the involvement of Trus in ribosomal biogenesis. In summary, our results highlight a role for the highly conserved ribosomal assembly protein Trus/PDCD2L in the control of mitotic proliferation, tissue growth, and oogenesis during *Drosophila* development. When the levels of this protein are reduced by mutation, the Xrp1-Dilp8 stress signaling pathway is triggered which slows development through inhibition of ecdysone production in an attempt to correct the cell proliferation and tissue growth imbalance.

646T Identifying Novel Regulators of Food Consumption through Genome-Wide Association Study and Chemoconnectome Screening in *Drosophila.*

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Food consumption plays a fundamental role in physiological and behavioral processes like aging, health, reproduction, and many more. These processes are affected by the quality and quantity of food ingested. Overconsumption of food, especially sugar, can lead to obesity, a precursor to diseases such as type 2 diabetes, heart disease, and reproductive deficiencies. Understanding the genetic and neuronal mechanism by which food consumption is regulated is essential to prevent these diseases. To elucidate these mechanisms, we performed Genome-wide Association Studies (GWAS) of ~200 Drosophila Genetic Reference Panel (DGRP) lines– a set of fully sequenced inbred lines derived from a natural population -, and screening of ~150 genes in the chemoconnectome (CCT)– the entire set of neurotransmitters, neuromodulators, neuropeptides, and their receptors underlying chemotransmission in an animal. We identified many key genes regulating food consumption in Drosophila. Among these genes are many G protein-coupled receptors like methuselah-like genes (mthl), metabotropic GABA-B receptor subtype 2 (GABA-B-R2), adenosine receptor (AdoR), adipokinetic hormone receptor (AkhR) and neuropeptides like prothoracicotropic hormone (ptth), CCHamide-1 (CCHa1) and adipokinetic hormone (Akh). Our findings provide more insight into the highly polygenic genetic architecture of food consumption.

647T Metabolic resilience and developmental timing: Arc1 requirement during dietary challenges

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Maintaining energy balance requires precise coordination between the nervous system and peripheral metabolic tissues to match energy intake with expenditure. *Drosophila melanogaster* provides a powerful genetic model to dissect the mechanisms behind energy balance, particularly during the larval stage, when rapid growth demands tight regulation of energy storage and usage. Through unbiased genetic screens, we previously identified Arc1, the fly homolog of mammalian ARC, as a neuronal regulator of energy balance. Our results reveal additional roles for Arc1 in the timing of developmental progression in response to dietary yeast content. We find that Arc1 expression in specific brain regions changes in response to dietary conditions. Specifically, the number of larval brain cells expressing Arc1 increases when we decrease the nutritional content of the diet. We further find that Dilp2, but not Dilp3 or 5, modulate Arc1 expression. Developmental progression, measured by the timing of pupariation and adult eclosion, showed that Arc^{115} animals – which have the *Arc1* locus intact and are used as a genetic background control – largely resisted dietary effects on larval development rate, whereas $Arc1^{18}$ mutants developed faster on a high-yeast diet with no differences in low-yeast diet. This difference in developmental timing was not due to altered PTTH levels, as *Arc1* mutation had no effect on PTTH expression in either fed or calorie-restricted larvae. We propose that dietary caloric content induces changes in Dilp2 levels, which promote changes in *Arc1* levels to enable metabolic adjustments that allow larvae to buffer against changes in diet-induced developmental timing. Our work provides insights into how dietary changes are sensed and highlights a potential role for Arc1 as a coupling molecule required for developmental and energetic homeostasis.

648T Intestinal barrier dysfunction promotes brain and muscle aging phenotypes in young flies

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Recent experiments have revealed that altered expression of intestinal epithelial junction proteins in *Drosophila melanogaster* can lead to various hallmarks of aging, including modulation of intestinal homeostasis, variations in microbial dynamics, changes in immune activity, stem cell perturbation, and alterations in lifespan. Loss of a specific occluding junction protein, 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, improves intestinal barrier function during aging, limits dysbiosis, and extends lifespan. 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. This project is investigating the impact of intestinal barrier modification on tissue outside of the gut and address communication between the gut and the brain and muscles in disease models. Results show that perturbing the gut barrier leads to an increase in age-related changes in the muscles and the brain, with accompanying changes in behavior. Current work utilizes cellular and molecular biological methodologies to build upon current knowledge to address crucial questions at the intersection between microbial dysbiosis, epithelial integrity, inflammation, protein aggregation, neurodegeneration, and disease, with the ultimate goal of discovering novel therapies that may enhance barrier function, healthspan, and lifespan.

649T Distinct Chromatin States Coordinate Genome-Wide Rhythmic Gene Expression in Drosophila Photoreceptors

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Biological rhythms are prevalent across organisms, and disruptions to these rhythms are associated with neurodegeneration, metabolic disorders and aging. At the molecular level, many genes exhibit cyclic expression that align biological functions with the 24-h day-night cycles. The canonical model for daily rhythms centers on a transcription-translation feedback loop (TTFL) driven by clock transcription factors and repressors. Additionally, environmental zeitgebers (time giver"), such as light, play crucial roles in rhythmic transcription. While the TTFL is well established for core clock gene expression, the mechanisms that directly govern the rhythmicity of the rest of the transcriptome are less clear. Using *Drosophila* photoreceptors, we identified ~6,000 genes rhythmically expressed under 12L:12D conditions. To understand the mechanisms underlying the widespread rhythmic transcription, we performed CUT&RUN to identify Clock and Cycle bound genes, and profiled the 24-hour dynamics of H3K4me1/2/3, H3K27ac, H3K27me3, H4K16ac, Pol II, and chromatin accessibility (ATAC-seq). Strikingly, only 171 genes were bound by Clock and Cycle, suggesting that most rhythmic genes are not directly regulated by two TFs but may rely on additional transcription regulators. Moreover, oscillations in histone modifications, Pol II occupancy and chromatin accessibility were largely uncoupled from gene expression rhythms, even at Clock targets. What, then, determines the timing of gene expression? Leveraged from chromatin state analysis, we noticed that genes marked by distinct histone modifications tend to peak at characteristic times. Specifically, H3K27me3-marked genes preferentially peak at ZT4, H4K16ac-marked genes at ZT12, and, to a lesser extent, genes marked by H3K4me1 and H3K27ac tend to show troughs at ZT12. These patterns suggest that histone marks may be timekeepers for daily transcription. Footprint analysis further showed that while the top TFs are shared across chromatin states, their binding preferences shift with specific histone marks: heterochromatin-associated TFs (e.g., nom, crol) are enriched at H3K27me3-marked regions, whereas activity-dependent TFs (e.g., Mad, Adf1) preferentially bind H4K16ac-marked regions. Together, our results supported a model in which pre-existing chromatin states collaborate with top TFs to drive phase-specific gene expression across the transcriptome.

650T Glut1 functions in the *Drosophila* intestine to regulate growth and food consumption

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Obesity is a major global health issue caused by an energy consumption to expenditure imbalance, where triglyceride storage is a key factor. Our lab primarily focuses on understanding the genes that are important for regulating triglyceride (TAG) homeostasis in a tissue-specific manner using the fruit fly, *Drosophila melanogaster*, as the model system. Recent research has identified *Glut1*, which encodes a glucose transporter in *Drosophila*, as a prospective regulator of obesity and TAG metabolism. However, its tissue-specific role in coordinating carbohydrate and lipid metabolism is unknown. This study focuses on the role of *Glut1* in *Drosophila* intestinal cells, which have a central role in the uptake of dietary sugar and lipids that have a direct impact on TAG storage and general energy homeostasis in the body. Here, we induced intestine-specific *Glut1* knockdown using the Gal4/UAS system and measured glycogen and TAG storage. Intestinal knockdown of *Glut1* resulted in decreases in glycogen, TAG, and overall protein content in whole animals. We hypothesized that intestinal *Glut1* transcriptionally impacts TAG and glycogen synthesis and/or breakdown, but after conducting qPCR studies of multiple TAG and glycogen metabolism genes, no results supported this hypothesis. However, decreasing *Glut1* in the fly intestine results in less food consumption, perhaps explaining the decreased storage of glycogen and TAG in these flies. Additionally, we observed that intestine specific *Glut1-RNAi* flies had reduced body size and weight. Since insulin-like peptides (ilps) are important for growth in flies, we hypothesized that *ilp* expression may be blunted when intestinal *Glut1* is knocked down. Interestingly, the expression of *ilp3* was increased in these flies suggesting a compensatory mechanism for the lack of nutrients induced by the absence of *Glut1*. Together these results indicate a role for Glut1 in the fly intestine to regulate organismal size as well as metabolic homeostasis and provide additional information about tissue specific regulation of growth and metabolism.

651T Neuronal 9G8 functions in NPF-expressing neurons to regulate organismal triglyceride storage

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Organisms store nutrients as fat to use for energy when food is scarce. In today's Western society where food is abundant, excessive fat is stored, resulting in metabolic diseases such as obesity. In order to better understand the pathogenesis of these diseases, understanding the full complement of genes involved in fat storage is essential. Genome-wide RNAi screens in *Drosophila* have been performed to identify several genes important for the synthesis and storage of triglycerides. One group of genes identified in these genetic screens includes proteins involved in mRNA splicing. Our lab has focused on studying the metabolic functions of 9G8, an mRNA splicing factor that helps establish intron and exon borders. In previous experiments, we found that inducing RNAi to *9G8* in the *Drosophila* fat body caused flies to store more fat. Given that the brain is a metabolic organ that orchestrates much of the body's functions, we wanted to know if 9G8 acts in *Drosophila* neurons to alter fat metabolism in the whole animal. To study this, we induced RNAi to *9G8* in all neurons and observed that these flies stored more triglycerides, suggesting that neuronal 9G8 influences fat metabolism. To further understand how 9G8 nonautonomously regulates fat storage, we analyzed the relative expression of genes encoding enzymes involved in fat synthesis and breakdown. We found that the acyltransferase *mdy* was upregulated, which may contribute to the increased fat phenotype we observed in these flies. Since our data suggests that 9G8 functions in the brain to control the communication with other organs to regulate fat metabolism, we hypothesized that 9G8 may be regulating the synthesis or secretion of neuropeptides to mediate lipid storage. To test this hypothesis, we analyzed the relative expression of neuropeptide genes and found that the NPY-like peptides, *NPF* and *sNPF*, were upregulated in the *9G8* knockdown flies. We then induced RNAi to *9G8* in *sNPF*- and *NPF*-expressing neurons and found that decreasing *9G8* in only *NPF*-expressing neurons caused these flies to store more fat and increase the relative expression of both *NPF* and *sNPF*. Together, these data suggest that 9G8 functions in *NPF*-expressing neurons to regulate the production of neuropeptides and organismal lipid homeostasis.

652T Drosulfakinin-CCKLR Signaling Coordinates Feeding Behavior and Lipid Metabolism in *Drosophila melanogaster*

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Neuropeptide signaling plays a key role in coordinating feeding behavior with energy storage in animal physiology. *Drosophila melanogaster* provides a tractable system to study the neuroendocrine mechanisms that are evolutionarily conserved to maintain energy homeostasis through coordinated control of feeding and metabolism. In *Drosophila*, the satiety-inducing neuropeptide drosulfakinin (Dsk) functions in feeding regulation, locomotor activity, and social behavior, but its role in metabolic homeostasis is not entirely understood. To determine how Dsk affects energy homeostasis, we quantified the storage of triglycerides and glycogen in *Dsk* mutants. Loss of *Dsk* results in an increase in triglyceride, but normal glycogen storage, indicating a selective effect on lipid metabolism. Previous studies have identified the G-protein-coupled receptors CCKLR-17D1 and CCKLR-17D3 as putative receptors of Dsk, which suggests it may signal through these receptors to coordinate metabolic responses. To test this hypothesis, we examined *CCKLR-17D1* and *CCKLR-17D3* mutants and found that loss of either receptor also resulted in an increase in triglyceride storage, suggesting that Dsk might act through both receptors to regulate lipid metabolism. Consistent with the increased triglyceride storage phenotype, *Dsk*, *CCKLR-17D1*, and *CCKLR-17D3* mutants all consumed more food than controls, suggesting that Dsk signaling is responsible for restricting feeding to maintain lipid homeostasis. To further understand this triglyceride accumulation phenotype, we performed qPCR to determine if changes in the expression of genes encoding enzymes important for regulating triglyceride metabolism also contributed to our lipid phenotype. In *Dsk*, *CCKLR-17D1*, and *CCKLR-17D3* mutants, expression of *Drosophila* fatty acid synthase gene (*FASN1*) was upregulated, consistent with enhanced lipogenesis. The expression of the gene brummer (*bmm*), which encodes a triglyceride lipase, was also upregulated, indicating a compensatory mechanism to restore lipid homeostasis. Together, these findings suggest that Dsk signaling restricts lipid accumulation by promoting satiety and through direct modulation of lipogenic and lipolytic gene networks. Our data establishes Dsk–CCKLR signaling as a link between neuropeptidergic regulation of feeding behavior to peripheral lipid metabolism, emphasizing its role in coordinating behavioral and metabolic processes in energy homeostasis.

653T Extreme QTL mapping of radiotolerance uncovers a connection with innate immune responses

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Ionizing radiation damages DNA via double-strand breaks, disrupts transmembrane processes, and modifies proteins. Individuals vary in their ability to repair damaged DNA and tolerate the insult of ionizing radiation, influencing their mutation rates and cancer susceptibility. Furthermore, because radiation is used as a frontline treatment for many tumors, individual responses to radiation may determine the ability to tolerate radiation therapy. Conventional mutant screens in model organisms have revealed the paramount role of DNA damage response and repair pathways in determining tolerance to ionizing radiation. However, natural genetic variation that underlies individual phenotype differences often occurs in distinct genes and pathways as compared to mutant screens. We therefore sought to isolate natural variation in radiotolerance of *Drosophila melanogaster* by performing extreme QTL mapping. We mapped genetic variation in radiotolerance at the 3rd instar larval stage to a large locus near the 3rd chromosome centromere. There are 15 genes within this region that are not previously known to influence radiation response. We therefore sought to uncover gene expression differences between the radiotolerant and radiosensitive lines in the absence of radiation, and 2 hours and 4 hours post radiation exposure. In regard to treatment response, both alleles show increased expression of, but not limited to, genes such as *mre11*, *Ku80*, *rad50*, *DNAlig4*, and *nbs*, consistent with the known functions of these genes in DNA damage response and repair. While the sensitive and tolerant alleles do not differ in expression of genes involved in these processes, we discovered that the sensitive lines are associated with upregulation of genes involved in enhanced DNA replication and cell division. These results align with the general observation that dividing cells are more sensitive to DNA damage. In contrast, tolerant lines show upregulation of innate immune pathways, especially the Toll pathway, in both the presence and absence of radiation. This aligns with observations from vertebrates, where radiation activates the innate immune system and triggers inflammatory pathways that promote tissue repair. Our results suggest that genotypes with higher constitutive expression of innate immune pathways may be radiotolerant. Our work reveals how natural genetic variation contributes to radiation sensitivity, including key processes not identified in forward genetic screens.

654T Functional Characterization of Hsp110 in Drosophila Reveals its Essential and Dosage-Sensitive Role in Nervous System Integrity

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The Hsp70 molecular chaperone system is the front line of defense in maintaining cellular proteostasis. In eukaryotes, ATP/ADP nucleotide exchange in the Hsp70 chaperone cycle is stimulated by Hsp110, a divergent member of the Hsp70 chaperone superfamily and co-chaperone of Hsp70. Hsp110 is also a known modifier of neurodegenerative and other protein misfolding-related disorders. Biochemical aspects of Hsp110 chaperone functions have been characterized in vitro, and cell biological and pathway interaction have been extensively characterized genetically in yeast model systems; however, a detailed understanding of its physiological roles in metazoans remains lacking, particularly in the nervous system. Taking advantage of the single Hsp110-encoding gene in the *Drosophila* genome, we conducted a comprehensive investigation of its expression and function in this animal model. Notably, *Drosophila* and human Hsp110 share significant similarity in their sequence, structure, and splicing variants. At the protein level, Hsp110 is ubiquitously expressed, with both cytosolic and nuclear distribution in a tissue-dependent manner. Functionally, while Hsp110 isdispensable for cell proliferation in developing larvae, it is essential for long-term cell survival and normal development of the nervous system, including non-autonomous effects on neuronal differentiation and glial cell migration. Furthermore, despite being isolated as a potent suppressor of protein aggregation and neurotoxicity in multiple neurodegenerative diseases, higher levels of Hsp110 are detrimental in flies, an effect that can be mimicked by the overexpression of Hsp40, another key co-chaperone of Hsp70, while simultaneous overexpression of both Hsp40 and Hsp110 does not further exacerbate their detrimental effect. Together, these results demonstrate a critical role of Hsp110 in neuronal development and cell survival, and further suggest that *in vivo*, the levels and activities of Hsp110 and Hsp40 co-chaperones need to be properly balanced. Furthermore, this work supports the contention that the Hsp70 chaperone network must be considered as a whole when targeted for potential therapeutic purposes to meet the complex pathophysiological demands in multicellular organisms.

655T Targeted Methuselah Antagonism in Insulin-Producing Cells Extends Healthspan in Drosophila melanogaster

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The emerging role of G-protein coupled receptors (GPCRs) and their ligands in aging has been exemplified by studies on Methuselah (Mth), where downregulation of mth or its ligand Stunted, through genetic or pharmacological means, extends lifespan in Drosophila. However, how Mth ligands mechanistically control aging remains unclear. A critical step toward understanding Mth signaling is identifying the molecular pathways and tissues essential for lifespan extension. Here, we report that selective targeting of Insulin-producing cells (IPCs) in the brain with a specific antagonist enhances lifespan, elevates stress resistance, and improves locomotion and memory. These long-lived, antagonist-targeted flies display reduced insulin-like peptide (dilp2) levels and elevated lipid and carbohydrate storage, while maintaining normal fecundity. Together, these findings suggest that Mth antagonists act within IPCs to modulate JNK and Foxo signaling, upregulate their targets Sod and Sirt4, and thereby play a pivotal role in determining lifespan. I further demonstrate that Stunted ligands can ameliorate both early and late stages of human Alzheimer's disease models.

656T The discovery of the *Drosophila* a key integrator between the microbiome and lipid absorption

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The gut tube is the major organ that digests, absorbs, processes, and secretes dietary lipids for all cells of the entire organism. Thus, the gut is heavily involved in regulating the whole organism's response to a meal and the progression of chronic metabolic diseases including metabolic syndrome, heart disease, and type II diabetes. In *Drosophila melanogaster*, the posterior midgut is thought to be the main site of dietary luminal lipid absorption. However, differing from functionally analogous intestinal cells in vertebrates, these posterior midgut cells in *Drosophila* lack the proteins necessary to secrete lipids into circulation, suggesting that an important role for other parts of the gut and the fat body in regulating the postprandial response to dietary lipid. The aim of the present study is to characterize the spatial and temporal specificity of *Drosophila* lipid absorption and secretion to identify essential key genes and proteins in the gut and other peripheral organs that control lipid physiology.

Towards this end, I measured the postprandial response of the *Drosophila* gut by conducting a time course with a fluorescent fatty acid (BODIPY-C12) as a metabolic tracer mixed into fly food containing a non-fluorescent non-absorbable dye as a control for feeding rate. Using HPLC and confocal microscopy, I found that the foregut is a previously unappreciated site of bulk lipid uptake, especially during early post-ingestion timepoints. Interestingly, feeding with other fluorescent lipids, such as BODIPY-cholesterol or the quenched phospholipid PED6, did not show uptake in the foregut, suggesting regional gut specificity for specific lipids. To determine which genes were involved in foregut lipid absorption, I performed post-prandial RNA-sequencing on germ-free and colonized foreguts. This analysis revealed many genes expressed in the foregut that are important for lipid-processing, including the *Drosophila* ApoB lipoprotein, *ApoLpp*, and the small exchangeable lipoprotein *nplp2*. These genes were not previously thought to be expressed outside of the fat body. Furthermore, the localization of these two presumptive foregut proteins (*ApoLpp* and *nplp2)* was confirmed with GFP fusion protein trap fly lines. Knockdowns of these genes in specific foregut cells showed impaired lipid export of lipids from the other major site of lipid absorption, the posterior midgut, suggesting an important postprandial role for foregut lipoproteins. Collectively, these findings are consistent with an underappreciated role for the foregut in *Drosophila* lipid metabolism, potentially as an organ for sensing meal contents and absorption of lipids. Additionally, given that the *Drosophila* foregut is the major site of bacterial colonization, these data also suggest a potential interplay of the microbiome with dietary lipids that promotes efficient nutrient uptake.

657T Does Drosophila have a mammalian LAT1 ortholog and why do we care?

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Dietary methionine restriction has been shown to extend lifespan in several model organisms, including *Drosophila*. Replication of methionine restriction study in humans is difficult, as adherence to a restrictive diet is not feasible in human test subjects. Therefore, pharmaceutical intervention that induces a state of methionine restriction would be a breakthrough clinical strategy. A collaborator in the University of Central Florida's College of Medicine has identified a compound that is capable of inducing methionine restriction and autophagy in mammalian cell culture. This compound targets the protein LAT1, inducing preferential export of methionine out of the cell. As longevity studies are not possible in cell culture, I am interested in determining if this compound can extend lifespan in *Drosophila*. The first step is to identify a mammalian LAT1 ortholog in *Drosophila* and determine if the ortholog is targeted by the compound. I have identified five candidate *Drosophila* genes that have high similarity to mammalian LAT1. In the initial experiments I am determining if the mammalian LAT1 inhibitor JPH203 is toxic in *Drosophila*, and if toxicity can be attenuated by RNAi or overexpression of each of the candidate genes. Once these experiments are concluded I will perform similar experiments with the methionine exporting compound followed by lifespan studies.

658T Absence of Chloride Intracellular Channels (CLICs) confers resistance to hypoxia via differential regulation of ERK and AKT pathways

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Ion channels are mediators of cellular excitability, ionic equilibrium, and signal transduction, and thus are indispensable for cellular adaptation to hypoxia. The modulation of ion channel activity under diminished oxygen availability exerts profound effects on cellular excitability and viability. Among these, the intracellular chloride channels (CLICs) constitute a distinctive subclass of chloride channels, predominantly localized within intracellular organelles. Given their roles in redox homeostasis and their transcriptional responsiveness to hypoxia, we investigated the hypoxic responses of *Drosophila melanogaster*, a species possessing a single CLIC homolog. We subjected null mutant flies for CLIC, to hypoxic stress and observed marked resistance to hypoxia-induced detriments relative to their wild-type counterparts. Notably, acute hypoxic exposure followed by reoxygenation failed to impair cardiac function in the mutants, in contrast to the deleterious effects observed in wild-type flies and the mutant flies also survived better overall. To elucidate the mechanism of these phenotypes, we examined molecular markers and discovered decreased expression of ERK at the transcript, total protein, and phosphorylated levels in CLIC mutants. Furthermore, histological analysis revealed that the myocardial fibers of mutants retained structural integrity, whereas those of wild-types exhibited disarray. Additionally, we detected an upregulation of pro-survival signaling pathways, particularly the AKT pathway, as evidenced by increased phosphorylation of AKT. This effect was abrogated upon pharmacological inhibition of the pathway using rapamycin. Our findings suggest that CLICs may potentiate fibrotic remodeling and suppress cytoprotective signaling under hypoxia. Thus, inhibition of CLIC function may confer cardioprotection and enhance organismal survival in hypoxic environments.

659T Whole organism single-nucleus RNA sequencing at unprecedented resolution reveals the systemic impacts of inflammaging

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Inflammaging, defined as a persistent, low-grade activation of the innate immune system during aging, drives progressive tissue dysfunction and contributes to age-related diseases. IL6-dependent activation of JAK/STAT signaling has been shown to be a major mediator of this process but from studies focusing solely on a single tissue. Therefore, a comprehensive understanding of how IL6 signaling and inflammaging systemically impacts different tissues across the whole organism has been lacking. To capture this systemic effect, we generated the Inflammaging Fly Cell Atlas (IFCA). This was done by inducing *Drosophila* IL6 ortholog *upd3* in the fat body, given its critical role in mediating the immune response during infection and aging. This novel whole-fly single-nucleus RNA sequencing (snRNA-seq) resource recovered an unprecedented 1.1 million cells spanning over 200 distinct cell types. The resolution covers 8 different ages to allow for sensitive tracking of IL6/*upd3* signaling and how this sequentially causes inflammaging across the whole fly. With this dataset, we simultaneously identified novel JAK/STAT pathway genes and improved our knowledge of how chronic inflammation drives aging. Our inclusion of males and females revealed novel sex-specific trajectories during inflammaging. This atlas offers a comprehensive map to decode how systemic inflammation orchestrates tissue decline and accelerates the aging process.

660T Precocious: a novel micropeptide regulator of Drosophila development, metabolism and stress response.

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This study describes a novel micropeptide encoded by the gene *CG46314*, which we have named **'precocious'**, which we characterize as a regulator of larval development and lipid metabolism. We show that *CG46314* encodes a 26 amino-acid micropeptide with several functions in *Drosophila*, particularly in the developing fat body to coordinate developmental timing. Overexpression of precocious in the larval fat body caused a complex developmental phenotype characterized by early (precocious) larval wandering followed by an unusually prolonged wandering phase and delayed pupariation. We observe that the early wandering behavior is acutely dependent on nutrient availability, occurring only under rich food conditions. This phenotype is underpinned by an altered metabolic state, notably a significant increase in lipid reserves despite a shorter foraging period. We are currently examining how precocious influences fat body integrin and insulin receptor levels as well as systemic insulin and steroid hormone signaling to regulate the foraging to wandering to puparium transitions. Interestingly, we find that precocious expression in the fat body results in decreased activity of the integrated stress response (ISR) transcription factor, ATF4 (encoded by *Drosophila crc*). Loss of *crc* has been previously demonstrated to result in decreased lipid reserves and developmental delay, suggesting that the metabolic effects of precocious expression may be mediated by its regulation of crc. Further, precocious expression resulted in reduced levels of several *crc* transcript isoforms. Based on this, we are investigating a potential molecular function of precocious in regulating *crc* transcription and transcript stability. Notably, *precocious* loss of function experiments have proven complex to interpret since the coding regions of *precocious* overlap with that of *crc*. However, we recently developed an antibody against precious that confirms that the micropeptide is indeed expressed the larval fat body, as well as other tissues. These findings highlight a role for precocious as a nutrient-sensitive coordinator of developmental transitions, contributing significantly to the emerging paradigm of functional micropeptides. We propose that precocious is a molecular lever that regulates metabolism to ensure that developmental duration is optimally aligned with organismal metabolic status and environmental cues.

661T Identification and functional characterization of a potent inhibitor of ecdysteroid biosynthesis

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Steroid hormones are critical endocrine factors that regulate diverse physiological functions in animals. In insects, ecdysteroids control molting and metamorphosis to coordinate growth and maturation, while also regulating various adult physiological processes, including metabolism, immunity, longevity, and reproduction. Thus, ecdysteroid signaling represents a promising target for manipulating insect physiology.

In this study, an *in vivo* chemical screening was performed in *Drosophila melanogaster* to identify small compounds that inhibit ecdysteroid signaling. Feeding one compound at nanomolar concentrations markedly delayed pupariation, prolonged the larval period, and consequently increased body size. The ecdysteroid titer was significantly reduced in compound-fed larvae, and concurrent feeding of 20-hydroxyecdysone rescued the pupariation defect, indicating that the developmental delay results from suppressed ecdysteroid biosynthesis. Further analyses revealed that this compound caused a strong reduction in cell size, but not cell number, in the prothoracic gland (PG), the ecdysteroid-producing organ. Under normal conditions, PG cells undergo endoreduplication-dependent hypertrophy during larval development, a process that depends on the TOR signaling pathway. Forced activation of TOR signaling in the PG to induce endoreduplication substantially alleviated the pupariation defect caused by compound feeding. These findings suggest that the compound inhibits ecdysteroid biosynthesis by suppressing endoreduplication in the PG, thereby disrupting *Drosophila* developmental transitions. We are currently investigating the molecular target and mode of action of this compound, as well as extending the findings to mammalian cell systems to explore its conserved biological effects.

662T Maternal diet influences the embryo's developmental trajectory and offspring phenotype in *Drosophila melanogaster*

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Parental diet significantly impacts offspring development, with prenatal factors like maternal nutrition and stress influencing adult offspring phenotypes. This concept aligns with the Developmental Origins of Health and Disease (DOHaD) hypothesis, which posits that maternal conditions during pregnancy can shape offspring's health and development. Intriguingly, in *Drosophila melanogaster*, where eggs develop independently of the mother after laying, these effects suggest a pre-encoded environmental influence within the egg. Our study investigates this phenomenon by exposing female flies to low-, medium-, and high-sugar diets for 10 days before mating with males on a control diet. We examined body weight, lifespan, fecundity, and triglyceride (TAG) levels in both mothers and their F1 offspring. Notably, a low-sugar maternal diet shortened lifespan, whereas a high-sugar diet increased maternal TAG levels. In the F1 generation, the female offspring from both low- and high-sugar diets displayed enhanced starvation resistance compared to medium-sugar controls. To explore the underlying mechanisms, we utilized single-embryo RNA sequencing and metabolomics techniques developed in our lab. One of the frequent hits was the Kynurenine (Kyn) pathway, and the approaches we use to test the role of Kyn are via supplementation and inhibitor. We see metabolite deposition differences in the embryos from varied diet fed mothers. We intend to use single-cell sequencing techniques to explore the underlying process's details further. Our findings highlight that maternal nutrition imposes lasting metabolic challenges on offspring, resulting in phenotypic changes, and support the DOHaD hypothesis even in species like *Drosophila*, where maternal influence ceases post-laying.

663T ERR and HSF cooperatively regulate cellular metabolism

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Metabolism must be precisely regulated to provide appropriate energy and building blocks for cellular homeostasis, growth, and survival. Inappropriate alterations in metabolic flux underlie a wide range of human diseases and even represent a hallmark feature of cancer cells. In this regard, one of the most notable metabolic alterations observed in various cancer cells is known as the Warburg effect (aerobic glycolysis), characterized by elevated glycolytic flux and aerobic lactate production. The resulting glycolytic program is suited to rapidly metabolize carbohydrates for biomass production in tumors, thus supporting cell growth and proliferation. While aerobic glycolysis is commonly associated with cancer cells, this form of glycolytic metabolism can also promote normal growth and development. In *Drosophila melanogaster*, aerobic glycolysis is activated prior to the onset of larval development, thus promoting a glycolytic state that supports rapid larval growth. This observation establishes the fly as an ideal model for studying the endogenous molecular mechanisms that regulate the Warburg effect. Previous studies demonstrated that the *Drosophila* Estrogen-Related Receptor (dERR), an orphan nuclear receptor, is a key activator of aerobic glycolysis. In *dERR* mutants, late-stage embryos fail to activate aerobic glycolysis, rendering larvae unable to use dietary carbohydrates to support biomass accumulation. While the role of dERR in regulating the larval glycolytic program is well-documented, the mechanisms that control dERR activity during this time remain poorly understood. In our ongoing studies of dERR regulation, we found that heat shock causes increased dERR nuclear localization in the larval fat body. This result raises the possibility that ERR activity is coordinated with the cellular heat response, and implicates heat shock transcription factor (HSF), which is the master regulator of cellular heat response, in mediating environmental regulation of dERR activity and cellular metabolism. Consistent with this idea, our multi-omics data suggested that mutations in the *Drosophila* ortholog of the HSFs (*dHsf*) result in larval lethality and induce defects in glycolytic processes and gene expression, similar to those observed in *dERR* mutants. Moving forward, our goal is to identify the molecular mechanisms by which dHsf regulates dERR activity in the context of glycolytic metabolism. By leveraging the special insights offered by *Drosophila*, our long-term aim is to not only identify the conserved mechanisms linking cellular stress response, metabolic regulation, and developmental growth but also reveal new metabolic vulnerabilities within cancer cells.

664T Establishment of *Drosophila* Intestinal Cell Lines to Expand *In Vitro* Models Supporting New Approach Methodologies

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Advancing *Drosophila melanogaster* as a New Approach Methodology (NAM) requires well-characterized *in vitro* models that represent diverse and key tissue types. While the *Drosophila* intestine provides a robust system for studying stem cell regulation and epithelial biology, no intestinal-derived cell lines have been available.

We report the establishment and characterization of three independent *Drosophila* intestinal cell lines generated by expression of oncogenic Ras exclusively in the embryonic intestine. These lines exhibit continuous proliferation and stable recovery after cryopreservation. Single-cell RNA sequencing confirmed their intestinal origin and cellular heterogeneity with multiple clusters with unique gene expression profiles. Notably, these lines form three-dimensional spheroids, which generate an outer media exposed polarized epithelial sheet polarity. Importantly, the arrangement of the apical and septate junctions in these cells are like the intestine epithelium observed *in vivo*. These intestinal cell lines provide the first scalable *in vitro* models of the *Drosophila* intestine. They are amenable to phiC31 site-specific transgenesis, transfection, and therefore offer a tractable platform for investigating intestinal biology, compound screening for toxins and pesticides. By extending the diversity of *Drosophila* cell types available to the research community, this work strengthens the utility of *Drosophila* as a premier genetic model system within the broader NAM framework.

665T Superoxide dismutase knockdown has sex-specific effects on lifespan and healthspan

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Superoxide dismutase, SOD, is a mitochondrial enzyme that removes toxic superoxide anion radicals generated through electron transport leakage by metabolizing them into hydrogen peroxide and oxygen. SOD dysfunction is associated with several disease conditions such as premature aging, amyotrophic lateral sclerosis, and cancer. In *D. melanogaster,* there are two paralogs of this enzyme, SOD1 and SOD2, which differ in their cellular localization. Previous studies have shown that *Sod1* and *Sod2* knockdown reduces lifespan in mice, flies, and nematodes, and *Sod1* overexpression increases lifespan. In contrast, *Sod2* overexpression increases lifespan in flies and nematodes but not in mice. Here, we investigate the sex-specific effects of *Sod1* and *Sod2* knockdown on health- and lifespan. Ubiquitous *Sod1* and *Sod2* knockdown affects lifespan in a sex-specific manner. Males with decreased SOD1 levels have a shorter lifespan than control animals and females with decreased SOD1 levels. Females with lower SOD1 levels also show a reduced medium lifespan, but their maximum lifespan is similar to that of controls. Males with lower SOD1 levels show decreased activity levels, while in females with lower SOD1 levels activity was similar to controls. In contrast, lower levels of SOD2 lead to increased median lifespan compared to control animals in both sexes. Males with lower SOD2 levels showed increased activity levels, while in females were similar to control animals. These results suggest that females and males respond differently to genetic manipulation of SOD1 and SOD2 and thus increased levels of reactive oxygen species. Ongoing experiments explore the molecular mechanisms that control these sex-specific effects of *Sod1* and *Sod2* knockdown on health- and lifespan.

666F GATOR2 regulation of MiTF

Richard Garcia, Chun-Yuan Ting, Shu Yang, Mary Lilly NICHD, NIH

The Target of Rapamycin Complex 1 (TORC1) signaling pathway regulates the balance between anabolic and catabolic metabolism. TORC promotes cell growth by supporting the synthesis of complex molecules and, conversely, facilitates energy production through autophagy by degrading macromolecules and organelles. MiTF/TFE transcription factors (MITF, TFEB, TFE3, TFEC) are downstream targets of this pathway and play a key role in activating the transcription of autophagy-related genes. It was previously thought that this transcription factor family was regulated primarily by TORC1 complex activity. However, we recently identified a novel role of the Gap Activity Towards Rags (GATOR 2) complex in protecting MiTF/TFE proteins from ubiquitin-mediated proteolysis in mammalian cells. Using the Drosophila ovary as a model, we aimed to investigate this new pathway and other regulatory mechanisms of MiTF regulation, the sole MiTF/TFE family member in Drosophila to better understand the role of GATOR2 complex in the regulation of metabolic homeostasis.

667F The function of the TORC1 regulator GATOR2 in the Response to Nutrient Stress

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The multiprotein Target of Rapamycin (TOR) Complex 1 (TORC1) is a serine/threonine kinase that stimulates anabolic metabolism and suppresses catabolism. Deregulation of TORC1 is implicated in various human pathologies, including cancer, epilepsy, and neurodegenerative disorders. The Gap Activity Towards Rags (GATOR) complex contains two subcomplexes: GATOR1, which inhibits TORC1 activity in response to nutrient starvation; and GATOR2, which counteracts GATOR1's function. Structural and biochemical studies have elucidated how GATOR1 regulates TORC1 activity by acting as a GTPase activating protein for Rag GTPase. However, while cryogenic electron microscopy has determined that the structure of the multi-protein GATOR2 complex is conserved from yeast to humans, how GATOR2 inhibits GATOR1 remains unclear. We will present our biochemical, genetic and computational analysis exploring how the GATOR2 complex inhibits GATOR1 to control growth, development and the response to stress in the Drosophila ovary.

668F Using *Drosophila* to determine precise exercise timing and regimentation for maximum benefit in Kennedy's Disease

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Endurance exercise is a potent intervention with widespread benefits proven to reduce disease incidence across species. While endurance exercise supports neural plasticity, enhanced memory, and reduced neurodegeneration, less is known about the effect of exercise on the initiation and progression of movement disorders like Kennedy's Disease (KD). We performed a thorough, unbiased characterization of exercise impact on a recently published Drosophila KD model expressing polyglutamine (polyQ)-expanded, full-length human Androgen Receptor (AR). Exercise imparts conditional improvements in mobility and longevity that are dependent on AR protein localization and exercise timing and intensity.

We found that exercise improves lifespan and mobility in KD flies with muscle-specific AR expression only during the training period. In contrast, when AR is restricted to neurons, mobility and longevity improve after exercise training is complete. Interestingly, chronic endurance exercise does not improve disease in flies with ubiquitous AR expression, but short-duration exercise enhances both lifespan and mobility in early life, similar to flies expressing KD protein only in muscle. Finally, to see whether pharmacological activation of exercise like pathways could improve KD outcomes, we combined short duration exercise with precisely timed resveratrol administration and found additive lifespan benefits. We propose that KD phenotypes are separately triggered in neurons and muscle during exercise, and that exercise training improves KD phenotypes in specific tissues, at distinct periods of disease progression. It is increasingly apparent that therapeutic regimens crucially depend on both dosage and timing of exercise-induced factors. Leveraging the tissue-specific mechanisms at play during these critical windows will be transformative for therapeutic implications, maximizing benefits while minimizing or fully negating adverse tradeoffs.

669F Reduced levels of Methylenetetrahydrofolate reductase impact mortality and climbing behavior after exposure to hypoxia in Drosophila melanogaster

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Hypoxia is a key factor in the pathophysiology of human diseases, and one example is ischemic stroke. Worldwide, ischemic stroke is the leading cause of death and disability. Nutrition is a risk factor for the onset of ischemic stroke as well as impacting outcomes after a stroke. Methylenetetrahydrofolate reductase (MTHFR) is an enzyme involved in the metabolism of folic acid, choline, and other B-vitamins. In humans, a polymorphism in MTHFR increases risk of ischemic stroke, as well as worse outcomes for patients, however the exact mechanisms remain unknown. We are using the Drosophila melanogaster model as they are an effective model for studying hypoxia pathways due to their centralized brain to determine possible mechanistic insights.

For the present study, we used *in vivo* RNAi and w1118 male and female flies. The RNAi flies were obtained from the DRSC/Trip Functional Genomics Resources Center at Harvard University. The *in vivo* RNAi model had the fly equivalent of MTHFR enzyme knock out, we refer to as *CG7560* .The RNAi fly was backcrossed to the w1118 background. At five to six days of age flies were exposed to hypoxia (1% oxygen) for a period of two hours after which flies were returned to normoxic conditions. The survival rate of flies was recorded after hypoxia treatment starting at 24 hours until 90 days. In a separate group of flies, we measured climbing behaviors at 24 and 48 hours, as well as 7 days post- hypoxia.

We confirmed that flies were exposed to hypoxia by measuring escape latency of larvae from food in vials during hypoxic conditions. Our data shows that hypoxia-exposed MTHFR (CG7560) RNAi flies had high mortality levels 24 hours post-hypoxia exposure. Climbing behavior in flies at 24 hours through to seven days post-hypoxia, showed a reduction in the motivation to fly in MTHFR-deficient flies. Furthermore, these flies also show a reduction in the number of movements at 24- and 48-hours post-hypoxia. The reduced movement disappeared by 7 days post-hypoxia.

The data generated thus far suggests that hypoxia negatively impacts climbing behaviors long after hypoxia in Drosophila melanogaster with reduced levels of MTHFR. We are investigating apoptosis in brain tissue of these flies.

The rates of ischemic stroke are increasing both in younger and older populations around the world. Further understanding the role of nutrition on mechanisms of ischemic stroke will make significant contributions to the field.

670F Reduced levels of Methylenetetrahydrofolate reductase reduced apoptosis and increased phagocytosis after exposure to hypoxia in *Drosophila melanogaster*

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Methylenetetrahydrofolate reductase (MTHFR) is an enzyme involved in the metabolism of folic acid, choline, and other B-vitamins. In humans, a polymorphism in *MTHFR* increases risk of ischemic stroke, as well as results in worse outcomes for patients, however the exact mechanisms remain unknown. Worldwide, ischemic stroke is the leading cause of death and disability. Hypoxia is a key factor in the pathophysiology of human diseases, and one example is ischemic stroke. Nutrition is a risk factor for the onset of ischemic stroke as well as impacting outcomes after a stroke. To determine possible mechanistic insights, we are using the *Drosophila melanogaster* model as they are an effective model for studying hypoxia pathways due to their centralized brain. Using the *CG7560* RNAi fly which is equivalent of MTHFR enzyme knock out we have shown reduced motivation to fly and number of total movements post-hypoxia.

For the present study to investigate changes in cellular signaling in *CG7560* female and male flies we stained cerebrum tissue 24 hours post-hypoxia. We measured levels of apoptosis, using active caspase-3, and phagocytosis, using Draper 5D14. We imaged immunofluorescence staining using the Echo Revolve microscope and collected Z-stacks. For analysis of staining we used automated quantification in Image J. The data analysis included the number of cells, volume, and surface area of staining.

We confirmed that flies were exposed to hypoxia by measuring escape latency of larvae from food in vials during hypoxic and normoxic conditions. In the cerebrum we report decreased volume of active caspase-3 in both female and male *CG7560* RNAi hypoxia-exposed flies. There was no difference in the number of cells or surface area. We see increased cell number of Draper 5D14 in female *CG7560* RNAi hypoxia-exposed flies compared to WT controls. There was no difference in the surface area or volume of Draper 5D14.

The data generated thus far suggests that when MTHFR levels are reduced hypoxia induces decreased levels of apoptosis in both female and male flies. Females with reduced levels of MTHFR have increased levels of phagocytosis. We are conducing follow up experiments including staining for other markers, as well as RNA sequencing measurements in brain tissue.

Ischemic stroke incidence is rising worldwide in both younger and older individuals. Clarifying the mechanistic links between nutrition and ischemic stroke will provide critical insights into disease prevention and progression.

671F Investigating the Roles of *Drosophila melanogaster* Seminal Fluid Components in Male Reproductive Senescence

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Male reproductive senescence, marked by declining ejaculate performance, is widespread across organisms and can greatly limit reproductive success. However, most studies focus on the decline in sperm quality, while how changes in the seminal fluid (SF) during aging can affect fertility remains understudied. The age-related deterioration of SF, including changes in proteins, lipids, and other molecular components, may play a critical role in fertility loss. To investigate how aging-related changes in SF components affect fertility, we determine how transcriptomic and metabolic changes between young and old *Drosophila melanogaster* ejaculatory bulb (EB), which produces SF independently of sperm. RNA sequencing of the EBs from young and old males identified 110 genes showing age-dependent downregulation and 201 genes showing upregulation. Preliminary EB-specific RNAi screen in young males shown that 12 age-downregulated genes are involved in fertility decrease. These genes include *MTF-1* (Metal regulatory transcription factor 1) and *Aldh-III* (Aldehyde dehydrogenase type III). Our metabolomic and trace element analysis of EBs found that fatty alcohols (fatty acyl alcohols) and copper levels decreased significantly between young and old males. Together, our findings suggest that male reproductive senescence may be due to changes in lipid metabolism and metal regulation during aging. Our research contributes to the understanding of the genetic mechanisms underlying male reproductive aging, offering broader insights into the aging process in diverse organisms.

672F During starvation calcium signaling across the *Drosophila* fat body is required for the release of lipids

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In *Drosophila* larvae, starvation triggers the release of the neuropeptide AKH and initiates the propagation of intracellular calcium waves across the fat body. Yet, the mechanism of this process and its role in lipid release remain unclear. This study aims to identify specific genes involved in calcium wave initiation and propagation, and to determine how calcium waves impact the release of lipids.

Using calcium imaging in the AKH neurons and the fat body, we show that pulsatile release of AKH from the AKH neurons leads to the generation of calcium pulses in the fat body. In the fat body, the calcium pulses require the release of calcium from the ER as RNAi knockdown of the *Inositol triphosphate receptor* (*Itpr*) gene in clones blocks elevation of intracellular calcium and the propagation of calcium waves.

To understand how the calcium signal is propagated between cells, the role of genes encoding gap junction proteins was also examined. Flip-out clones were generated in which CRISPR/Cas-9 was used to target either the *Inx1* (*ogre*) or *Inx2*. Targeting *Inx1* leads to lower levels of calcium in the mutant clones and slower propagation of the calcium waves. Targeting *Inx2* blocks elevation of intracellular calcium in the mutant clones and blocks wave propagation.

To study the relationship between calcium waves and the release of lipids, larvae were starved for 48 hours to deplete lipid stores. Clones in which the *Itpr* gene was knocked down or the *Inx2* gene targeted by CRISPR/Cas-9 retained abundant lipid droplets, whereas the wild-type cells that received the calcium signal lacked lipid droplets. These findings suggest that release of calcium from intracellular stores and communication through gap junctions play essential roles in the Drosophila starvation response and the release of lipids from the fat body.

673F Investigating the Fas Apoptosis Inhibitory Molecule (FAIM) in *Drosophila melanogaster*

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Protein aggregation is a hallmark of neurodegenerative diseases such as Alzheimer's, Parkinson's, and Amyotrophic Lateral Sclerosis (ALS). No cure exists for neurodegeneration, however the ability to dissolve protein aggregates could open potential ways to combat these diseases. The evolutionarily conserved Fas Apoptosis Inhibitory Molecule (FAIM) can inhibit formation and disrupt protein aggregates such as amyloid beta, which is linked to Alzheimer's disease, and mutant SOD1 aggregates, which are involved in ALS. The *faim* gene is conserved across species throughout evolution, and the FAIM protein has a very similar tertiary structure across species. *Drosophila melanogaster* have one *faim* gene whose protein product is 38% similar to human FAIM, and structural models generated by AlphaFold suggest a very similar tertiary structure. We generated a complete FAIM-knockout in flies using CRISPR-Cas9 and found that, despite being evolutionarily conserved, flies lacking FAIM are homozygous viable under normal culture conditions. Other studies have found that FAIM plays a role in protein homeostasis during cellular stress. We investigated the lifespan of flies lacking FAIM under various stressors, including heat stress and starvation. We found that male flies lacking *faim* have shorter lifespans than wildtype controls at both 25°C and 29°C, whereas female flies lacking *faim* have longer lifespans than wildtype controls at both temperatures. We also tested motor function over time with a climbing assay and found no drastic loss of climbing ability in *faim* knockout flies as they age. Additionally, we expressed and purified the *Drosophila* FAIM protein and future experiments will test its function against aggregating proteins *in vitro*. Further investigation of the localization and function of FAIM in *Drosophila* will increase our understanding of the unique biochemistry and cellular function this protein plays in protein homeostasis and protein aggregation.

674F Genotoxic effects of lead, cadmium, and arsenic combinations in *Drosophila*

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Approximately 78 million Americans live within three miles of a designated Superfund site, areas that the Environmental Protection Agency designates as "the most contaminated land in the nation," many of which contain toxic heavy metals. Exposure to heavy metals is associated with an increased risk of various types of cancer. At the molecular level, exposure to lead (Pb) increases reactive oxygen species (ROS) that induce DNA damage. Exposure to arsenic (As) and cadmium (Cd) disrupts DNA repair pathways. Specifically, Cd displaces zinc (Zn) from DNA repair enzymes and downregulates the expression of DNA repair genes. Published studies have characterized the genotoxic effects of individual metals. However, heavy metal exposure often occurs concurrently in the environment. Few published studies have explored the consequences of combined metal exposures. We aim to fill this knowledge gap by studying the combined effects of Pb, Cd, and As in *Drosophila melanogaster* using environmentally relevant concentrations. We hypothesize that combinations of heavy metals could have additive, synergistic, or antagonistic effects, as observed with drug-drug combinations. We will identify the organismal consequences of metal combinations by measuring developmental timing, viability, and fertility. We have observed a dose- and combination-dependent delay in larval pupation, with some combinations extending development time to 7 days for initial pupal formation. Surprisingly, once pupae form, we observe no significant difference between the treated and untreated groups, supporting the idea that the most severe effects occur during the larval developmental stage. We have preliminary data suggesting that Pb and Cd exhibit antagonistic interactions, in which Cd appears to mitigate Pb-induced toxicity. Additionally, using inductively coupled plasma mass spectrometry (ICP-MS), we will quantify the accumulation of toxic metals in *Drosophila* tissues after ingestion of contaminated food that matches environmental levels. We have observed significant differences in metal accumulation between male and female larvae using full-body ICP-MS. After determining which tissues accumulate heavy metals, we will use gamma-H2Av immunostaining to quantify DNA damage, specifically double-stranded DNA breaks. We will then investigate long-term chromosomal changes induced by heavy metal exposure using a genetic construct to measure loss of heterozygosity (LOH). LOH can be especially damaging if the lost allele is for a tumor suppressor gene, which can cause cancer if the only remaining copy is defective. Understanding the combined impacts of toxic heavy metals will provide critical insights into the actual risk of exposure at sites such as Superfund sites, helping inform scientifically motivated safety standards and influence cleanup priorities for contaminated land.

675F Exercise benefits are inherited inter- and trans-generationally in Drosophila

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The benefits of exercise extend beyond individual health, and evidence is accumulating that the effects of exercise are heritable. Recent data indicate that parental exercise can transmit favorable developmental and metabolic effects to several generations of offspring, but mechanisms driving these benefits is unclear.

To date, the majority of clinical studies focus on parental exercise and its effects on fetal development and metabolism, while model organisms have identified heritable changes in genes and target tissues. We previously showed that replacing the mitochondrial genome (mtDNA) of several Drosophila strains with mtDNA from other strains impacts exercise performance, indicating that exercise capacity is influenced by both the mitochondrial and nuclear genomes.

Here, we found that the progeny of exercised flies demonstrate improved mobility and longer lifespan than age-matched flies whose parents were never exposed to exercise. Furthermore, while most lifespan-extending interventions come at reproductive cost, we found that exercised and mated male and female flies beyond their reproductive prime (adult day 50), yielded progeny with shorter developmental time and more eggs successfully emerging as adults. Excitingly, these benefits persist in the F2 generation.

To define the inherited epigenetic factors that extend healthy organismal physiology, we performed ATAC-Seq and transcription profiling on exercised and unexercised parental and F1 flies. Integration of the differential expression and pathway analyses with open chromatin signatures will allow us to establish exercise-induced downstream targets that extend healthy lifespan inter- and trans-generationally. Drosophila provide the most expedient genetic system in which to perform longitudinal physiological and biomolecular studies for the entire lifespan of parents, F1 progeny, the F2 generation and beyond. Similar studies in humans would take several generations of investigations, whereas Drosophila allow us to identify shared factors rapidly and cost-effectively, making significant progress towards future identification of therapeutic strategies.

676F *Lamp1* deficiency alters lipid metabolism in larval midgut

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Lysosomes play essential roles in maintaining cellular homeostasis and metabolic regulation. These organelles act as nutrient signaling hubs inside the cell that integrate nutritional signals and regulate energy balance, including lipid metabolism. We previously characterized *Drosophila* Lamp1, an evolutionarily conserved lysosomal membrane-associated protein (LAMP). *Lamp1* mutant larvae show excessive endo-lysosomal acidification (EELA). In addition, *Lamp1* deficiency results in increased medium chain diacylglycerols (DAG) levels in whole larvae, suggesting defects in interorgan lipid transport.

We hypothesized that defects in lipid transport should cause additional, tissue-specific cellular and molecular phenotypes. BODIPY staining of wild type and *Lamp1* mutant larvae was used to identify tissue-specific differences in lipid accumulation. Confocal microscopy showed increased accumulation of neutral lipids in the anterior midgut of the mutants. Increase in lipid droplet size in the mutant midguts was confirmed by TEM. We then deployed a multi-omics approach to dissect the effect of Lamp1 defects on lipid metabolism, comparing midgut and fat bodies (FBs) of wild type and mutant larvae. Proteomics analysis identified many proteins associated with lipid metabolism that differentially accumulated in FBs and midgut of mutant larvae. Protein levels suggest upregulation of lipid biosynthesis in *Lamp1* FBs an increase in lipid catabolism in the mutant midgut. A significant increase in the levels of the regulatory subunit of the AMPK complex, SNF4Aγ, in *Lamp1* mutant midgut and decrease of this protein in *Lamp1* mutant FBs suggested that changes in lipid metabolism in these tissues are regulated by AMPK. Western blots showed no significant changes in the total AMPKα or p-AMPKα level, suggesting that allosteric regulation by γ subunit and not phosphorylation-dependent regulation controls AMPK activity in the mutant. Lipidomics analysis of larval guts and FBs showed significant increase in several TAG species in the mutant midgut, consistent with BODIPY and TEM analyses. Excess lipids could lead to lipotoxicity and mitochondrial disfunction, a known trigger for AMPK-mediated regulation of lipid metabolism. Interestingly, no significant differences in midchain DAG levels were observed between wild type and mutant guts. Our results show that Lamp1 is necessary to maintain lipid homeostasis and may mediate lipid transport from the midgut to other tissues.

677F Age-dependent decline of Pex5 ubiquitination underlies peroxisome dysfunction in *Drosophila*

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Peroxisomes play an indispensable role in cellular and metabolic homeostasis. The Pex5 protein is the main matrix protein carrier, transporting peroxisome matrix proteins into the peroxisome during the peroxisome biogenesis. Monoubiquitination of the Pex5 protein enables its recycling back to the cytosol, allowing it to initiate another round of protein transport. We have previously shown that peroxisomal protein transport/import declines with age. However, the mechanisms underlying age-dependent decline of peroxisome import remains largely unclear. To address this knowledge gap, we investigated the changes in Pex5 ubiquitination levels in aging flies using a FLAG knock-in strain that we recently generated through CRISPR/Cas9-mediated homologous recombination repair technology. We immunoprecipitated the Pex5-FLAG protein from three age groups (1-week, 4-week, and 7-week) of the knock-in strain and examined the ubiquitination levels of the isolated target protein. Our results indicate that Pex5 ubiquitination levels decrease with age, revealing a potential molecular mechanism for age-related decreases in peroxisomal protein import.

678F Functional characterization of *CG5577* in Drosophila melanogaster as a potential ortholog to *PDXP/CIN*

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Many metabolic genes in *Drosophila melanogaster* remain uncharacterized. One such gene, *CG5577*, is predicted to encode a member of the haloacid dehalogenase (HAD) superfamily of non-protein phosphatases. Sequence similarity suggests that *CG5577* is the ortholog of human pyridoxal phosphatase (*PDXP*), which desphosphorylates vitamin B_6 (pyridoxal 5'-phosphate, PLP) (1). *PDXP* is also known as chronophin (*CIN*) and, additionally, dephosphorylates Ser-3 on cofilin/ADF family proteins, a modification that regulates actin dynamics during mitosis and cytokinesis. This dual specificity arises from conformational changes that enable *PDXP/CIN* to bind both substrates. Dysregulation of vitamin B_6 metabolism has been linked to several disorders, including neurodegenerative diseases such as Alzheimer's and Parkinson's, as well as skeletal disorders like hypophosphatasia. Likewise, disruption of actin dynamics can cause severe cell division defects. The goal of our project is to determine whether *CG5577* functions orthologously to human *PDXP/CIN*, which would illuminate mechanisms of PLP and actin regulation while establishing Drosophila as a powerful genetic model for studying vitamin B_6 and cytoskeleton-related diseases. Knockout of *PDXP/CIN* in mice leads to decreased muscle performance but enhanced cognitive learning, while loss of active cofilin stabilizes F-actin structures and causes severe cell division defects. We have previously found that CRISPR-Cas9 knockout of *CG5577* produced larval lethality and reduced muscle performance, providing preliminary evidence that it acts similarly to *PDXP/CIN*. To further elucidate the muscular phenotype, we are currently visualizing larvae skeletal muscles microscopically to identify specific muscular defects. We are also determining whether *CG5577* directly acts on PLP or cofilin at the molecular level through enzymatic characterization to test whether this ortholog recapitulates the functions of human *PDXP/CIN*. Successfully characterizing *CG5577* would provide a platform to study vitamin B_6 and actin-related disorders in a genetically tractable model, while broadly advancing knowledge of genomic and biochemical mechanisms involving vitamin B_6 and cofilin.

679F Determining the mechanisms that underly Alzheimer's Disease induced changes in circadian behavior.

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Circadian rhythms are 24-hour cycles that regulate daily processes and are controlled by a molecular clock that is conserved across species. Disrupted circadian rhythms are a hallmark of aging and Alzheimer's Disease (AD), as sleep becomes fragmented and sleep/wake patterns change. AD pathogenesis has been extensively studied, however the underlying mechanisms of circadian rhythm disruptions in aging and AD remain unknown. *Drosophila* have been used as a model for both AD and aging. AD models in *Drosophila* show circadian disruptions similar to those observed in AD patients. A potential mechanism that could explain altered circadian behavior in both aging and AD is changes in H3K4 methylation, which is involved in the regulation of gene expression. We hypothesize that decreased histone methylation in the aging eye and brain results from lower methyltransferase activity. Methyltransferases use S-adenosylmethionine (SAM) as a substrate, generating S-adenosylhomocysteine (SAH), which in turn inhibits activity of methyltransferases. The ratio of SAM and SAH is referred to as the methylation index. This ratio becomes imbalanced with age, altering histone methylation and thus negatively impacting methionine metabolism. Glycine N-methyltransferase (Gnmt) controls the ratio of SAM and SAH by methylating glycine to form sarcosine. Gnmt is cell-autonomously upregulated by FOXO in response to inflammation. Similar to Gnmt, in humans Nicotinamide N-methyltransferase (NNMT) is an enzyme responsible for controlling the methylation index. In brain samples from AD patients, NNMT is expressed at higher levels due to inflammation. Studies have also reported increased SAH levels in brains of AD patients, while concurrently decreasing activity of methyltransferases. Thus, our working model is that an age-dependent decrease in the SAM:SAH ratio leads to decreased histone methylation that can alter circadian-regulated gene expression. As a response we predict there is a decrease in methyltransferase activity causing changes in rhythmic gene expression. Here I propose a model to explain the circadian rhythm changes observed during aging and in *Drosophila* AD models. We hypothesize that inflammation due to aging and AD associated tau expression upregulates Gnmt, disrupting histone methylation, leading to changes in circadian rhythmic gene expression of clock cells.

680F Investigating chromosomal rDNA instability and extrachromosomal rDNA amplification during aging

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Aging is characterized by the gradual and irreversible decline in physiological integrity that leads to impaired function and ultimately death. The genomic instability of essential ribosomal DNA (rDNA) loci is a key molecular effector of aging and replicative lifespan (RLS) in *Saccharomyces cerevisiae*, though it is unclear how rDNA impacts the aging of multicellular organisms. rDNA is comprised of tandemly repeated cistrons encoding the ribosomal RNA genes required for ribosome structure and function. This repetitive genomic architecture ensures that the high rRNA transcriptional demand for ribosome biogenesis is met, but also predisposes rDNA to sporadic intrachromatid exchange, excising stretches of rDNA copies from the genome as extrachromosomal rDNA circles (ERCs). This rDNA instability causes chromosomal rDNA loss in the *Drosophila* germline during aging, but it remains unclear how rDNA instability impacts somatic cells or organismal aging. Here, we use parallel *Drosophila* and mouse embryonic fibroblast (MEF) models to observe rDNA stability during aging in the somatic cells of multicellular organisms. Surprisingly, we find that aged *Drosophila* have increased rDNA copy number (CN) compared to young *Drosophila*. Similarly, we observe that rDNA CN increases occur in MEFs after an initial gradual loss during aging. Furthermore, we find that *Drosophila* with chromosomal rDNA deficiencies have reduced lifespan, suggesting rDNA abundance limits lifespan despite the observed rDNA amplification during aging. Intriguingly, these observations are consistent with aged yeast, which have increased cellular rDNA CN as a result of the replication of ERCs, despite chromosomal rDNA reduction. The accumulation of ERCs in yeast can trigger cellular senescence and limit RLS. These observations suggest that ERC amplification may be a conserved somatic response to compensate for chromosomal rDNA loss, but ultimately compromises cellular function, potentially underlying functional decline during aging. We are now investigating the molecular mechanism underlying our observed rDNA amplification to determine if it is the result of ERC replication, and to understand the source and consequences of somatic rDNA amplification. By uncovering the molecular link between rDNA instability and aging, we can investigate regulators of rDNA maintenance as a potential target to be manipulated to extend lifespan.

681F Touch and Go: Mechanoreceptor activation in *Drosophila melanogaster* induces context-dependent dynamic valence responses and lifespan modulations

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Sensory perceptive events slow the aging process across taxa, illustrating a remarkable phenomenon that is poorly understood. Mechanosensation, an ancient sense utilized across all kingdoms of life and vital for human development and social behaviors, provides a promising opportunity to uncover conserved mechanisms of sensory perception-modulated aging. In *Drosophila melanogaster,* we have found that touch perception alters aging and is experienced as positive or negative depending on context, suggesting that touch input is interpreted within an organism's broader frame of reference to create a sensory experience and modulate its internal state, and that this state change may drive changes in aging.

It is our hypothesis that positive or aversive valences, or hedonic tones, of touch inputs play causal roles as experiential aspects of touch that shape neural processes to modulate aging. To better understand the modulatory relationship between touch input and the animal's state, we interrogated the valence associated with optogenetic activation of touch neurons. We discovered that strong optogenetic activation carries a negative valence, while activation that mimics gentle touch carries a positive valence. This positive valence only emerges after flies have been group housed; previously isolated flies have no associated valence. We have also found that activation of touch neurons in a manner that induces positive valence modulates lifespan and that these effects on lifespan depend on social context, suggesting a causal relationship between the experiential aspects of touch and its effects on aging. Lastly, we identified transcriptional changes in the brain that are associated with the differential valence responses, revealing mechanistic genetic candidates underlying these state-driven differential behavioral and aging responses. Our findings imply that mechanosensory input is sufficient to induce molecular, behavioral, and physiological responses that reach far beyond the input stimulus, implying a complexity of effects in the brain and periphery not previously demonstrated in *Drosophila* that mirrors the physiological and physical effects of human touch. We aim to expand upon these findings to dissect the mechanisms through which touch modulates behavior and lifespan, thus allowing for identification of molecular therapeutic targets and touch-based interventions that translate to promote human longevity and health at advanced ages.

682F Bacterial association drives distinct diet-dependent responses to growth in *Drosophila melanogaster*

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Organismal growth is achieved through the complex interplay of many regulatory networks, which include insulin/insulin-like signaling and ecdysone signaling in Drosophila. These pathways integrate multiple inputs including diet, genetics, and microbial contributions.

We have shown that the microbially-sensitive gene *Arc1* collaborates with the microbiota to promote growth and sensitizes flies to their diet. Similar to mammals, axenic Drosophila larvae grow slower and are smaller than their wild-type counterparts. These effects are exacerbated in *Arc1* mutants. Interestingly, elevating yeast concentration suppresses growth defects in axenic wild-type and *Arc1* mutant animals. To understand the connection between microbes, host genotype and diet, we investigated the extent to which the microbiota and/or *Arc1* buffers animals against extreme dietary conditions. We found that high sugar or low yeast diets significantly increase mortality in axenic *Arc1* mutants compared to wild-type axenic animals. Overall, our findings suggest that animals prioritize faster development over increasing size on more extreme diets. Interestingly, we uncovered a glucose-yeast formulation that enhances the speed of growth while increasing pupal size.

Specific bacterial contributions to growth are not fully understood. Bacteria can enhance growth by modifying the host diet through metabolism of existing dietary compounds or by supplementation with bacterially-derived molecules. We previously showed that *Acetobacter aceti*-conditioned diet can promote larval growth though less robustly than live bacteria. Using metabolomics, we discovered that histidine and arginine were significantly enriched in *A. aceti* conditioned diet suggesting a potential role in growth promotion. We supplemented our standard cornmeal-molasses diet with these two amino acids together and independently. Surprisingly, the two amino acids supplemented together inhibited growth. This effect was not observed when supplemented independently. Generally, animals raised with histidine and arginine supplementation grew slower and were smaller. Axenic Arc1 mutants were also slower but were larger under those conditions. These effects appear to be dose-dependent and specific to histidine and arginine. Together, our results suggest that the synergistic interactions between host genotype, the microbiota and diet are finely tuned to produce the optimal balance of growth period length and final animal size.

683F Role of plasmalogen in non-autonomous regulation of cardiac health in *Drosophila*

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Plasmalogens are ether phospholipids that play critical roles in mitochondrial integrity, oxidative stress resistance, and aging. The heart is among one of the tissues enriched for plasmalogens. However, it remains unclear whether cardiomyocytes rely on locally synthesized plasmalogens or import them from peripheral tissues. To address this knowledge gap, we used *Drosophila* to investigate whether the heart functionally depends on locally synthesized plasmalogens or circulating plasmalogens derived from other organs. We used TinC-Gal4 to drive heart-specific RNAi knockdown of major plasmalogen biosynthesis genes, CG5065/Far1, Gnpat, Agps, and Kua. Cardiac performance was assessed using the semi-automated optical heartbeat analysis (SOHA) system. Cardiac-specific knockdown of plasmalogen biosynthesis genes did not significantly alter cardiac contractility or rhythmicity, suggesting that local plasmalogen synthesis is not essential for maintaining heart function. Currently, we are exploring a potential non-autonomous mechanism underlying plasmalogen-regulated cardiac function. We noticed that Kua, the plasmanylethanolamine desaturase involved in final step of plasmalogen biosynthesis, expressed highly in Malpighian tubules (the fly renal tubules). Our preliminary results showed that renal-specific knockdown of Kua significantly impaired cardiac function. Together, our study highlights a cell-non-autonomous mechanism by which kidney-derived plasmalogens are required for maintaining cardiac health.

684F The impact of gut health on brain and muscle decline

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Previous research in fruit flies has discovered a relationship between increasing age and a deterioration in gut health, with developing intestinal barrier dysfunction tightly linked to mortality. Because of this tight association between organismal health and gut function, several markers of aging have been investigated in order to determine the role of barrier integrity on aging phenotypes. This study examines whether intestinal barrier deterioration can impact protein aggregation within the brain and muscles. Protein aggregates are a specific aging phenotype associated with several diseases of aging and neurodegeneration, such as Parkinson's Disease and Alzheimer's Disease, with an increase in age associated with elevated protein aggregate levels. This research utilizes the knockdown of the protein Snakeskin (Ssk) in the midgut of Drosophila melanogaster, with Ssk knockdown inducing intestinal barrier permeability and numerous hallmarks of aging in young flies. Ssk is a septate junction protein, equivalent to mammalian tight junctions, important for maintaining the integrity of the connections between adjacent gut epithelial cells. Ssk mislocalizes in aging flies, perturbing these junctions and causing intestinal permeability. Knocking down its expression in the gut, using the GeneSwitch Gal4/UAS system in young flies, causes a decrease in barrier function and a severely reduced lifespan, in addition to other markers of aging such as gut microbiota dysbiosis, inflammation, stem cell overproliferation, and metabolic dysregulation. These experiments investigate whether manipulating Ssk expression in the gut can impact protein aggregate formation in tissues outside the gut in order to understand whether unhealthy or aging guts exhibiting barrier dysfunction may be associated with neurodegenerative diseases linked with protein aggregation in the brain and muscles. Behaviors associated with these aging phenotypes are also analyzed. Ideally, these insights will fuel a focus on intestinal health in the studies of several diseases with accompanying protein aggregation.

685S The effect of perfluorooctanoic acid (PFOA) on lifespan, locomotor behavior, and immune gene expression of aging *Drosophila*

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Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic chemicals utilized for their waterproof and grease-resistant properties. However, these substances are persistent drinking-water contaminants. Perfluorooctanoic acid (PFOA), a specific PFAS, poses risks to organisms near heavily contaminated sites. We evaluated PFOA's effect on a set of physiological traits in *Drosophila melanogaster.* We hypothesized that continuous PFOA exposure at concentrations matching previously reported concentrations at contaminated sites, will affect lifespan, locomotor behavior, and immune gene expression. Our results show dose-dependent reduction in lifespan at concentrations of 100 mg/L, 1000 mg/L but not at 10 mg/L for both sexes. These dose-dependent effects demonstrate PFOA's toxicity and support the growing need to assess PFOA risks to environmental health. Further locomotor video tracking analysis revealed a significant decrease in the distance traveled in 15 minutes by 5-day-old flies of both sexes exposed to 100 mg/L of PFOA in comparison to control-treated groups. There was not a significant change in the distance traveled in 15 minutes for older flies of either sex suggesting a stronger neurological effect of PFOA treatment in younger individuals. Because previous studies have linked the activation of innate immunity to neurological impairment including reduced locomotion and decreased lifespan, we next tested if 100 mg/L PFOA exposure influenced immune gene expression. We measured the expression of two NF-kB target genes, *Diptericin B* (*DiptB*) and *Drosomycin* (*Drs*), in both *Drosophila* heads and headless bodies. Our results show no clear pattern of significant gene expression change in both genes for both males and females, but we did observe an upregulation of *DiptB* in 15-day-old female bodies and 15-day-old male heads of PFOA-treated flies in comparison to the control group. We also observed that PFOA treatment resulted in a significant downregulation of *DiptB* in 30-day-old female heads and of *Drs* in 50-day-old male bodies. Together these results suggest that 100mg/L PFOA treatment potentially impacts neurological activity in younger individuals, but this effect does not correlate with significant changes in the expression of the immune genes *DiptB* and *Drs*. More analysis on other aspects of *Drosophila* biology may need to be addressed to determine how PFOA reduces fly lifespan.

686S *Nepl15* Affects Lifespan, Aging, and Physiological and Cytological Traits in *Drosophila melanogaster*

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The M13 family of peptidases is involved in blood pressure and inflammatory response and expressed in a variety of human tissues. The *Neprilysin-like* 15 (*Nepl15*) gene, a member of this family, found in *Drosophila melanogaster* is predicted to encode a protein that is secreted, catalytically inactive, and is involved in the control of glycogen and triglyceride storage. When this gene is knocked out of the flies ($Nepl15^{KO}$), mutant male flies store significantly less glycogen and lipids while mutant female flies accumulate more glycogen and a modest reduction in lipid concentration, indicating a clear sex-specific difference. *In situ* hybridization of the embryo also suggests that this gene is expressed starting from early embryonic stages and have displayed no detrimental effect on the fertility or fecundity of mutant females. Functionally, *Nepl15* mutants show age-dependent health benefits with mutant females having improved longevity and retained intestinal barrier function at older ages while mutant males showed no significant changes in either of these variables. Furthermore, *Nepl15* mutants maintain normal climbing ability, and survival under exercise, suggesting preserved energy efficiency. Optical Coherence Microscopy (OCM) of the adult hearts revealed significantly reduced contractility in males *in vivo*. The heart rates in older flies (week 5 after eclosue) did not decline as in wild-type control flies, suggesting stronger heart performance. At the molecular level, *Nepl15* mutant flies display an overall reduction in mTOR expression and maintained levels of ATP. Additionally, only female mutants show a significant reduction in oxidative stress, which is consistent with upregulated *Sod2* and while male mutants displayed no significant alterations in oxidative stress, which is consistent with the conserved levels of *Sod2*. Further analysis revealed that mutant females display upregulated *SIRT6* expression while mutant males showed a reduction in *SIRT6* expression, which may assist in explaining the improved longevity seen in mutant females. Collectively, these findings illustrate the role of *Nepl15* as a molecular instrument into the fitness, physiological function, and cytological traits, governing sex-dependent metabolic stability and lifespan variation in *Drosophila melanogaster*.

687S RalA/exocyst mediates Nf1-regulated metabolism through a neuronal mechanism in *Drosophila*

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Neurofibromatosis type 1 (NF1) is a monogenetic neurocutaneous disorder that produces various tumors, as well as metabolic alterations such as increased resting energy expenditure, low fasting glucose level, increased insulin sensitivity, and reduced muscle function. This disorder is caused by mutations in *NF1* gene, which encodes a protein called neurofibromin (Nf1). Nf1 has a GTPase-activating protein-related domain (GRD) that promotes the activity of RAS GTPase and affects multiple signaling pathways downstream of Ras. When NF1 is mutated, the activity of several major signaling pathways downstream of Ras is upregulated, including Raf/MEK/ERK, Akt/mTOR, and RalGEF/RalA. Which of these downstream signaling pathways mediate Nf1 effects on metabolism is a major open question. Here we tested the role of one of these pathways, the RalGEF/RalA, in metabolism in *Drosophila*. In flies, loss of Nf1 alters metabolic rate via effects in a subset of metabolism-regulating neurons labeled by a particular genetic driver (PCB-Gal4), allowing *in vivo* genetic analysis of Nf1 metabolic effects. RalA belongs to the RAS GTPase family proteins and is a downstream effector of Nf1/Ras. In normal physiological conditions, RalA regulates membrane trafficking by facilitating the assembly of the exocyst complex to mediate various biological processes. However, the concrete mechanism underlying the regulation and if RalA/exocyst mediates the Nf1-regulated metabolism needs to be further investigated. The exocyst is an octameric protein complex. Intriguingly, except for forming the exocyst complex to regulate cellular functions, each exocyst subunit has separate function, which makes it difficult to investigate exocyst-mediated biological processes. Here, we manipulated the RalA/exocyst expression in PCB+ neurons using Gal4-UAS system. We employed respirometry to measure the CO_2 production and O_2 consumption rate of flies. First, we expressed the constitutively active form of RalA in PCB+ neurons to test whether activation of RalA was sufficient to affect the metabolism of flies. We found the activation of RalA in PCB+ neurons increased the CO_2 production of flies. Second, we expressed the dominant negative form of RalA in the context of Nf1 deficiency in PCB+ neurons. The results showed loss-of-function of RalA in PCB+ neurons rescued the increased CO_2 production and O_2 consumption rate. RalA interacts with sec5 and exo84 to facilitate the assembly of exocyst complex. Therefore, we knocked down sec5 or exo84 in PCB+ neurons in the context of Nf1 deficiency which showed a rescue effect. In summary, our results demonstrated that neuronal RalA/exocyst plays a critical role of Nf1-regulated metabolism, which may contribute to our understanding of how Nf1/RalA control the metabolism of *Drosophila* through a neuronal circuit and the pathology of the metabolic symptoms in NF1 patients.

688S Identifying the ion transporters mediating manganese toxicity in adult *Drosophila*

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Manganese (Mn) is an essential divalent cation involved in many cell functions, including oxidative stress (ROS) and protein glycosylation. Flies fed diets with elevated Mn display a shortened life span with locomotion defects mimicking aspects of neurodegenerative disease. Recently, using a cornmeal diet with known amounts of divalent cations, we determined that the toxic effects of dietary Mn are affected by the levels of other divalent ions in the diet, especially calcium and magnesium (Ghosn, 2024). This suggests that Mn absorption and/or homeostasis may involve several different kinds of ions transporters. To test this, we added 10 mM $MnCl_2$ to this diet and assessed the life span of adult flies harboring mutations in a variety of ion transporters. We predict that mutations in transporters handling Mn would alter the toxic effects of dietary Mn on life span. Mutant males were outcrossed to virgin *w* females acclimated to the normal cornmeal diet and F1 progeny were collected to assess the life span of both male and females on this same diet now with 10 mM $MnCl_2$. Control *w* adult males or females exhibit a median life span of 16-19 days in the Mn diet. This compares to ~26 days for adults with loss-of-function alleles of Zip99c and ~29 days for ZnT63c mutants, suggesting these two Zn-Fe transporters, both expressed in the gut, help absorb dietary Mn. Consistent with the diet effects of calcium and magnesium, adults harboring TrpM mutations, known to effect magnesium homeostasis (Hofman, 2010), partially rescue Mn toxicity with a median life span of 21-28 days depending on allele and sex, while mutations in 3 mitochondrial calcium or magnesium transporters also improves life span on Mn diet. We are presently characterizing the role of the ubiquitous divalent metal transporter (DMT; *Malvolio*) in Mn toxicity. Clearly, Mn is handled by several different classes of cellular transporters suggesting that when seriously elevated, its competition with other divalent ions, including calcium and magnesium, may initiate a generalized disruption of metal ion homeostasis.

689S Sex-specific differences in the *Drosophila melanogaster* response to arsenic exposure

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Sex is a key biological determinant of an organism's susceptibility to toxicant exposure. While sex-specific differences are apparent in epidemiological studies linking environmental toxins with human disease outcomes, the mechanisms underlying such sex divergence remain largely unexplored. Even as modern toxicology builds high-throughput experimental and computational pipelines to examine the backlog of untested manmade chemicals, sex remains a major confounding factor unaddressed by current *in vitro* and *in silico* approaches. Moreover, with governments across the globe limiting the use of mammalian systems for chemical safety studies, few *in vivo* options remain to study sex-specific differences in toxicology. In this study, we address these emerging issues by demonstrating that the fruit fly *Drosophila melanogaster* can serve as a powerful genetic model for studying sex-specific differences in the response to toxicant exposure. Utilizing a combined metabolomic and transcriptomic approach, we find that flies mount sex-specific responses to sodium arsenite ($NaAsO_2$) exposure. Specifically, we discover that the majority of genes and metabolites differentially expressed upon $NaAsO_2$ exposure are unique to either the male or female datasets, with sex the primary determinant of expression regardless of arsenite dose. We identify largely non-overlapping co-expression modules between the sexes and characterize their molecular functions. While the sexes share a common set of core heavy metal and stress response genes, we find their baseline levels and kinetics differ widely. Finally, we demonstrate a machine learning approach simultaneously identifying arsenite response gene signatures and classifying arsenite exposure levels therewith, as a potential method to compare uncharacterized toxicants to an expanding library of known compounds. Overall, our investigation demonstrates that males and females mount a sex-specific response to a pervasive environmental toxicant and establish *Drosophila* as a powerful model to identify and study the sex-specific molecular responses to toxicant exposure.

690S Proline metabolism serves a protective role against chemical stress in *Drosophila melanogaster*

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Proline metabolism plays important roles in multiple biological processes, including energy regulation, interorgan metabolic trafficking, mitochondrial metabolism, and the stress response. Regarding this latter function, although the role of proline in the stress response has been extensively studied in plants, this function in animals is poorly understood. We are addressing this knowledge gap by determining how genetic and pharmacological manipulation of proline metabolism affects the sensitivity of adult flies to chemical stress. Our work has revealed that *sluggishA*^{1} (*slgA*^{1}) mutants, which harbor altered proline dehydrogenase (PRODH) activity, are resistant to a variety of toxicants, including sodium arsenite, cadmium chloride, lead acetate, DMSO, and ethoprophos. Similarly, males and females fed the proline analog, thiazolidine-2-carboxylic acid (T2C), are resistant to sodium arsenite. In contrast, loss-of-function mutations in *Δ*^{1}*-pyrroline-5-carboxylate synthetase* (*P5CS*), which is needed for normal proline synthesis, render adult males and females more sensitive to sodium arsenite. Similarly, male and female flies fed DL-propargylglycine (DL-PPG), which we verified reduced proline levels, resulted in increased sensitivity to sodium arsenite. Together, these findings indicate that proline metabolism serves a central role in the *Drosophila* chemical stress response.

691S miR-10 Overexpression Phenocopies Huntingtin Loss to Drive Hyperphagia in *Drosophila*

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CAG expansion in the huntingtin (HTT) gene causes Huntington's disease (HD), which, in addition to neurological dysfunction, also presents with metabolic disturbances, including hyperphagia and altered energy balance. HTT is a conserved protein essential for neuronal stability, intracellular trafficking, and synaptic function, and evidence indicates that specific miRNAs can regulate HTT expression, suggesting post-transcriptional control of HTT levels. A key unresolved question is whether environmental exposures can drive miRNA-dependent epigenetic changes in HTT that persist across the lifespan and increase vulnerability to neurological dysfunction.

Here, to evaluate the functional consequences of altering neuronal levels of miRNAs or *htt,* we used the *Drosophila* model system, as these pathways are conserved. To evaluate the functional consequences of altering neuronal Huntingtin levels employing *Drosophila* genetic tools, we manipulated *htt* expression using the pan-neuronal driver Elav-GAL4. Under control diet conditions, HTT overexpression (*Elav*>HTT) markedly suppressed feeding, reducing food intake by 50-55% in females and 45-50% in males ($p < 0.05$), indicating a stronger sensitivity to elevated HTT in females. Although we did not see a significant difference in feeding with *htt* overexpression, a trend of increase in feeding was noted. In contrast, pan-neuronal knockdown of *htt* (*Elav*>*htt*-RNAi) produced a robust hyperphagic phenotype, with food consumption increasing by ~50% in males and 55-60% in females, accompanied by significantly reduced locomotor activity and increased resting time ($p < 0.05$; $n = 16$).

Given that miR-10 is predicted to target *htt* mRNA, we explored whether altering this miRNA could replicate the HD phenotype. Strikingly, pan-neuronal miR-10 overexpression phenocopied *htt* deficiency. Elav>miR-10 flies showed increased feeding by 30% in males ($P<0.05$), disrupted day/night locomotor rhythms, and elevated rest time ($p < 0.05$; $n = 16$). Importantly, immunostaining reveals that miR-10 overexpression results in fewer BiP puncta, which is a regulator of endoplasmic reticulum stress. This pattern mirrors the cellular stress response seen in htt-deficient neurons, which also exhibit reduced BiP puncta. These findings suggest that miR-10 may downregulate htt or pathways dependent on htt stress response.

Our data suggest that overexpressing miR-10 mimics the metabolic and behavioral effects seen in *htt* KD, implying they might be involved in pathways related to HD. Exploring this interaction further could lead to new miRNA-targeted treatments for HD and other metabolic conditions.

692S MEI-P26 links paternal Western diet exposure to altered neuronal metabolism and mitochondrial function in *Drosophila melanogaster*

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Paternal nutrition can shape offspring metabolism and behavior, but how dietary experience reprograms neuronal energy balance remains unclear. Our previous transcriptomic analysis of offspring from Western diet (WD) fathers revealed significant upregulation of *mei-P26* in the brain, suggesting a potential role in diet-induced neuronal remodeling. However, the molecular mechanisms by which paternal dietary experience reprograms neuronal energy balance and whether translational regulators such as MEI-P26 drive the resulting mitochondrial changes in fathers and their offspring, remain unknown. Building on this finding, we used *Drosophila melanogaster* to investigate how short-term paternal WD exposure influences MEI-P26–mediated regulation of neural metabolism and mitochondrial function in fathers and their offspring.

We first observed that WD-fed fathers exhibited a 3-fold increase in food consumption measured by FLIC ($p < 0.001$), ~40% higher locomotor activity ($p < 0.001$), and a two-fold rise in CO_2 output ($p < 0.05$), indicating elevated metabolic drive. Biochemical assays showed ~30% higher TAG content and a significant reduction in glycogen stores (25-33% decrease, $p < 0.05$-0.01), consistent with increased nutrient utilization. WD fathers also exhibited a 1.8-fold higher $NADH/NAD^+$ ratio ($p < 0.01$), indicating a shift toward a more reduced redox state and altered oxidative metabolism.

We next asked whether metabolic function is altered in their offspring. Male offspring of WD fathers (WF_0) showed reduced respiration across all OXPHOS-linked states ($p < 0.01$), with glycerophosphate-linked respiration preserved, while female offspring exhibited 30-40% reductions ($p < 0.001$) across most states. These mitochondrial deficits were accompanied by elevated TAG and glucose levels ($p < 0.001$), consistent with systemic metabolic reprogramming.

Transcript analysis revealed upregulation of *mei-P26* (2.1-fold, $p < 0.05$) and diet-responsive microRNAs (*miR-277*, *miR-10*, *miR-305*). Since MEI-P26 regulates growth and translational control, we tested its role directly. Overexpression of MEI-P26 in insulin-producing cells (dilp2 > MEI-P26 OE) suppressed feeding and glucose levels ($p < 0.05$), whereas RNAi knockdown enhanced both, supporting its function as a neuronal metabolic regulator.

Together, these results indicate that MEI-P26 may serve as a mediator by which paternal diet influences neuronal energy metabolism, alters mitochondrial function, and passes metabolic memory to offspring.

693S Proline does more than put kinks in your peptides

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Proline metabolism plays diverse and essential roles in animal physiology, including mitochondrial ATP production, osmotic regulation, biosynthesis of key molecules, and interorgan metabolite shuttling. Despite the importance of this amino acid, questions remain as to how the enzymes involved in proline metabolism are regulated in animal cells. In this regard, *Drosophila* provides the ideal model for dissecting the molecular mechanisms that control proline homeostasis. Jeff Hall previously identified a viable mutation in the gene *sluggish A* (*slgA*), which encodes the fly proline dehydrogenase homolog and is essential for proline catabolism. We are leveraging this classic mutant strain to dissect the mechanisms that control proline synthesis and degradation. Using untargeted metabolomics, we have characterized the metabolic consequences of the *slgA¹* mutation in adults. In parallel, we are analyzing loss-of-function alleles of *Δ¹-Pyrroline-5-carboxylate synthetase* (*P5CS*), which is essential for proline biosynthesis, and using pharmacological inhibitors to target multiple enzymes in the pathway. Our approach have revealed a feedback loop in which *P5CS* gene expression is modulated by changes in *slgA¹* function, highlighting previously unappreciated regulatory mechanism that we hypothesize fine-tunes proline metabolism.

694S Cellular remodeling of citrate metabolism shapes tissue plasticity during enteric infection

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Metabolism and immunity are ancient cellular functions which have co-evolved to produce coordinated responses to threats such as infectious micro-organisms. Because of their evolutionary lineage, invertebrate systems are well-suited for dissecting the fundamental metabolic pathways that shape infection responses. Using *Drosophila*, we've uncovered a putative role for citrate metabolism through the evolutionarily conserved metabolic enzyme ATP Citrate Lyase (ACLY) in cellular immune-metabolic integration. ACLY catalyzes the conversion of citrate to acetyl-CoA and oxaloacetate in both the cytosol and nucleus of many cell types. Through its production of acetyl-CoA, ACLY has a central role in dictating cellular function. Our new evidence suggests that ACLY's subcellular location is important for local immune-metabolic responses to enteric bacterial infection by impacting midgut tissue plasticity. We discovered that ACLY changes subcellular compartments in specific midgut cells during enteric bacterial infection, relocating from the cytoplasm to the nucleus of regenerating cells. Enteric infection likely triggers ACLY movement through stimulating AKT signaling and other immune signaling pathways. This re-localization of citrate metabolism reflects metabolic rewiring that is needed to shape tissue plasticity post-infection, shifting the location of acetyl-CoA production from the cytosol for use in energy production, to the nucleus for chromatin remodeling during maturation into a functional immune-responsive cell. This nuclear pool of acetyl-CoA facilitates acetylation-dependent transcription programs that may promote both immune activation and differentiation in the regenerating cells. We propose that the regulation of citrate metabolism through ACLY serves as a crucial metabolic cycle during enteric infection. ACLY thus dictates tissue plasticity through its subcellular location and ultimately influences infection responses, illustrating the ancient molecular ties between immunity and metabolism.

695S Dissecting Rapamycin-sensitivity across diverse genetic backgrounds of Drosophila melanogaster

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Downregulation of the mechanistic target of rapamycin (mTOR) complex has been shown to extend lifespan and delay development across multiple organisms, including Drosophila melanogaster. Rapamycin, an inhibitor of mTOR complex 1 (mTORC1), is currently undergoing FDA-approved clinical trials as a promising anti-aging therapeutic. Despite its conserved effects, rapamycin sensitivity varies widely across individuals and species, and the genetic basis of this variation remains unclear. Previous work from our collaborators revealed that over 140 genetically diverse Drosophila Genetic Reference Panel (DGRP) strains exhibit striking variation in pupation time after rapamycin treatment. Surprisingly, this variation does not correlate with genetic differences in or near the mTOR gene itself, suggesting that polymorphisms affecting downstream signaling may underlie these phenotypic differences.

To explore this, we analyzed the proteome and phosphoproteome of first-instar larvae from sensitive and resistant strains. Larvae were treated with rapamycin for 12 hours, and a parallel cohort was monitored for 72 hours to validate strain-specific growth responses to rapamycin. Sensitive DGRP strains 348 and 517 showed a twofold reduction in length when treated with 20 μM rapamycin compared to controls ($p < 0.0001$), whereas resistant strain 441 displayed no significant change. Mass spectrometry data were processed using sorting pipelines and linear modeling in R, identifying several differentially expressed and phosphorylated proteins, including translation regulators, kinases, and metabolic enzymes involved in growth control and stress response. Notably, Calcium/calmodulin-dependent protein kinase II (CaMKII) and an amino acid transporter showed consistent abundance differences between sensitive and resistant strains, suggesting divergence in nutrient-sensing and signaling activity. We are now initiating functional validation using RNA-interference (RNAi) lines crossed with nos-Gal4 to knock down candidate genes in germline stem cells. Since mTOR inhibition promotes stem-cell quiescence, these experiments will test whether specific candidates modulate quiescence entry under rapamycin treatment.

Understanding the mechanisms driving rapamycin sensitivity is critical for its clinical translation. Our findings highlight how natural genetic variation in Drosophila can reveal molecular diversity in mTOR-dependent drug responses, guiding future precision therapeutic strategies.

696S Alkylglycerol Supplementation Boosts Plasmalogen Levels and Extends Lifespan in *Drosophila melanogaster*

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Plasmalogen depletion is strongly associated with age-related pathologies, chronic inflammation, and the loss of tissue homeostasis. Plasmalogens and related ether phospholipids in the plasma membrane modulate cell signaling, the behavior of membrane proteins, and can play a role in cell-cell connections. Associations between phospholipid depletion, inflammation, and lifespan are well-documented, but the mechanisms connecting these observations remain hazy. We demonstrate that phospholipids in the plasma membranes of epithelial cells contribute to maintenance of the gut barrier, a vital line of defense against chronic inflammation and a strong predictor of lifespan.

We found that dietary supplements of the plasmalogen precursor batyl alcohol (BA) increased whole-body plasmalogen content, and both BA and plasmalogen supplements significantly extended lifespan in wild-type *Drosophila melanogaster*. Gene expression patterns suggest that BA suppresses age-related inflammation (inflammaging) and modulates tissue homeostasis. Electron microscope and immunofluorescent imaging revealed that glycerophospholipid content strongly affects the structure and permeability of the midgut. Plasmalogen knockdown mutants (Kua/Kua) have leaky guts, a phenotype consistent with premature senescence and chronic immune-system activation. BA supplements improve the integrity of the gut in aging wild-type flies, thereby minimizing contact between pathogens and the hemolymph. We propose that plasmalogens and related phospholipids in the plasma membrane reinforce gut barriers to block the systemic inflammation associated with aging. This suggests that dietary alkylglycerol supplements may have therapeutic potential in the treatment of age-related gut dysfunction.

697S Highly dynamic regulation of SLC2A carbohydrate transporters by developmental time and dietary nutrients in the *Drosophila* larval fat body

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Glucose import into organs is a critical first step in carbohydrate metabolism. The solute carrier 2 family (SLC2A) transporters are the primary facilitative glucose transporters in mammalian cells. The *Drosophila melanogaster* genome encodes 31 SLC2A carbohydrate transporters, but little is known about the tissue-specific roles of these genes. Using our previously published RNA sequencing data, we found eleven SLC2A carbohydrate transporters that are moderately or highly expressed in the third instar larval fat body: Tret1, nebu, sut1, glug, pippin, Glut1, CG15406, CG4607, CG8249, CG7882, and CG33281. We investigated expression of these transporters in the fat body during the final 24 hours of the third instar larval stage, using Sgs3-GFP expression to stage animals during this time period. The mRNA levels of five of the transporters were unchanged over time. However, we discovered that the expression of three transporters decreased before metamorphosis (*sut1*, *CG8249*, and *CG33821*), while the expression of three transporters increased before metamorphosis (*glug*, *CG4607*, and *Glut1*). To examine how dietary nutrients regulate SLC2A transcript levels, we fed third instar larvae standard laboratory food (cornmeal, molasses, and yeast extract), plain agar, or 10% sucrose for 24 hours, then isolated fat body RNA and performed RT-qPCR. We found that most sugar transporters are induced by starvation on plain agar, with the exceptions of *sut1*, *CG8249*, and *CG33821* that were suppressed by starvation. Transcript levels of *Tret1*, *glug*, *pippin*, *CG4607*, and *Glut1* in fat body were increased by the 10% sucrose diet, relative to standard food. In contrast, fat body mRNA levels of *nebu*, *sut1*, *CG8249*, and *CG33821* were decreased in response to the high sugar diet. The roles of these transporters in regulating fat body glucose uptake, nutrient storage, and circulating glucose levels are under investigation. Together, our findings suggest that complex, transporter-specific mechanisms tune the capacity for glucose transport in response to developmental time and nutrient availability.

698S TORC2 Regulation of Autophagy and Its Impact on Longevity

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Aging is the greatest risk factor for many chronic diseases, including neurodegenerative disorders, cancer, cardiovascular disease, and type 2 diabetes. Nutrient-based interventions such as time-restricted feeding (TRF) consistently extend lifespan and improve healthspan across model organisms, yet the molecular mechanisms driving these benefits remain unclear. Autophagy, a conserved cellular recycling process is crucial for lifespan extension under dietary interventions, and its decline contributes to age-related diseases. While the mechanistic Target of Rapamycin Complex 1 (TORC1) is well known to inhibit autophagy, emerging evidence from our lab revels that its counterpart, TORC2, plays an active and essential role in promoting autophagy and longevity. In *Drosophila,* loss of the TORC2-specific component Rictor blocks lifespan extension from TRF dietary restriction, or TORC1 inhibition, and disrupts autophagy and proteostasis. Notably, rictor null flies subjected to TRF exhibit impaired canonical autophagy signaling, including defective lipidation of *atg8a*, indicative of compromised autophagosome maturation. In contrast, RICTOR overexpression alone is sufficient to extend lifespan and recapitulate key benefits of TRF. Collectively, these findings identify TORC2/RICTOR as a central effector of nutrient-dependent longevity pathways, demonstrating that TORC2 is not merely permissive but is an essential regulator of autophagy and organismal responses to nutrient restriction.

699S The Effects of Longterm Sialic Acid Exposure on Lifespan Longevity within *Drosophila melanogaster*

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Sialic acids are a family of carbon sugars with a 9-carbon backbone. They are found on the surface of vertebrate cells covalently attached to membrane phospholipids and membrane-associated proteins. Sialic acids are prevalent as glycolipids and glycoproteins in neurons where they help to regulate neuronal sprouting and plasticity, axon myelination and myelin stability, and the remodeling of mature neuronal connections. Sialic acid is integral in the nervous system and those with Alzheimer's disease (AD) have been found to have higher serum sialic acid levels, an indication of inflammation and disease. Sialic acids are naturally produced by mammals. The most common sialic acid produced in humans is N-acetylneuraminic acid (Neu5Ac). Neu5Ac is found within the nervous system of *Drosophila* and plays an important role in later sialyation within the cell. We synthesized Neu5Ac over a 16-hour period and subsequently ensured its purity using NMR. To determine the effect of Neu5Ac on longevity of *Drosophila*, 5 μM, 10 μμM, 50 μM of Neu5Ac were administered to adult control flies. The lifespan of these animals was observed and recorded. These data will be used to assess whether Neu5Ac may alleviate the phenotypic characteristics of AD in flies.

700S Measuring water consumption after sleep deprivation in *Drosophila melanogaster*

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Metabolic syndrome, a diagnosed cluster of risk factors leading to the progression of severe cardiovascular and endocrine disorders, affects an estimated one third of American adults. Development of the condition is exacerbated through poor sleep, dietary choices, and exercise levels. In addition to increased efforts in researching mind-to-gut connections and higher rates of diagnosis of the disease, our work adds to the understanding of interactions between body systems. Our research focuses on studying the relationship between extreme sleep deprivation and water consumption in *Drosophila melanogaster.* Using flies from the *Drosophila Genetic Resource Panel* (DGRP), we mechanically sleep deprive three strains that require different amounts of sleep-short sleepers (<400 minutes of rest), medium sleepers, and long sleepers (>600 minutes of rest). The sleep deprivation period is supported through the Trikinetics *Drosophila* Activity Monitor where the flies are vigorously shaken every minute for five seconds for five days uninterrupted. After sleep deprivation, we measure water consumption through a Capillary Feeding Assay to find trends of consumption by strain. We hypothesize that individuals with longer sleep needs will likely drink more water in order to help regulate their bodily functions. The results will help uncover the dual effects of dehydration and sleep deprivation on the individual and reveal the plasticity necessary to recover from such events. With the conservation of important homeostatic pathways for sleep and hydration cycles in humans to fruit flies, we hope that our research can bring more awareness on the impacts of dehydration coupled with poor sleep habits, especially in individuals affected by metabolic syndrome. Further research can bring about identification of genes involved in recovery after periods of stress related to sleep deprivation.

701S Characterization of Adiposyn, a possible fat-to-synapse circulating signal that regulates metabolism

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Organs can communicate to one another by releasing hormones into the bloodstream. Learning where these inter-organ factors go, where they come from and their function, is fundamental to learning how organs communicate and how these factors may play roles in disease. One of the current barriers in discovering and studying hormones is the limited ability of existing methods to identify their sources, targets and receptors. To overcome these barriers, our lab separately performed *in vivo* and *in silico* screens to discover novel tissue-secreted proteins in *Drosophila* 3rd instar larvae. *In vivo* screens involved tissue specific labeling using the proximity labeling enzyme TurboID and mass spectrometry, while *in silico* screens involved using AlphaFold-Multimer. From both screens, our lab discovered and is currently investigating a novel circulating factor that we named Adiposyn. Using GFP-tagged Adiposyn *in vivo* and in S2R+ cells, we show that it is secreted from the adipose tissue into the hemolymph and physically interacts with synaptic cell adhesion proteins Neuroligin and Neurexin. Adiposyn KO flies are viable but exhibit a rescue of developmental delay on stressful food types such as high-sugar. Therefore, we hypothesize that Adiposyn is a fat-to-synapse signal that regulates metabolism. We will also discuss the progress related to other novel circulating factors discovered in our lab.

702S Exploring individual variation in toxic response using wild, isogenic, and constructed heterozygous flies

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With all due apologies to Forest, life is not like a box of chocolates, unless your box contains an unpredictable mix of toxins and nutrients. Living things interact with their environment and, often, detoxify it. Given this fundamental nature of toxic responses, we know surprisingly little about their variability between individuals. What is the impact of genetic variability on toxic responses? *Drosophila melanogaster* is a strong model for examining how genetic variation impacts biological responses - but we can ask this question in different ways. In this study, we characterized the biological response of *D. melanogaster* to environmental stress using three different types of fly lines, each with different expected genetic variability: pseudo-wild fly lines, isogenic lines, and constructed isogenic heterozygous lines. Wild lines reflect the genetic variability in natural populations, but change over generations as they adapt to the lab environment. Isogenic lines are stable over generations, but multiple lines need to be examined to capture natural phenotypic variation. Could heterozygous lines offer a stable, diverse alternative? We exposed flies to environmental nickel (Ni) and characterized five phenotypes in both sexes across four lines each of three types of genetic backgrounds. We expected the wild lines to show the most intraline variability and the least interline variability, the isogenic lines to show the opposite variability pattern, and the heterozygotes to fall in between. Overall, internal Ni increased with exposure concentration and negatively impacted most, but not all, phenotypes we examined. Ni exposure reduced survivorship, food consumption, and total lipid concentration in a dose-dependent manner, but did not affect NADP(H) enzyme activities. Effect patterns were reasonably consistent across the three line types, but differed in magnitude and substantially in variation. The pseudo-wild lines had similar effect magnitudes with low variation between lines. The isogenic lines had larger effects than the pseudo-wild, with greater magnitude, and much greater variation between lines. Surprisingly, the isogenic and heterozygous lines were not substantially different in effect magnitude or variation. Counter to our expectations, the heterozygotes no more resembled wild lines than the isogenic lines. Capturing realistic variation in phenotypic responses requires examining multiple lines regardless of whether they are isogenic or heterozygous.

703S Age-associated sarcomere branching is a conserved feature of skeletal muscle remodeling

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Aging adversely affects skeletal muscle, and muscle health in turn serves as an important indicator of lifespan. Skeletal muscle undergoes extensive structural remodeling with age, ultimately contributing to muscle atrophy and sarcopenia. Using serial block-face scanning electron microscopy (SBF-SEM), we previously demonstrated that aging is also accompanied by increased sarcomere branching in mammalian skeletal muscle, independent of fiber type, in both mice and humans. These findings suggest that aging is associated with enhanced connectivity within the contractile networks of skeletal muscle.

To investigate the mechanisms underlying age-induced sarcomere branching, we used *Drosophila melanogaster* indirect flight muscles (IFMs) as a model system. IFMs are the largest muscle group in *Drosophila*, located in the thorax and essential for flight. They represent a highly specialized fibrillar muscle type characterized by a contractile apparatus that lacks lateral connectivity and exists as distinct, individual myofibrils. Despite this specialized architecture, analysis of IFM myofibrils from 30-day-old flies revealed an onset of sarcomere branching, indicating that age-associated sarcomere branching is a conserved phenotype across species.

We further observe that increased branching is accompanied by alterations in thick- and thin-filament protein expression, as well as changes in the expression of *Neurochondrin (NCDN)*, a known regulator of branching in IFMs. Misexpression of *NCDN* in developed IFMs led to an increased frequency of sarcomere branching in aged flies, suggesting that aging-related branching is modulated through established myofibrillar branching pathways. Notably, inducing increased sarcomere branching through overexpression of *NCDN* did not affect fly lifespan, indicating that branching alone is not a negative indicator of muscle health. Together, these findings suggest that age-associated sarcomere branching may represent an adaptive remodeling process rather than a degenerative outcome. Collectively, this work establishes *Drosophila* IFMs as a powerful model for studying age-associated myofibrillar branching and provides a foundation for identifying genetic regulators of myofibrillar connectivity during muscle aging.

704S Evaluating the Effects of ZYN Nicotine Exposure on Fetal Development in Drosophila Melanogaster

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Nicotine use from traditional cigarettes, and electronic cigarettes have steadily declined among younger populations; however, smokeless products such as ZYN have emerged as popular alternatives to adolescents and young adults. Nicotine pouches are oral, smokeless products that deliver nicotine through absorption in the gums without inhalation. Numerous studies have highlighted the negative effects of nicotine use during pregnancy such as impairing fetal and adolescent development, birth defects, and developmental delays. To evaluate the effects of ZYN on fetal viability and development, we investigated the impact of nicotine pouch exposure on pregnant Drosophila melanogaster. Drosophila melanogaster serves as an ideal model organism for studying nicotine toxicity due to its genetic tractability, short life cycle, and similarities in fundamental biological processes to humans. In addition, the development of embryos externally allows for precise observation of how maternal nicotine exposure affects embryo development and morphology at early stages through adulthood. We conducted lethal dose assays to assess ZYN effects on viability. The effects on fetal development were assessed by exposing virgin female flies via ZYN product (ZYN powder mixed in fly food) before mating. Then, we monitored embryos emergence time and sex ratio on ZYN products and controlled food. Additionally, physical deformities are measured in surviving adult flies post-exposure. This study will provide new insights into physiological and developmental effects of nicotine use through ZYN products, contributing to an understanding of health complications associated with modern nicotine use.

705T Transcriptional regulation of Bithorax Complex genes defines adipocyte heterogeneity in *Drosophila*

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The Bithorax complex (BX-C) homeobox proteins are essential for specifying segmental identities along the anterior-posterior axis during *Drosophila* embryogenesis and for preserving these spatial regulatory programs to sustain segmental organization and tissue-specific spatial identities throughout post-embryonic development. We have previously shown that two *BX-C* genes, *abdominal A* (*abd-A*) and *Abdominal B* (*Abd-B*), define regional differences in larval fat body, with abdominal adipocytes exhibiting distinct characteristics compared to thoracic adipocytes. These differences are further enhanced by active Wnt/Wingless signaling. However, the mechanism underlying the region-specific expression of *abd-A* and *Abd-B* in the fat body remains poorly understood. In this study, we identified cis-regulatory elements and transcription factors that control *abd-A* and *Abd-B* expression in abdominal, but not thoracic, adipocytes. Promoter bashing of the *Abd-B* locus revealed a 627bp fragment responsible for its adipocyte-specific activity. Yeast one-hybrid screening using this fragment, followed by *in vivo* genetic analyses, uncovered several novel regulators: Lola (Longitudinals lacking), Lolal (Lola like), and Cg (Combgap) act as repressors, while Prg (Piragua) and Svp (Seven up) function as activators of *Abd-B* expression. Moreover, depletion of Lola/Lolal expanded active Wnt-induced adipocyte defects from the abdominal to the thoracic region, further supporting their role in region-specific gene regulation. In addition, Lolal represses the expression of both *Abd-B* and *abd-A* in abdominal adipocytes. Together, these observations reveal a complex regulatory network that fine-tunes *BX-C* gene expression in postembryonic adipose tissue. This work reveals how enhancer elements and transcription factors establish region-specific adipocyte identities along the anterior-posterior axis, advancing our understanding of the developmental origins of adipocyte heterogeneity in *Drosophila*.

706T Validating evolutionarily conserved functions of human genes in the *Drosophila* melanogaster egg chamber system

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It is estimated that as many as 75% of human disease-causing genes have a *Drosophila* homolog, meaning many cancer-associated genes are evolutionarily conserved between the species. We conducted a screening of 64 human cancer-related genes which were previously identified as causing a phenotype in the *Drosophila* eye, indicating a conserved function, and overexpressed them in the *Drosophila* egg chamber's somatic cells. Because the *Drosophila* egg chamber is composed of all three epithelial cell types, as well as a transitional type called semi-mesenchymal, it serves as a great model to further investigate cell development and epithelial morphology. Additionally, the egg chamber is semi-transparent and goes through 14 defined stages of morphological change, making deviations from the norm easily identifiable. Given the conserved functions and signaling pathways between *Drosophila* and human genes, we hypothesized that multiple of the 64 would play an oncogenic role in the egg chamber's somatic cells, leading to abnormal phenotypes. TJ-Gal4 was used to express UAS-human genes in all somatic egg chamber cells, and the Gal80ts system was used to selectively control expression timing. Egg chambers were stained with DAPI and Br, a downstream target of Notch, ecdysone and JAK/STAT signaling, and imaged with confocal microscopy. We observed phenotypes such as early- and late-stage degeneration or apoptosis, squamous cell accumulation, abnormal egg chamber shape and Broad expression defects. Our findings provide a foundation for further dissecting the molecular mechanisms and conserved signaling pathways underlying the oncogenic roles of these 64 human cancer-related genes.

707T Traffic Jam activates the *Flamenco* piRNA cluster locus and the Piwi pathway to ensure transposon silencing and *Drosophila* fertility

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Flamenco (Flam) is a prominent piRNA locus expressed in *Drosophila* ovarian follicle cells that silences *gypsy/mdg4* transposons to ensure female fertility. Promoter-bashing reporter assays in OSS cells uncover compact enhancer sequences within *Flam*. We confirm the enhancer sequence relevance in vivo with *Drosophila Flam* deletion mutants that compromise *Flam* piRNA levels and female fertility. Proteomic analysis of proteins associated with *Flam* enhancer sequences discover the transcription factor Traffic Jam (TJ). *Tj* knockdowns in OSS cells cause a decrease in *Flam* transcripts, *Flam* piRNAs, and multiple Piwi pathway genes. TJ ChIP-seq analysis confirms TJ binding at enhancer sequences deleted in our distinct *Flam* mutants. TJ also binds multiple Piwi pathway gene enhancers and Long Terminal Repeats of transposons that decrease in expression after *Tj* knockdowns. TJ plays an integral role in the on-going arms race between selfish transposons and their suppression by the host Piwi pathway and *Flam* piRNA locus.

708T Defining the contributions of transcription factor-RNA interactions to coregulated gene expression in dosage compensation.

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Coordinated gene expression relies on precise regulatory mechanisms to function efficiently, and disruption of these highly intricate coregulatory mechanisms can lead to devastating diseases and developmental outcomes. Despite the extensive research done on gene regulation, the mechanisms involving how transcription factors (TFs) interact with long non-coding RNAs (lncRNAs) to facilitate coregulated gene expression remain unclear. To address this gap in knowledge, I am investigating how TFs and lncRNAs work in combination to achieve precisely orchestrated gene expression by examining coordinated gene regulation during *Drosophila* dosage compensation (DC). DC involves a conserved upregulatory mechanism where both TFs and lncRNAs function together to coregulate genes on the X-chromosome. In *Drosophila*, DC is mediated by the X-specific MSL (male-specific lethal) complex, composed of five proteins and one of two functionally redundant *roX* (RNA on the X) lncRNAs. The MSL complex interacts with X-linked chromatin via the genome-wide GA-binding pioneer TF, CLAMP (Chromatin Linked Adaptor for MSL Proteins). The MSL complex nucleates at X-linked CLAMP binding GA-rich elements, then spreads and deposits H4K16ac for the upregulation of ~1000 genes on the male X-chromosome. CLAMP and *roX2* are necessary but not sufficient on their own for X-specificity; therefore, neither explains the specificity of the MSL complex as a whole. Considering these findings, I hypothesize that the interaction between CLAMP and *roX2* promotes X-specificity and that RNA-factor interactions are necessary to accomplish X-linked gene upregulation in DC. To explore the impact of the CLAMP-*roX2* interaction, we developed endogenous CLAMP mutants where the predicted *roX2* interaction domain is disrupted. Using these mutants, I performed RNA-seq to measure changes in X-linked and autosomal gene expression in both female and male third-instar larval wing discs and brains. I also performed CUT&Tag in these tissues to assess both CLAMP occupancy and H4K16ac deposition as a readout for efficient DC. Together, these experiments will define how the CLAMP-*roX2* interaction affects DC in cell-cycling and specialized tissues, as well as provide insights into the overall impact of TF-lncRNA interactions on coregulated gene expression.

709T Global map of Bicoid properties reveals cooperative DNA binding is enhanced by nucleosome competition

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The mechanism by which target genes interpret the information provided by transcription factor (TF) concentrations remains a fundamental question in gene regulation. Recent advances suggest that TFs form DNA-bound hubs, which aid transcriptional regulation, as well as dynamic mobile clusters. As an example, Bicoid (Bcd), which forms an exponential-like gradient to pattern the anterior-posterior (AP) axis of the blastoderm stage embryo, has been shown to exhibit both transcriptional hubs and mobile clusters in the nucleus—populations that have been posited to enhance the transcriptional activity of Bcd. Additionally, it has been proposed that cooperative binding of Bcd to DNA can explain the sharpness in target gene expression. To understand how the Bcd concentration gradient conveys positional information to target genes by the proposed mechanism of cooperative DNA binding despite the barrier posed by nucleosomes, bulk-level *in vivo* investigation of populations essential for gene regulation is required. However, the mechanisms connecting the bulk nuclear concentration of Bcd to the formation and spatiotemporal distribution of DNA-bound Bcd and hubs remain largely uninvestigated.

To address this gap in knowledge, we used Raster Image Correlation Spectroscopy (RICS), a type of scanning Fluorescence Correlation Spectroscopy, in live embryos to quantify the absolute concentrations of three sub-populations of Bcd: (1) freely diffusing, (2) DNA-bound (including monomers and hubs), and (3) slowly-moving clusters. Each of these subpopulations has distinct diffusivities and binding properties, allowing us to quantify the spatiotemporal dynamics of these disparate populations along the AP axis from nuclear cycles 10 to 14. We also found that, unlike Dorsal, another blastoderm patterning factor, the DNA-binding of Bcd is driven mainly by its bulk concentration, as reflected in an almost linear dose/response relationship between the concentrations of free Bcd and the DNA-bound population. The shape of the dose/response relationship indicates cooperative binding that can be explained by a mathematical model of nucleosome-mediated cooperativity between Bcd molecules. In this model, Bcd at high concentration can compete with nucleosomes leading to cooperative DNA binding and cooperative eviction of nucleosomes. These results enhance our understanding of the transcriptional interpretation of the positional information provided by the Bcd concentration gradient on a bulk-level.

710T Angel Wing: a class of alternative splicing regulators in animals

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Alternative splicing (AS) regulates the diversity and level of the proteome. The specificity in AS is in turn regulated by RNA binding proteins, but our understanding of how they act is far from complete. Here we identify the Angel wing (Anw) protein, previously CG10948, as a novel AS regulator. Loss of Anw in *Drosophila* disrupts splicing in muscle genes and subsequently muscle function. Based on a mini-gene assay in which Anw and its RNA targets are co-expressed in cultured cells, we demonstrated orthologous splicing regulation of the mini-gene transcripts, interaction between Anw and its RNA targets, and a remarkable functional conservation among Anw homologs. Anw forms nuclear foci, and genetic ablation of Anw domains suggests that maintaining distinctive features of these foci is important for its function. The evolution of Anw is dynamic with gene gains and losses, but as others suggested, preserves a cross-phyla “ultra conserved element” as an alternative exon that potentially regulates its own level by non-sense mediated mRNA decay. As the human *anw* homolog is a candidate gene for myasthenia gravis, our work suggests a mechanism for cellular dysfunction in this disease.

711T Use of a *Drosophila* continuous mesodermal/muscle cell line to study *Hox* regulation in cardiac development

Grace Martin, Sumaiya Islam, Sayeed Rayhan, Kristopher Schwab Biology, Indiana State University

The *Drosophila* embryonic heart tube (dorsal vessel) is an excellent experimental system to study the molecular and genetic mechanisms regulating heart development. Our laboratory has identified an important role for the *trithorax* Group (*trxG*) and *Polycomb* Group (*PcG*) genes controlling heart development. The *trxG* and *PcG* genes encode conserved chromatin regulatory proteins that antagonize one another to control developmental genes, such as the *Homeotic (Hox*) genes. *Hox* gene activity partially controls the regional patterning of the developing heart in both insects and mammals. However, the precise regulation of cardiac *Hox* expression during heart development has yet investigated. These investigations are hampered by the small number of cardiac cells and microscopic anatomy of the heart tube which limits the feasibility important experimental techniques used to study the mechanisms of chromatin and transcriptional regulation without the use of technically challenging procedures to obtain sufficient quantities of purified cell populations.

Continuous *Drosophila* mesodermal/muscle cell lines established from developing embryos may offer an opportunity to study these mechanisms. Several cell lines developed by the Simcox laboratory were generated via the expression of *Ras*^{V12} under the control of the *24B-Gal4 (held out wings [how])* driving expression within the mesoderm, cardiac muscle, and somatic muscle during embryonic development. The 24B>Ras-attP-G1-CloneG1, hence referred to as the G1 cell line, was selected for further investigation from the three cell lines offered by the Drosophila Genomic Resource Center. The G1 cell line expresses several mesodermal and muscle lineage markers confirming its derivation from the mesodermal lineage. While the mesodermal-derived S2 and Kc cell lines are devoid of *Hox* gene expression, the G1 cell line expresses the *Hox* gene *abdominal-A (abd-A)* allowing the experimental study of *Hox* regulation by *trxG* and *PcG* genes via standard genetic modification techniques, such as dsRNA-induced RNAi knockdown, providing the opportunity to study *Hox* gene regulation. Our preliminary studies demonstrates that the G1 cell line is amenable to gene expression knockdown via the use of standard dsRNA techniques. This result demonstrates the potential use of this cell line to study mesodermal-specific chromatin and transcriptional regulatory mechanisms.

712T *trithorax (trx)* and *trithorax group (trxG)* regulation of *Hox* gene expression and anterior-posterior patterning within the *Drosophila* embryonic heart tube.

Sumaiya Islam, Sayeed Rayhan, Adam Farmer, Grace Martin, Shaad M. Ahmad, Kristopher Schwab Biology, Indiana State University

The *trx* and *trxG* genes encode conserved chromatin regulatory proteins that positively control developmental genes, such as the *Homeotic (Hox*) genes. *Hox* genes control the regional patterning of the developing heart in both insects and mammals. However, the precise regulation of cardiac *Hox* expression and activity has yet to be explored. Our previous findings identify two roles for *trx* regulation of cardiac patterning via Hox activity: 1) heart-proper region development requires Abd-A activity within the posterior cardiac hemisegments and 2) the development of the anterior-most two Svp CC pairs requires Antp activity at the boundary of the anterior and posterior aorta.

To further define cardiac *trx* and *trxG* activity, we investigated additional hypomorphic *trx* and *trxG* mutants for *Hox* expression and aberrant morphology within the embryonic heart. Two hypomorphic *trx* strains that disrupt the C-terminal SET-domain encoding H3K4 HMT activity were evaluated for Hox expression and patterning defects. The trx^{B21} allele truncates the Trx protein prior to the SET-domain, while the trx^{B27} allele encodes a point mutation located near the SET-domain substrate binding site. Unlike the amorphic trx^{E2} mutant, the trx^{B21} and trx^{B27} homozygous mutants have normal posterior *abd-A* expression and heart-proper development indicating that H3K4 methylation activity is dispensable. However, trx^{B21}/trx^{E2} and trx^{B27}/trx^{E2} transheterozygous mutants possess a moderate to severe reduction of *abd-A* expression and abnormal heart-proper morphology which result suggests sufficient Trx non-catalytic dosage is necessary for *abd-A* expression and cardiac patterning. Additionally, the trx^{B21} and trx^{B21}/trx^{E2} transheterozygous embryo showed reduced A2 *Svp* cell number and altered *Antp* expression within the anterior-posterior boundary. Together, these results indicate that while the catalytic activity of the SET domain is dispensable for maintaining posterior *abd-A* expression, it plays a key role in regulating Antp activity and proper Svp patterning within the anterior embryonic heart tube.

Finally, several *trxG* genes were evaluated for Hox expression and cardiac defects. Although cardiac cell division defects were observed for several mutants, the normal *abd-A* expression and cardiac patterning that the activity of these genes may be dispensable for cardiac patterning. Overall, these results indicate that *trxG* genes have unique and diverse roles in regulating heart development.

713T *Polycomb (Pc)* and *Pc Group (PcG)* genes repress *trithorax (trx)*-mediated *Hox* expression and patterning within the *Drosophila* embryonic heart tube

Sayeed Rayhan, Sumaiya Islam, Grace Martin, Adam Farmer, Shaad M. Ahmad, Kristopher Schwab Biology, Indiana State University

PcG genes repress the expression of important developmental genes, such as the *Hox* transcription factors, and antagonize the transcriptional activity of *TrxG* genes. Recently, we identified *trx* as a positive regulator of *Hox* expression and anterior-posterior patterning within the *Drosophila* embryonic heart tube. The wildtype heart tube is organized into two distinct anatomical regions: the narrow aorta and the wide posterior heart-proper regions. *trx* maintains the expression of *abdominal-A (abd-A)* in the posterior heart tube which is necessary for heart-proper patterning, while *Pc* antagonizes *trx* activity by repressing *abd-A* expression within the anterior heart tube maintaining the aorta fate.

To determine the precise roles for *trx* and *Pc* in *Drosophila* heart development, a *trx, Pc* recombinant strain possessing amorphic alleles of both genes was generated and crossed to generate homozygous *trx*, *Pc* mutant embryos. Remarkably, the homozygous *trx, Pc* null embryos recapitulated the homozygous *trx* mutant phenotype consisting of the absence of *abd-A* expression and heart-proper patterning. This data suggests that *trx*-mediated transcriptional activation and maintenance is absolutely required for cardiac *Hox* activity and patterning, while *Pc* activity mediates repression of cardiac *Hox* expression to prevent abnormal heart patterning.

Additionally, other PcG genes may be necessary for proper cardiac *Hox* activity. *PcG*-mediated repression of gene expression is mediated by several complexes, including the Polycomb-repressive complex 1 (PRC1), PRC2, and other noncanonical repressive complexes. Our screen of *PRC1* mutant strains for aberrant cardiac *Hox* expression and patterning similar to the *Pc* mutant phenotype has successfully identified the *Sex combs on midleg (Scm*) and *Polyhomeotic* (*Ph*) as key PRC1 members responsible for repressing anterior cardiac *abd-A* expression. In contrast, our screen of PRC2 genes indicate that these genes may be dispensable for *abd-A* repression since *abd-A* expression remains restricted to the posterior heart-proper region similar to the wildtype phenotype. These preliminary findings identify distinct roles of *PcG* regulation in embryonic heart development suggesting that PRC1 activity, rather than PRC2, is necessary for cardiac *Hox* repression and normal heart tube patterning.

714T Transcriptional Regulation of Stochastic Cell Fate Specification in the *Drosophila* eye

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Cell fate specification is generally considered to be a tightly controlled process, guided by lineage and signaling mechanisms that lead to reproducible outcomes. However, some cell fate decisions rely on stochastic mechanisms in which cells randomly choose between two or more fates with a set probability. Stochastic cell fate specification arises from inherent molecular noise in gene expression. Little is known about how variability in gene expression drives fate decisions during metazoan development.

Here, we investigate a stochastic, binary cell fate decision in the developing visual system of Drosophila melanogaster. The fly eye consists of a mosaic of two R7 photoreceptor subtypes that are defined by their expression of light-detecting Rhodopsin 3 (Rh3) or Rhodopsin 4 (Rh4) proteins. The choice to express Rh3 or Rh4 is controlled by a transcription factor called Spineless (Ss), which is randomly expressed in 67% of R7 photoreceptors (SsON R7s).

ss is expressed in two distinct phases during eye development. The early and late expression of *ss* is regulated by two enhancers (the “early” enhancer and “late” enhancer). Early expression is noisy, with R7 precursor cells expressing *ss* at different levels. Interestingly, about 67% of precursors express *ss* at high levels while 33% express at low levels, which corresponds to the proportion of terminally differentiated SsON/ SsOFF R7s. Manipulating DNA elements (silencers and enhancers) and the transcriptional repressor Klu changes the level of early *ss* expression and the proportion of SsON/SsOFF R7s.

From these data, I hypothesized that variable expression of *ss* early sets the ratio of SsON/SsOFF R7s late. Based on this model, I am investigating how regulation of transcription produces variable *ss* expression to determine the stochastic R7 fate choice. I am using the MS2/MCP system to monitor and characterize *ss* transcription and regulation in live developing cells in conjunction with our custom-made spot detection software that quantifies and tracks transcription in individual *ss* expressing cells over time. These experiments will establish the link between early *ss* transcription and terminal fate by monitoring endogenous expression from the precursor stage to terminal differentiation. Additionally, I am exploring how manipulating *cis* and *trans* regulatory elements affects *ss* transcription in the precursors and the ultimate stochastic fate choice in R7s. This work will uncover generalizable mechanisms that regulate stochastic cell fate specification.

715T The phenotypic nonspecificity of cell to cell signalling in *Drosophila melanogaster*

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The redundancy of transcription factor (TF) function results in the frequent phenomenon of phenotypic nonspecificity where the lack of the resident TFa expressed from the *TFa* locus is complemented by the expression of a non-resident TFb. As most cell to cell signal transduction pathways end with a change in the activity of a TF that alters a cell’s pattern of gene expression, we hypothesize that these terminal TFs would also be redundant. One expectation of this hypothesis is the phenotype due to the lack of a resident signalling factor, SFa, expressed from the *SFa* locus, being rescued by the expression of a non-resident SFb due to the phenotypic nonspecificity of terminal TF function of signalling pathways. We tested this expectation by screening a *decapentaplegic* (*dpp*) hypomorphic mutant combination (dpp^{d5}/dpp^{d6}) for rescue by the expression of 39 non-resident SFs using *UAS* constructs driven by the *dppGAL4* driver. We identified 3 partial rescues of wing development by expression of either Hedgehog (HH), Semaphorin 1a (SEMA1A) or Wnt oncogene analog 2 (WNT2). The expression of WNT2 partially rescues both male and female wing development but SEMA1A only rescues female wing development. If the SF does not play a role in wing development, it is then likely substituting for/complementing DPP function. HH is required for wing development, but wings developed normally in *Wnt2* null mutants, and normally when *SEMA1A* mRNA was knocked down using RNAi. This suggests that we found two examples of the expectation of the hypothesis that terminal TFs are functionally redundant. However, this does not prove the hypothesis as the redundancy of the terminal TFs was not assessed directly. We screened only 39 of the 107 presumed non-resident Drosophila SFs, and therefore, there is the potential for additional examples (1-4) of rescue of the dpp phenotype by the expression of a non-resident SF.

716T Multiple isoforms of CELF homolog Bru1 are required for indirect flight muscle development in *Drosophila*

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CELF family members are key regulators of RNA processing in muscle. CELF proteins direct alternative splicing and the expression of structural and signaling proteins that determine fiber-type specific contractile properties and sarcomere morphology. Misregulation of RNA processing and the alternative splicing associated with increased CELF activity triggers the neuromuscular disease Myotonic Dystrophy Type I (DM1), and the muscle weakness and atrophy observed in patients illustrate that CELF proteins are crucial to promote muscle development and homeostasis. Interestingly, CELF proteins are themselves alternatively spliced, but little is known about their functional relevance in muscle. We have previously shown that CELF homolog Bruno1 (Bru1) is required for indirect flight muscle (IFM) development in *Drosophila melanogaster*. Bru1 promotes cytoskeletal reorganization necessary to assemble myofibrils and during muscle maturation is required for production of IFM-specific splice isoforms that support sarcomere growth and myosin contractility. Here we report that multiple Bru1 isoforms are required during IFM myogenesis. IFMs express three distinct Bru1 isoforms. During myogenesis there is a transition from a short, two RRM-containing isoform D, to a longer three RRM-containing isoform. As compared to pan-isoform $bru1^{del3}$ mutants where sarcomere development is defective, when the short isoform Bru1-isoD is present in isoform-specific mutant $bru1^{del4}$, IFM sarcomeres develop normally but hypercontract in the adult. Interestingly, the majority of misregulated exons are affected in both *bru1* mutants, but the fold-change is much larger in the pan-isoform mutant $bru1^{del3}$. IFM are also more sensitive to overexpression of Bru1-isoA than Bru1-isoD. Strikingly, sarcomere structure and muscle function can be rescued only when Bru1-isoD is present with expression of the long, three-RRM Bru1 isoform from around 56 h APF driven by Fln-Gal4. We conclude that a short, two-RRM Bru1 isoform is sufficient early in myogenesis, but muscle maturation requires expression of a long, three-RRM isoform. We propose a model where the three-RRM isoform has a higher splicing activity which is required during later phases of myogenesis. Our data suggest that muscles utilize different CELF isoforms to modulate the level of CELF activity during developmental transitions to fine-tune the acquisition of mature, fiber-type specific properties.

717T Molecular mechanisms underlying neural-specific splicing and 3'UTRs

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The conserved family of ELAV/Hu RNA binding proteins (RBPs) regulates global mRNA processing in neurons. They expand transcriptome diversity by inducing alternative splicing (AS) of internal exons and alternative polyadenylation (APA) to lengthen 3'UTRs. Not only are ELAV/Hu RBPs necessary for both programs of neuronal isoforms, ectopic assays show that they are sufficient to confer neural-like splicing and 3'UTR isoforms in a non-neural setting. Several lines of evidence suggest that ELAV/Hu RBPs act locally to modulate splicing choices and suppress proximal cleavage and polyadenylation sites, via cognate U-rich motifs near sites of regulated mRNA processing. However, available data do not rule out whether ELAV/Hu RBPs might act via more distant regions of target transcripts, or to what extent some genes are indirectly affected via a regulatory cascade. Since AS and APA are co-transcriptional events, ELAV/Hu could act co-transcriptionally with either promoters, spliceosome complex and/or cleavage and polyadenylation (CPA) factors. Alternatively, ELAV/Hu factors might also alter isoforms by post-transcriptional effects, such as by stabilizing neural isoforms. To distinguish these possibilities, we generated stable cell lines that inducibly express wildtype and mutant ELAV/Hu RBPs. We are employing both long-read direct RNA-sequencing (DRS) and dynamic 4sU-nascent sequencing to characterize the effects of ectopic ELAV/Hu RBPs on native transcripts with temporal precision and single molecule connectivity. This allows to assess the contributions of transcription, processing, and stability to the alternative transcriptome induced by ELAV/Hu RBPs. We are also using reporter assays to test if cis-acting binding sites mediate direct regulation of splicing and 3' UTR targets of ELAV/Hu RBPs.

718T Direct and indirect regulation of target gene expression by JAK/STAT signaling during epithelial patterning in Drosophila

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A relatively small number of signaling pathways drive a wide range of developmental decisions involving distinct target genes. This versatility points to a role for cellular context in generating diversity in signaling outcomes, but how targets are chosen is not well understood.

Context-dependent signaling outcomes occur during patterning of the Drosophila follicular epithelium, where activation of epidermal growth factor receptor (EGFR) signaling by its localized ligand Gurken (Grk) induces distinct cell fates depending on its location. Posterior follicle cells respond to Grk by expressing the T-box transcription factors Midline (Mid) and H15, while anterior cells respond by expressing the homeodomain transcription factor Mirror (Mirr). The choice between these alternative EGFR targets depends on input from additional localized ligands. At the posterior, activation of JAK/STAT signaling by the ligand Upd is required for Mid/H15 expression, while at the anterior activation of BMP signaling by the ligand Dpp is required for Mirr expression. This choice is reinforced by mutual repression between Midline/H15 and Mirror, and through the ability of both Dpp and Upd to repress their "opposing" target (i.e. *mid/H15* and *mirr*, respectively).

The integration of these multiple localized positional cues suggests that crosstalk between these signaling pathways generates mutually exclusive EGFR signaling outcomes, but at what level this occurs remains unclear. To approach this question, we identified genomic regions from the *mid* and *mirr* loci that, in a transgenic reporter, reproduce the expression patterns and regulation of the endogenous *mid* and *mirr* genes. Focusing here on the posterior follicle cells, where Upd positively regulates mid but represses *mirr*, we use these reporters to study this opposing regulation of these two downstream targets.

Here we show that *mid* reporter expression does not require putative STAT92E binding sites, suggesting that its positive regulation Upd is indirect. Indeed, we find that Upd positively regulates *mid* by increasing levels of the EGFR effector Pointed (Pnt), suggesting crosstalk in which Upd ensures levels of Pnt adequate to allow Grk to induce mid expression in the posterior region of the epithelium. Putative STAT92E binding sites in a *pnt* transcriptional reporter are required for expression suggesting that, for this target, positive regulation by Upd is direct. Interestingly, Upd-mediated regulation of the *mirr* reporter in these cells also requires intact STAT92E binding sites. Upd therefore influences expression of Grk target genes through both direct and indirect mechanisms.

719T Mito-Nuclear Signaling in Mitochondrial Biogenesis and Cellular Stress Responses

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The process by which cells sense and respond to mitochondrial abundance in order to meet developmental and tissue-specific energy demands remains largely unknown. We employed a *Drosophila* model expressing MitoXhoI, a mitochondrially targeted restriction enzyme that depletes mtDNA, to investigate the nuclear response to mtDNA deficiency. MitoXhoI expression led to compensatory upregulation of the nuclear-encoded ETC subunit succinate dehydrogenase A (SDHA), as demonstrated through quantitative imaging of an endogenous tagged SDHA-mNeonGreen fusion protein in *Drosophila* midgut monolayer epithelium. SDHA upregulation appears to be a transcriptional response, as SDHA mRNA levels were also elevated in MitoXhoI-expressing clones. CG15011, the fly homolog of the human transcription factor NFXL1, and a top-layer regulator in a transcriptional network governing mitochondrial biogenesis, was essential for this response, as CG15011-RNAi completely abolished MitoXhoI-induced SDHA overexpression. CG15011 has a single transmembrane helix. Full-length GFP-tagged CG15011 was ER-localized, while a truncated protein lacking a transmembrane domain was exclusively nuclear-localized. Notably, overexpression of this truncated variant was sufficient to upregulate SDHA expression, suggesting that CG15011, an ER-anchored transcription factor, might be cleaved and translocated from the ER to the nucleus under mtDNA deficiency to activate nuclear-encoded mitochondrial genes. We are currently using the midgut system to assess the role of numerous candidate ER and nuclear-encoded mitochondrial proteases in this process. Thus far, Afg3l2, a mitochondrial mAAA protease, as well as calpains, calcium-activated proteases associated with the ER, have emerged as the strongest hits, for which RNAi knockdown markedly diminished compensatory SDHA upregulation. Furthermore, RNAi knockdown of calpains or Afg3l2 inhibited MitoXhoI-induced CG15011 cleavage in cultured cells. We propose that Afg3l2 and calpains may regulate CG15011 through mitochondria-ER calcium flux-- mtDNA deficiency impairs mitochondrial Ca^{2+} uptake, leading to its accumulation at the ER-mitochondria interface, where it activates ER calpains which cleave CG15011. Our work reveals a novel cellular stress response induced by mtDNA deficiency to boost mitochondrial biogenesis. Ongoing work aims to further elucidate the nature of this pathway and explore the potential for a conserved mechanism in mammals.

720F Identification of direct Bruno 1 RNA targets and protein interactors supports Bruno 1 isoform-specific function in D. melanogaster indirect flight muscle (IFM)

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CUGBP Elav-Like Factor (CELF) family proteins are important fiber-type specific splicing regulators in muscle and are implicated in diseases such as Myotonic Dystrophy Type 1 (DM1). While vertebrate genomes encode six CELF family members which are partially redundant, a single CELF homolog Bruno1 (Bru1, aret) has been shown to be a key RNA regulator during development of the indirect flight muscles (IFM) in *D. melanogaster*. Bru1 has been shown to regulate cytoskeletal rearrangements supporting myofibrillogenesis, promote a maturation switch in the splicing of IFM-specific isoforms, and influence sarcomere growth and myosin contractility. Although Bru1 and vertebrate CELF proteins are themselves alternatively spliced, little is known about isoform-specific CELF function. Our transcriptomic and Western blot data demonstrate that multiple Bru1 protein isoforms (A, I, and D) are expressed in IFM and that isoform-specific mutants have different phenotypic severity, which motivates investigation into Bru1 isoform-specific functions. To identify Bru1 isoform-specific direct RNA targets, we performed enhanced UV crosslinking and immunoprecipitation (eCLIP) from fly thoraces. UAS constructs expressing individual GFP-tagged Bru1 isoforms were driven selectively in IFM with Flightin-Gal4 and compared to both control UAS-GFP and endogenously tagged Bru1-eGFP that labels all Bru1 isoforms. We identified hundreds of high-confidence peaks that identify direct RNA targets of Bru1 in IFM, notably including sarcomere protein transcripts. We further tested if Bru1 isoforms A and D have distinct protein interactors using immunoprecipitation-mass spectrometry. Our data reveal nuclear as well as cytoplasmic proteins that interact with Bru1 in IFM, consistent with localization of Bru1 to the nucleus as well as cytoplasmic granules during IFM myogenesis. Our data are consistent with a model where Bru1 isoforms have different levels of splicing activity and suggest overlap in direct RNA targets and Bru1 isoform function. Future experiments will validate CLIP results with splicing reporters and use structure-function analysis to confirm functional motifs and binding domains. These data provide new insight into the regulation of RNA-binding protein activity through alternative splicing and expand our understanding of CELF protein function in the RNA regulatory network in muscle.

721F Identification of a candidate akirin enhancer sequence

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Akirin, a small nuclear protein with conserved function across eukaryotes, is a critical determinant in the development of functional, robust cardiac and skeletal patterning and musculature. Akirin serves as a transcription cofactor by acting as a link between transcription factors such as Twist. Akirin uses chromatin remodeling complexes to ensure that Twist functions appropriately during transcription. If Akirin function is impaired, the resulting muscle patterning and structure is greatly impacted. Despite this importance, the biology of *akirin*, and the regulation of this critical genetic locus remains unknown. We have identified a number of highly conserved DNA sequences among closely related *Drosophilid* species as likely candidate control elements for the *akirin* locus. We are currently evaluating these candidate sequences through a variety of *in vivo* and *in vitro* techniques, both in live *Drosophila* embryos, as well as in cultured S2 cells. We have determined that several of these sequences do indeed have enhancer activity *in vivo* and are analyzing their embryonic expression patterns through reporter techniques. We are hopeful that these sequences will shed light on the regulation of this enigmatic yet critical locus.

722F Uncovering Sage's Collaborative Role in Salivary Gland Gene Regulation

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Transcription factor (TF) networks are complex systems that require coordination among multiple TFs to drive organogenesis and tissue-specific gene expression programs. Within the model system of the Drosophila embryonic/larval salivary gland, the basic helix-loop-helix TF known as Sage interacts with a TF that is more broadly expressed – Fork head (Fkh) - to regulate expression of salivary gland genes encoding secretory cargo proteins, the enzymes that modify these cargos, pro-apoptotic factors, and morphogenetic proteins. To elucidate the mechanisms of TF interactions and gene regulation, we are utilizing the embryonic salivary gland to discover how these two TFs interact with each other and with their endogenous target genes to establish and maintain salivary gland-specific functions. Using tissue-specific ChIP-seq, we are mapping the complete repertoire of genes bound by each TF in the SG, and through microarray and single-cell RNA sequencing (scRNA-seq), we are identifying the salivary gland target genes affected by their loss. The combination of these two techniques allows us to build a complete interaction map for Sage and Fkh. By studying the correlations between TF binding site configurations and TF-dependent gene expression, we will develop and test models for coordinate gene regulation in the context of a functional organ. Understanding the molecular details of how Sage and Fkh coordinate binding and gene regulation will illuminate how tissue-specific gene expression is achieved in this system and will provide paradigms for how transcription factors network in more complex organisms.

723F Motif-driven nucleosome positioning represses chromatin accessibility

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Nucleosomes serve as a general repressor of transcription in the eukaryotic cell and thus have to be repositioned or removed for transcriptional regulation to occur. However, how nucleosomes are regulated by transcription factors genome-wide and how this affects gene regulation is not well understood. Here we used a two-model deep learning strategy to systematically predict how transcription factor binding motifs affect MNase-seq nucleosome maps in the early Drosophila embryo. This revealed that nucleosome depletion and positioning are separable effects with sometimes opposing effects on gene regulation. Nucleosome depletion is driven by pioneer transcription factors like Zelda and promotes chromatin accessibility and transcriptional activation. In contrast, nucleosome positioning, driven by architectural transcription factors like Su(Hw) over ranges of up to a kilobase away, frequently has a repressive effect on transcription. The repressive effect is dominant over Zelda's ability to bind its motif and make chromatin accessible, suggesting that nucleosome positioning can override the action of pioneer transcription factors. Collectively, our results suggest that nucleosomes can be effective repressors when well positioned by transcription factors and that the rules by which transcription factor motifs affect nucleosomes can be uncovered with deep learning sequence models.

724F Mapping Stop Codon Readthrough in the Adult *Drosophila* Brain at Single-Cell Resolution

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Under specific conditions, ribosomes recognize stop codons as sense codons and continue translating downstream regions of mRNA transcripts. This phenomenon, known as **stop codon readthrough (SCR)**, enhances proteomic diversity and function in organisms ranging from bacteria and viruses to yeast and mammals. In *Drosophila*, over 400 genes have been documented to undergo SCR. Notably, *Drosophila* SCR efficiency varies among tissues and cell types, with neurons in the central nervous system exhibiting disproportionately high readthrough efficiency. Nonetheless, the cell-type specificity and spatial distribution of SCR remain poorly understood. To overcome these limitations and elucidate SCR patterns at the single-cell level, we are using **STARmap** (Spatially-resolved Transcript Amplicon Readout mapping) and **RIBOmap** (ribosome-bound mRNA mapping) techniques—originally developed in Xiao Wang's lab—to visualize SCR events in the adult *Drosophila* brain. We are designing RIBOmap probes specific for coding regions 3' of stop codons to detect ribosome-coupled mRNAs, thereby revealing active translation and SCR events. We successfully detected SCR signals for multiple genes in the adult *Drosophila* brain, in cryosections and whole brains. Probes produced highly specific SCR signals compared to negative controls. As expected, STARmap captured more signals, while RIBOmap revealed a subset representing active translation in the same tissue. To our knowledge, this is the first successful implementation of these techniques in the adult *Drosophila* brain. Continued protocol optimization and benchmarking against endogenous labeling data will further validate these results. Ultimately, we aim to build a spatially resolved atlas of SCR activity across neuronal cell types and uncover new insights into the functional roles and regulatory mechanisms of SCR in the *Drosophila* brain.

725F Exploring histone locus body initiation mechanisms throughout development

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Histone genes encode proteins required to organize DNA into chromosomes in all eukaryotes. In many metazoans, expression of these genes is regulated by the histone locus body (HLB), a nuclear body composed of various proteins and RNAs responsible for histone mRNA production and processing.

The HLB initiates at histone genes prior to zygotic genome activation (ZGA) and is thereafter maintained throughout the organism's life. However, it is unclear whether HLB initiation is restricted to embryogenesis. We explore the potential for HLB initiation in maturing cells using transgenics and immunofluorescence assays.

In D. melanogaster, replication-dependent histone genes are clustered in a repetitive array, which is repeated about 100 times at a single locus. HLB initiation at this locus supports embryonic development and is vital to the organism's survival. By inserting an engineered histone transgene into the genome, we are able to manipulate a secondary locus to delay HLB initiation without organismal fatality. Our engineered histone array contains a large ~3kb reporter sequence that interrupts elements critical for initiation in the H3/H4 promoter. The reporter is flanked by FRT sequences, allowing Flippase to inducibly create an uninterrupted, intact H3/H4 promoter after typical HLB initiation. Using DNA fluorescence in situ hybridization and immunohistochemistry, we test for HLB initiation at the transgene.

We expected that the HLB would be unable to initiate at the transgene prior to Flippase induction due to the large interruption. However, we found that the HLB is still able to initiate, indicating the initiation mechanism is more flexible than we expected. Following this surprising finding, we are developing new transgenic arrays to explore the initiation mechanism at the interrupted promoter. We also designed a new experimental transgene to further interrupt H3/H4 promoter.

We hypothesize that the importance of HLB initiation to organism survival is reflected in its flexibility and resistance to disruptions. Through exploration of HLB initiation throughout development, we hope to further explore the abilities of the HLB to initiate despite changes in the developmental environment, alluding to the latent potential of maturing cells to develop and change.

726F Single-Cell Chromatin Accessibility Landscape of the *Drosophila simulans* Ovary and Egg Reveals Regulatory Dynamics of Gene Expression and Transposon Activity

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Chromatin accessibility plays a fundamental role in regulating gene expression and preserving genome stability during germ cell development. In *Drosophila simulans*, the relationship between chromatin organization, transcriptional regulation, and transposon activity during oogenesis remains poorly characterized. This study aims to construct a high-resolution chromatin accessibility landscape of the *D. simulans* ovary and egg using single-cell ATAC sequencing (scATAC-seq). scATAC-seq, a state-of-the-art genomic technique designed to map chromatin accessibility at the single-cell level, presents unique analytical challenges due to limited sampling and data sparsity; however, it enables precise identification of open chromatin regions and active regulatory elements across heterogeneous cell populations. Ovarian and egg tissues will be dissected, and nuclei isolated for transposition and sequencing to capture accessible chromatin states across germline cell types. The resulting data will be processed through advanced bioinformatics analyses, including motif enrichment, clustering, and integration with transcriptomic datasets to reveal correlations between chromatin accessibility and gene expression. This integrative approach is expected to identify key transcription factors, regulatory networks, and transposon associated chromatin features defining distinct stages of germline differentiation. Ultimately, the study will provide comprehensive insights into how chromatin accessibility shapes gene expression dynamics and transposon regulation during oogenesis in *Drosophila simulans*, contributing to a broader understanding of genome regulation in the reproductive *Drosophila*.

727F Transcriptomic analysis of Drosophila models of Retinitis Pigmentosa reveal dysregulation of redox homeostasis and protein folding

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Retinitis pigmentosa (RP) is an inherited retinal degeneration (IRD) in which the rod photoreceptors die, followed by loss of cone photoreceptors. Mutations in at least 60 genes cause dominant RP including mutations in *SNRNP200*. The *SNRNP200* encodes an RNA helicase that is an essential component of the spliceosome that processes pre-mRNAs into mature mRNAs. Drosophila has a single orthologue of *SNRNP200*, *Brr2*, that has 86% similar and 74% identical amino acids. We refer to this orthologue as *Snrnp200*. There are currently limited treatments for RP. The goal of our study is to determine the molecular basis of *SNRNP200*-associated RP to inform on treatments. Towards this end, we developed a Drosophila model containing a point mutation in *Snrnp200* that causes human RP using CRISPR gene editing. This mutation results in dominant photoreceptor dysfunction associated with abnormal morphology and enlarged mitochondria at the cellular level. Expanding on this model, we expressed an RNAi against *Snrnp200* specifically in the developing eye using the Gal4/UAS system. This resulted in a rough eye phenotype that was partially rescued by treatment with the antioxidant N-acetyl cysteine, suggesting a role of oxidative stress in disease pathogenesis. To reveal the effects of *Snrnp200* mutations on the transcriptome, total RNA was isolated from adult heads and RNA-seq was performed. Differential gene expression and alternative splicing analyses were performed, comparing results among the *Snrnp200* models and controls. Over 2,000 differentially expressed genes with adjusted p-value threshold <0.05 were identified in both models. Pathway analysis revealed that both models exhibited similar dysregulation of RNA splicing, protein folding, mitochondrial function, and redox homeostasis, as well as down-regulation of retinal-specific genes, which is consistent with the mutant phenotypes. The *Snrnp200* knock-down model showed up-regulation of small nuclear RNAs, which are typically encoded in introns, suggesting intron retention. Supportive of this finding, splicing analysis revealed altered splice site selection and intron retention in both models. These data suggest that treatment with antioxidants and methods that restore protein homeostasis are avenues for therapy.

728F Genetic mechanisms underlying independently evolved gene expression patterns in distantly related species

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Highly specific gene expression patterns can evolve independently across distantly related species. However, whether these independently evolved similar gene expression patterns are the result of the change in the same enhancers and transcription factors are less elucidated. *bond*, a fatty acid elongase gene, is expressed in a highly specific pattern in the *Drosophila melanogaster* ejaculatory bulb (EB), and is involved in pheromone production in tissue. The same highly specific independently evolved in the distantly related *Scaptodrosophila lebanonensis*. In this study, we showed that *bond* expression in the *D. melanogaster* EB is regulated by a 285 bp enhancer and requires the transcription factor *Abdominal B (Abd-B)*. In *S. lebanonensis,* we showed that the enhancer driving bond expression in the EB is larger and do not did not require *Abd-B.* This suggests possible different evolutionary mechanisms underlying the evolution of this similar gene expression pattern across these distantly related species. We are currently investigating *cis*-regulatory changes and the transcription factors that underlie the independent evolution of this gene expression. These results suggest possible co-option of existing enhancer by evolving new transcription binding sites can alter gene regulation and underlie independently gained EB *bond* expression.

729F Understanding the role of metazoan histone H3 lysine-36 methylation in suppression of cryptic transcription

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Accurate transcription initiation is fundamental to cellular identity and genome integrity. Eukaryotic chromatin must allow productive elongation while simultaneously suppressing spurious initiation within gene bodies. In *S.cerevisiae*, methylation of histone H3 lysine-36 (H3K36me) by Set2 couples elongating RNA polymerase II (Pol II) to histone deacetylation through recruitment of the Rpd3S complex, thereby erasing promoter-like acetylation and preventing intragenic cryptic transcription. This elegant mechanism directly links transcription elongation to chromatin modification, ensuring that only bona fide promoters remain accessible for Pol II recruitment. Despite the conservation of Set2-mediated H3K36me across eukaryotes, previous studies cast doubt as to whether this anti-cryptic initiation function operates similarly in multicellular organisms.

Here, we address this question by integrating high-resolution nascent-transcription profiling (PRO-seq), steady-state RNA expression analysis (polyA-selected RNA-seq), and chromatin profiling (Cut&Run) to define how Set2 and H3K36me control transcriptional precision in the third-instar larval wing imaginal disc. This tissue contains relatively homogeneous populations of diploid proliferating epithelial cells that give rise to the adult wing, providing an ideal system to detect tissue-specific transcriptional phenotypes without the confounding heterogeneity of other, largely polyploid, tissues. We analyzed three different mutant lines (*Set2*Δ*, H3.2*^{K36R} *and H3.3*^{K36R}) along with their respective controls (*yw, H3.2*^{HWT} *and H3.3*^{Ctrl}).

Across all six genotypes genome-wide, PRO-seq accurately identified ~10,000 previously annotated transcription start sites (TSSs) along with >150,000 unannotated sites (uTSSs). These uTSSs were further classified as potential intragenic TSSs (piTSSs) located within gene bodies and potential extragenic TSSs (peTSSs) located outside annotated gene regions. Differential analysis using edgeR identified hundreds of peTSSs that were upregulated in *Set2* and *H3.2*^{K36R}relative to controls, whereas piTSSs that were differentially utilized were fewer in number. Overall, very few events were detected in the *H3.3*^{K36R} mutant. A multi-omic integration of PRO-seq and poly(A) RNA-seq identified 711 piTSSs upregulated in Set2 vs yw, and 210 piTSSs upregulated in K36R vs HWT, irrespective of the expression level of their host genes. Notably, many of these cryptic initiation events coincide with transposon de-repression arising from both extra- and intra-genic regions, indicating that H3K36 methylation safeguards genome integrity by preventing transposon activation. Collectively, our findings demonstrate that H3K36 methylation suppresses cryptic initiation primarily within extragenic regions of the genome, including expression of retrotransposons.

730F Inter-dependent SUMOylation uncovers novel interactions within the piRNA pathway

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The piRNA pathway is an essential and widely described mechanism for transposon silencing in Drosophila germline and somatic cells. This process, from piRNA cluster transcription to target suppression, involves multiple steps including piRNA maturation, modification, and transport. While many proteins in this complex chain of events are actively studied, a significant number of interactions are not fully understood.

Previously, it was shown that several piRNA pathway-related proteins are modified by SUMO (small ubiquitin-like modifier). While potential interactions between SpnE, Mael, and Piwi have been described, the hierarchy of this complex's assembly and the role of SUMOylation remain unknown. In our current study, we demonstrate interlinked SUMOylation events that point to the existence of a modified Mael-SpnE-Piwi complex. We found that Mael and SpnE, proteins involved in different steps of piRNA biogenesis, interact with Piwi, and this interaction is accompanied by SUMOylation. This supports a model where SUMOylation plays a significant role in the formation of a stable Piwi-Mael-SpnE complex. Based on these data, we propose a new, unifying model where SUMOylation acts as a critical regulatory step in piRNA biogenesis, common to both germline and somatic cells.

731F Multisite phosphorylation is required for ERK-dependent downregulation of Capicua

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The receptor tyrosine kinase (RTK)/extracellular signal-regulated kinase (ERK) signaling pathway regulates essential cellular processes, including proliferation, growth, and differentiation. Downstream outputs of this pathway involve downregulation of the transcriptional repressor Capicua (Cic), which is required for the expression of RTK/ERK target genes during development. Although Cic has been recognized as a conserved ERK substrate, the specific phosphorylation sites responsible for its regulation remain undefined. We identified ERK-dependent phosphorylation sites in Cic using affinity purification-mass spectrometry (AP-MS) and examined their functional significance. Multiple ERK phosphorylation sites are distributed throughout the length of the Cic protein. Their roles were tested using transgenic flies expressing mutant Cic variants, all of which retained normal levels of ERK binding. Mutation of twenty high-confidence sites resulted in a dramatic increase in Cic protein stability and repressor activity in vivo, suggesting that this mutant variant was largely resistant to ERK-mediated inactivation. Mutating subsets of the identified phosphorylation sites led to only a partial increase in Cic repressor activity, indicating that Cic downregulation requires simultaneous phosphorylation at multiple residues. Our results reveal that multisite phosphorylation is required to promote Cic inactivation and turnover, uncovering a mechanism by which ERK signaling inhibits Cic activity and ensures proper developmental gene expression in *Drosophila*.

732F Ribosome Profiling of Minute Wing Imaginal Discs

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Ribosomal protein (Rp) gene haploinsufficiency, a hallmark of Minute mutants in Drosophila melanogaster, triggers a stress response mediated by the transcription factor Xrp1 and drives cell competition between wild-type and Rp-deficient imaginal disc cells. The ribosomal protein RpS12 controls Xrp1 activity in Minute genotypes, and Xrp1 in turn regulates bulk translation rate through PERK-dependent phosphorylation of eIF2α. Thus, Rp gene haploinsufficiency could potentially affect translation through multiple mechanisms: altered ribosome abundance, RpS12 activity, and global inhibition of translation initiation via eIF2α phosphorylation. However, the specific translational effects remain uncharacterized. To address this gap, we adapted ribosome profiling to small Drosophila imaginal disc samples and profiled wing discs from RpS3, RpS12, and Xrp1 mutants and their combinations. This genome-wide approach reveals actively translated transcripts, enabling us to identify translation-specific effects of Rp haploinsufficiency and determine their dependence on RpS12 and Xrp1. We will present these findings to elucidate the translational landscape of Minute mutants in Drosophila. These results may also inform our understanding of Rp gene mutations in mammals, including those in Diamond-Blackfan Anemia patients and many cancers.

733F Regulation of mitochondrial homeostasis in stress and disease by the RNA-binding protein Rbfox1

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Regulation of gene expression in response to stress occurs in specialized membraneless subcellular compartments composed of proteins and RNA, known as RNP granules. Rbfox1 is an RNA-binding protein known for its role in alternative splicing; however, it can also associate with stress-dependent RNP complexes and mitochondria, through its low-complexity sequence domains (LCDs) or the RNA recognition motif (RRM) (Kucherenko & Shcherbata, 2018).

In humans, RBFOX1/A2BP1(Ataxin2-binding protein 1) is dysregulated in several neurodevelopmental and neuropsychiatric disorders, where mitochondrial dysfunction plays an important role in disease progression. Rbfox1's mechanistic contribution to different neurological diseases is unclear, as is the significance of its association with mitochondria. *Drosophila* Rbfox1, the ortholog of human RBFOX proteins, serves as an excellent model to study its cellular functions.

Proteomic analysis identified several mitochondria-associated proteins containing LCDs as Rbfox1 interactors, suggesting that Rbfox1—similar to its role in the assembly of nuclear and cytoplasmic RNP granules—may also contribute to mitochondrial RNA granule assembly. This interaction appears to be stress dependent. We further found that Rbfox1 can bind mitochondrial RNA and regulate mitochondrial gene expression. Our data indicate that both Rbfox1 expression and cellular stress can affect mitochondrial homeostasis and biogenesis.

To examine the role of Rbfox1 in vivo, we generated CRISPR-Cas9 mutant flies lacking the conserved RNA-binding domain. These mutants exhibit severe mitochondrial fragmentation and morphological abnormalities in larval brains. RNA sequencing of larval brains revealed differential expressions of both mitochondrial and nuclear-encoded genes critical for mitochondrial structure and function. Further investigation of alternative splicing changes in the nucleus, as well as differences in mitochondrial RNA cleavage in these mutants, will provide insights into specific mechanism by which Rbfox1 dysregulation disrupts mitochondrial homeostasis and thereby exacerbates neurological diseases.

734S Testing histone locus body formation requirements

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Histones are proteins that DNA wraps around so that it can be compacted into the nucleus. Histones also serve as gatekeepers of the genome, controlling access to processes such as replication and transcription. Histone production is greatly increased during S-phase of the cell cycle to accommodate newly synthesized DNA. A specialized nuclear body known as the histone locus body (HLB) forms at the histone gene locus and coordinates expression and processing. This nuclear body has several known components, including the scaffolding factor Mxc, the histone mRNA processing factor FLASH, the negative regulator Mute, and the DNA binding protein CLAMP. HLB formation can occur when the poly-glutamine domain of CLAMP is artificially tethered to the H3-H4 promoter of a transgenic histone array. However, these HLBs are not functional: histone mRNAs are not produced from this transgenic array. Therefore, the mechanisms of HLB formation and requirements for full functionality remain an open question. To address these unknowns, we are continuing experiments with the artificial tethering system. We will expand the list of HLB factors that we tether to the transgenic histone locus. We are focusing on Mxc, FLASH, Mute, and CLAMP, as well as previously-tested domains of these proteins. In addition, we will determine if the context of the histone locus is necessary for HLB formation. We will tether HLB proteins to a separate site in the genome, outside of the histone locus. We are currently in the process of making transgenic fly lines to do these experiments. We will then stain polytene chromosomes to test for HLB formation. We will also test HLB functionality using an established PCR-restriction digest assay, which relies on restriction enzyme sites engineered into the transgenic histone genes. Understanding how the HLB forms and determining the requirements for its function furthers our knowledge of the regulation of histone gene expression. These experiments have broad implications for cell cycle regulation and nuclear body formation.

735S Understanding the Role of Gene Body Methylation in Zygotic Genome Activation in *Nasonia vitripennis*

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DNA methylation is a fundamental epigenetic modification involving the addition of a methyl group to cytosine residues, typically at CpG dinucleotides. It plays a vital role in regulating gene expression, development and maintaining genomic integrity. Methylation occurs in two main patterns. Promoter methylation (PM) takes place mainly in the regulatory regions and is associated with gene silencing. Gene Body methylation (GBM) takes place between the transcription start and end sites, and is associated with high, steady gene expression. GBM is broadly conserved among eukaryotes, but deeper characterization of its function is complicated by the pervasive presence and significant functional importance of PM in vertebrate models, which has obscured the function of GBM.

While *Drosophila* has lost most of the machinery required for CpG methylation, and has negligible genome methylation, most other insects have retained CpG methylation, almost exclusively in the GBM mode. An example of this is the wasp *Nasonia vitripennis*, which we have been using as a model to understand the function of GBM in the absence of confounding PM. Genome wide base-pair resolution methylation analyses revealed that approximately one-third of protein-coding genes are methylated, with GBM being enriched in the 5' coding regions and demarcating exon-intron boundaries. Functionally, GBM is associated with the constitutive expression of genes across developmental stages, typically those involved in basal cellular functions. Among *Nasonia's* three *Dnmt1* (DNA Methyltransferase 1) paralogs, maternally deposited *Nv-Dnmt1a* was shown to be essential for early embryogenesis, with knockdown leading to a significant reduction in global GBM and resulting in embryonic lethality marked by failures in cellularization and gastrulation, key events correlated with zygotic genome activation during Maternal-to-Zygotic Transition (MZT). We are currently investigating how GBM affects the chromatin state at methylated loci, how methylation and its loss affect the efficiency of RNA polymerase, and how GBM and Dnmt1a interact with other contributors to the MZT, such as Zelda, in ensuring proper activation and regulation of the zygotic genome. This study will advance our understanding of epigenetic regulation in non-vertebrate systems and shed light on the evolutionary diversity of ZGA control mechanisms.

736S Mettl3 mediated m6A-modification is required for the development and function of indirect flight muscle in *Drosophila*

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Muscles rely on the precise regulation of alternative splicing and gene isoform expression to promote the assembly of sarcomeres and fiber-type specific contractile properties. Many muscle diseases, from cardiomyopathies to muscular dystrophies, display misregulation of RNA processing and gene isoform expression which is thought to underly muscle structure and function deficits. N6-Methyladenosine (m6A) is an epigenetic RNA modification that regulates RNA processing, including alternative splicing, mRNA stability, and translation efficiency. Mettl3, the primary enzymatic writer of m6A, is reported to regulate muscle development in vertebrates, but the developmental mechanisms of Mettl3 function have yet to be understood. We report that m6A pathway function in myogenesis is conserved in *Drosophila melanogaster*. We show that Mettl3 is expressed and m6A is present on RNA in the fly muscle, and Mettl3 mutants have behavioural deficits in climbing, jumping, and flight ability. Muscle-specific RNAi knockdown of Mettl3 results in altered sarcomere dimensions and flightlessness, while neuronal-specific Mettl3 knockdown flies have defects in motor neuron axon branching. Both sarcomere and neuronal defects could be rescued by expression of Mettl3, indicating tissue-intrinsic Mettl3 function. We confirm defects in expression of sarcomere, mitochondrial and synapse-associated genes at the transcript and protein level, using mRNA-Seq, RT-qPCR, and proteomics. Notably, mitochondrial proteins are downregulated and we detect swollen mitochondria with altered network morphology in the absence of Mettl3 or the cytoplasmic m6A reader Ythdf1. Our experiments revealed significant changes in muscle specific alternative splicing, including isoform switches in key sarcomere proteins such as Titin-like gene bent (bt, Projectin), Myofilin (Mf), Zasp52 and Unc-89. These targets are differentially regulated in flies mutant for the nuclear Ythdc1 and cytoplasmic Ythdf reader proteins, reflecting distinct functions for m6A modification in specific subcellular compartments. Our results establish *D. melanogaster* as a model to study foundational principles of m6A function in myogenesis and reveal a conserved and muscle-intrinsic function for m6A modification in regulating alternative splicing and protein expression levels during myofibrillogenesis.

737S Silent Messengers of the Western diet: miRNAs Journey from Somatic Cells to Sperm, and to the Embryo

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Growing evidence across species indicates that transgenerational inheritance of metabolic traits is a conserved phenomenon, with small RNAs emerging as the most strongly supported carriers of heritable information. In mice, somatic cells of the epididymis have been shown to transfer RNAs to germ cells, providing a direct route for soma-to-germline communication; however, whether this mechanism is conserved across species or represents a mammal-specific process remains unknown. Here, we investigate whether the transfer of somatic RNAs into germ cells occurs in *Drosophila* following paternal exposure to a Western diet and how this influences early embryonic development. Using a somatic cell specific driver (c587-GAL4) to express uracil phosphoribosyltransferase (UPRT), we selectively labelled newly synthesized somatic RNAs with 4-thiouracil (4-TU) and tracked their movement into the germline. Time-dependent labeling revealed a progressive accumulation of thiolated RNA in sperm, with approximately 7.77 % ± 0.39 of total RNA recovered in the streptavidin-bound fraction after 24 hours 4-TU feeding, with Western diet-fed males showing distinct streptavidin-positive signals along the sperm tails as reported before in mice, suggesting enhanced soma-to-germline RNA communication under dietary stress. Furthermore, interestingly, miR-277-3p was upregulated in the Western diet-fed males' sperm, and this miRNA was detected to be transferred from the somatic cells into the germline. miR-277-3p is a conserved miRNA that has important role in regulating metabolic reprogramming by modulating TOR signalling pathway in *Drosophila*. Notably, embryos fertilized by this Western diet fed males also exhibited distinct miR-277-3p upregulation during the maternal to zygotic transition (MZT), a critical developmental window when zygotic transcription is first initiated especially at the 2-3 hours after egg laying. These findings suggest that diet-induced paternal RNAs actively modulate the early embryonic transcriptome, establishing a potential mechanism for epigenetic inheritance. Together, our results provide direct evidence that somatic cells in the *Drosophila* testis transmit RNA-based information to the germline and the next generation, supporting a model in which diet-responsive miRNAs act as molecular messengers that mediate soma-to-germline communication and reshape the epigenetic landscape underlying offspring development.

738S Engineering Synthetic Transcription Factors to Explore How Activation Domains Shape Shadow Enhancer Logic

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Gene expression during development is tightly controlled by enhancers, regulatory elements that recruit specific transcription factors (TFs). Many developmental genes are regulated by sets of shadow enhancers, or pairs of enhancers that bind overlapping TFs to drive similar expression patterns. These shadow enhancers are thought to buffer against mutations or environmental stress, ensuring robust transcription. While shadow enhancers have been identified across species, including Drosophila, the interaction between activation domains of bound TFs and their collective effect on transcription remains poorly understood. This raises a key question: how do activation domains work together to drive gene expression, and what mechanisms underlie their coordination? Understanding how these elements function is crucial for clarifying enhancer activity and advancing synthetic biology approaches to gene regulation.

Therefore, we propose a synthetic biology platform using engineered TFs to investigate how activation domains coordinate transcription in shadow enhancer systems. I constructed synthetic transcription factors by fusing custom-designed Zinc Finger (ZF) DNA-binding domains with a well-characterized activation domain. These constructs were expressed in Drosophila S2 cells and assayed in a high-throughput fluorescent reporter system to obtain quantitative readouts. The constructs successfully triggered reporter expression in the presence of ZF binding sites, validating both DNA-binding specificity and activation potency. This platform allows us to systematically test different domain combinations and measure their effects on gene activation.

Moving forward, future work will test combinations of Zinc Finger DNA-binding domains and activation domains, as well as vary the number of ZF binding sites on a reporter enhancer. This synthetic biology approach aims to characterize expression robustness, determine whether activation domains interact additively, sub-additively, or synergistically, and provide insights into the regulation of gene expression in development and disease.

739S Transcription factor binding site variability does not impact HLB formation at the H3H4p in Drosophila

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Metazoan replication-dependent (RD) histone genes must be coordinately expressed in large amounts during S-phase to maintain histone homeostasis and genomic stability. This is achieved in part through the presence of multiple copies of the histone genes and through the formation of the Histone Locus Body (HLB), a nuclear condensate essential for histone transcription and RNA processing. In Drosophila melanogaster, the histone genes are present at a single locus, comprised of ~100 copies of a tandemly arrayed 5kb repeat containing one copy of each of the four core histones and the linker histone H1. Deletion of all ~200 copies of the endogenous RD histone genes is lethal; however, only 12 copies of the histone array can rescue this lethality. We therefore hypothesized that the histone genes across the locus are differentially regulated. By utilizing long-read sequencing data, we found that these genes are nearly identical in sequence except for variability in the length of the GA-repeats in the H3H4 bidirectional promoter. The GA-repeats are bound by the transcription factor CLAMP, an interaction that is critical for HLB formation at the histone genes. Despite variability in GA-repeat length, we did not detect differential binding of CLAMP or other transcription factors. Although CLAMP targets histone gene loci in other drosophilid species, the GA-repeats are poorly conserved. We therefore compared HLB formation at the H3H4 promoter in four different species of Drosophila and found that, despite differences in sequence and GA-repeat length, HLB formation was observed across all species. To our surprise, even with substantial differences in the GA-repeat length, purified CLAMP was able to bind the H3H4 promoter in each species we examined. To determine whether GA-repeat length influences histone gene expression, we are creating luciferase reporter constructs controlled by the H3H4 promoter of different species, each harboring a variable repeat sequence length. These experiments will enhance our understanding of how GA-repeat length influences transcription factor binding and function. This will also shed light on the importance of functional conservation over sequence conservation alone.

740S Context-dependent role of Snail repressor in early *Drosophila* development

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Transcriptional repressors bind sequence-specific motifs within enhancers and counteract the effect of transcriptional activators, but the molecular mechanisms underlying this repression remain poorly understood. Similarly, how the arrangement of repressive motifs within enhancers influences repression is unclear. To address these questions, we used the deep learning model BPNet to investigate the role of the repressor Snail during dorsoventral patterning in the early *Drosophila* embryo. By training BPNet to predict ChIP-nexus data from sequence alone, we found that many Snail motifs prevent the binding of other transcription factors, though the mechanism remains unresolved. ATAC-seq and H3K27ac ChIP-seq experiments done in mutant embryos with ubiquitous Snail expression revealed that Snail binding reduces chromatin accessibility and H3K27 acetylation in a sequence context-dependent manner. Differential modeling of H3K27ac data in the ubiquitous Snail-expressing mutant vs. a control mutant where Snail targets are derepressed, further supports Snail's role in repressing this active enhancer mark. To elucidate these context-dependent effects, we are now investigating whether Snail influences nucleosome stability, given the high nucleosome occupancy observed at Snail-repressed enhancers.

741S Me31B is required during indirect flight muscle myogenesis in *Drosophila*

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During muscle development, RNA regulation determines the balance in expression of sarcomere proteins, enabling muscles to fine-tune their morphologies and contractile properties. RNA-binding proteins (RBPs) direct alternative splicing to produce multiple isoforms of myofiber-type specific proteins and regulate mRNA nuclear export, trafficking, stability, and translation within muscle cells. In a candidate screen, we identified a role for the DEAD-box RNA-helicase Me31B in *Drosophila* adult myogenesis. Me31B is the homolog of vertebrate DDX6, which associates with several protein complexes in cytoplasmic granules and functions in translational suppression and mRNA degradation. Misregulation of DDX6 is associated with intellectual disability, dysmorphic facial features, feeding difficulties, and cardiac defects, but little is known of the mechanism underlying these pathologies. Here we show that Me31B is expressed in *Drosophila* muscle and localized in granules throughout the myoplasm as well as in distinct punctate structures at the nuclear membrane throughout myogenesis, consistent with a role in muscle development. Muscle-specific RNAi knockdown of Me31B results in lethality and flightlessness in surviving escapers. We determine Me31B localization in IFM with respect to the nuclear pore complex, eIF4E, Bru1, and granule proteins Tral and Orb, noting a particularly strong co-localization with Tral in immunofluorescence. RNAi knockdown of Cup and Tral, but not Bru1, result in disrupted Me31B localization and a decrease in granule number. RNAi knockdown of dynein adapter proteins Egl and Dlic also alter Me31B localization, identifying microtubule-dependent trafficking of Me31B-containing granules in IFM. Using coIP-mass spectrometry, we identify additional Me31B interacting proteins in fly thorax. Several domains of Me31B are reported to interact with other granule proteins and using CRISPR structure-function alleles we show that a single functional helicase domain is sufficient to support muscle development but disrupts Me31B nuclear membrane localization. Our data reveals a role for Me31B in pupal and adult muscle development and provide insight into the constitution and dynamic localization of cytoplasmic RNP granules during myogenesis.

742S Novel RNA-binding protein, Pep, regulates RNA processing and development in Drosophila

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RNA-binding proteins (RBPs) regulate post-transcriptional processes, including alternative splicing, mRNA stability, localization, and translation. Their dysregulation contributes to the development of cancer, cardiovascular, neurodevelopmental, and neurodegenerative diseases. Despite their biomedical importance, many RBPs remain underexplored or uncharacterized. We recently identified the Protein on ecdysone puffs (Pep) as a novel RBP playing a key role in controlling Drosophila development. Pep is ubiquitously expressed across all Drosophila tissues and developmental stages and is localized in active loci that control development. Using molecular cloning techniques, we generated transgenic Drosophila flies expressing Pep-GFP and discovered that Pep co-localizes with Drosophila Hrp38, a known RBP and RNA processing regulator. Additionally, RNA immunoprecipitation followed by sequencing (RIP-seq) and mass spectrometry revealed that Pep binds to developmentally regulated RNAs and interacts with known RBPs, such as Hrp38, suggesting its key role in RNA processing regulation. We performed Pep knockdown using shRNA, and unlike many other previously described Drosophila RBPs, which are functionally redundant, Pep mutation causes developmental arrest and lethality at all stages, disrupting nuclear RNP complexes. Our findings strongly suggest that Pep functions as a hub regulating RNP complexes involved in post-transcriptional processes and controlling transcriptional profiles during development.

743S From Binding to Building: Characterizing MEF2-Mediated Gene Regulation During Myoblast Differentiation in Drosophila

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Muscle development in Drosophila provides an ideal model for dissecting the transcriptional networks that guide cellular differentiation and tissue morphogenesis. Central to this process is Myocyte Enhancer Factor 2 (MEF2), a conserved member of the MADS-box family of transcription factors that activates numerous genes required for muscle differentiation. Previous over-expression studies in adult myoblasts have shown premature activation of muscle structural genes such as Myosin heavy chain (Mhc) and bent (bt). However, the relationship between MEF2 protein abundance in adult myoblasts and its direct genomic binding activity remains incompletely understood.

In this study, we show that knocking down epigenetic regulators such as components of the Nucleosome Remodeling Deacytylase Complex (NuRD) results in the premature activation and expression of muscle structural genes, suggesting that in myoblasts MEF2 is bound to DNA but prevented from activating target genes . Next, we will identify genome-wide MEF2 binding sites within the wing imaginal disc myoblasts using a combination of protein-level analyses and chromatin immunoprecipitation sequencing (ChIP-seq). These studies will define how MEF2 abundance correlates with its DNA occupancy and transcriptional regulatory function. By integrating these results, this work will clarify how MEF2 promotes molecular binding into developmental outcomes and uncovers potential co-regulators that modulate its expression.

744S BMP signaling controls the metabolic state and neuromodulatory pathways in Ventral Nerve Cord

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Bone morphogenetic proteins (BMPs) are conserved regulators of neuronal development and synaptic plasticity, yet the cell-type–specific transcriptional programs directly controlled by this pathway remain incompletely defined. To identify transcriptional targets of BMP signaling in the nervous system, we performed single-cell RNA sequencing (scRNA-seq) on ventral nerve cords (VNCs) from third instar control larvae and *Mad*^{null} mutants, capturing >38,000 cells. We recovered all known major cell types populating the VNC (Nguyen and Vicidomini et al., *Dev Cell* 2024), indicating that overall lineage composition is preserved upon loss of BMP signaling. Our analysis revealed two principal forms of transcriptional variation: (1) a shared metabolic signature across cell types, characterized by increased glycolytic genes and reduced respiratory-chain, proteasome, and ribosomal transcripts; and (2) a motor neuron (MN) class–specific response affecting the release of neurotransmitters and neuropeptides and the neuromodulatory signaling.

For example, in Type III (peptidergic) MNs, neuropeptide mRNAs are broadly reduced. We identify novel transcriptional targets for BMP signaling, such as AstCC. AstCC transcript and peptide levels are decreased in *Mad*^{null} Type III MNs; also, in-silico analysis detects candidate Mad/Medea binding sites within this locus.

In Type II (octopaminergic) MNs, BMP signaling promotes the expression of vesicle monoamine transporter (Vmat)- which is reponsible for packaging the amine neurotransmitters into secretory vesicles- as well as expression of selected amine receptors, such as Oct-TyrR.

In Type I (glutamatergic) MNs, loss of BMP signaling negatively impacts VGlut transcript and protein levels, but appears to increase some components of the vesicle release machinery. Metabotropic pathways also show transcriptional changes: Gαs, Gαo, and Gαq transcripts are elevated in *Mad*^{null} mutants, whereas monoamine receptors (including Oct-TyrR, Oamb, CG12290) are differentially regulated. Since many of these genes harbor putative Mad/Medea binding motifs, they are probably direct targets of BMP signaling.

This study identifies multiple candidate BMP targets in distinct cell types and reveals that loss of BMP signaling preserves cell fate but alters metabolic, synaptic, and neuromodulatory gene programs in a MN class–specific manner.

745S Doa, but not “dead on arrival”: Investigating links between the Nab2 RNA binding protein (RBP) and Darkener of apricot/CKL2, a kinase that promotes Mettl3 activity in the fly brain

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N6-methyladenosine (m^{6}A) is the most abundant post-transcriptional mRNA modification across eukaryotes and is deposited by a complex containing accessory subunits surrounding a Mettl14/Mettl3 catalytic core. m^{6}A controls post-transcriptional processing of mRNAs and its enrichment in the central nervous system of multiple organisms has led to the hypothesis that m^{6}A plays a key role in regulating levels of important neuronal proteins. Our prior work has shown that loss of the highly conserved RBP, Nab2/ZC3H14, in flies produces a neurodevelopmental disease characterized by altered brain structure and impaired learning and memory; in human patients, Nab2 loss leads to a form of intellectual disability. Within the fly nervous system, we determined that Nab2 loss alters transcript levels and splicing of a group of neuronal mRNAs, with the most common defect being retained first introns in 5’UTRs. Molecular analyses also found evidence that Nab2 loss alters m^{6}A methylation of some of these mis-spliced mRNAs, suggesting a potential link between m^{6}A deposition and these mis-splicing events. While probing links between Nab2 and the m^{6}A machinery, we found that Nab2 loss causes an intron retention in the 5’UTR of the mRNA encoding the serine-threonine kinase Darkener of apricot (Doa; human CLK2), which seems to elevate levels of Doa protein. Critically, the Fl(2)d subunit of the m^{6}A deposition complex is phosphorylated by Doa and this increases “writer” activity of the complex. The goal of current work is to test interactions between Nab2 and Doa, with a focus on the hypothesis that Doa is downstream of Nab2 in a brain m^{6}A regulatory pathway. A recent Nanopore Direct RNA sequencing analysis has defined a set of Nab2-regulated m6A sites in the brain transcriptome that provides a pool of potential RNA targets of this Nab2-Doa-Mettl3 pathway. Intriguingly, the *Doa* mRNA is within this group, suggesting that Nab2 is part of an inhibitory feedback loop that limits Mettl3 activity in vivo. To extend from these findings, we are also beginning to analyze how Nab2 loss affects translation of individual mRNAs using the Direct Analysis of Ribosome Targeting (DART) technique. Overall, this work will provide further insight into the mechanism behind the increased deposition of m^{6}A on transcripts in Nab2 mutants, and how the essential functions of Doa coincide with the phenotypes of the Nab2 mutant flies.

746S Cis-regulatory competence and combinatorial repression shape anterior pair-rule stripe formation in *Drosophila*

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Background. Knowledge of the molecular mechanisms underlying differential transcription, particularly how transcription factors act together within regulatory networks to generate specific expression patterns, remains elusive. We address this problem by studying the segmentation cascade that specifies the anterior–posterior axis of the *Drosophila* embryo, focusing on the establishment of the seven-striped expression pattern of pair-rule genes during the syncytial blastoderm stage. Each stripe, or pair of stripes, is controlled by a cis-regulatory module (CRM) that responds to specific combinations and concentrations of maternal and gap transcription factors. Our main interest is the regulation of the anterior-most pair-rule stripes (stripes 1), which form at the transition between regions that either do not form or do form canonical stripes in the embryo. We test a model in which the CRMs of these stripes are broadly competent for activation, while combinatorial repression restricts their activity by delimiting the borders of stripes and preventing their ectopic expression in anterior regions. **Methodology.** We combine genetic analyses, ectopic expression assays, in silico prediction of transcription factor binding sites, and in vitro DNA–protein interaction assays. **Results.** The simultaneous removal of Huckebein (Hkb), Tailless (Tll), Sloppy-paired 1 (Slp1), and the forkhead protein fd19B (CG9571) contributes additively and differentially to the positioning of anterior stripes of *hairy* (*h*), *even-skipped* (*eve*), *runt* (*run*), and *fushi-tarazu* (*ftz*). Combined misexpression of Slp1 and fd19B confirms their additive repressive activity on target stripes. Moreover, predicted binding sites for these factors are enriched in anterior stripe CRMs, and *in vitro* assays show that fd19B and Slp1 directly bind fragments of the eve stripe 1 CRM enriched for canonical forkhead sites.

747S Glial EMC4 expression levels during development determine the severity of adult-stage physiological and behavioral phenotypes in Drosophila melanogaster

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The endoplasmic reticulum (ER) is a central site for membrane protein biogenesis, where nascent transmembrane proteins undergo folding, modification, and insertion. The endoplasmic reticulum membrane protein complex (EMC), composed of eight to ten subunits, mediates these processes. To assess the cell type-specific function of this complex, we reduced expression of EMC subunit four specifically in glial cells of *Drosophila melanogaster* using RNA interference through the GAL4-UAS system. Glial-specific EMC4 knockdown driven by *repo-GAL4* caused severe phenotypes, including an approximately one-day delay in developmental timing, reduced developmental viability, complete loss of climbing ability, and a shortened adult lifespan of only 5-6 days. To determine whether adult phenotypes result from acute loss of EMC4 in glia, we restricted the knockdown to adulthood using drug- and temperature-inducible *GAL80* transgenes and found that lifespan was nearly normal and climbing ability was fully rescued, indicating that glial EMC4 depletion is most detrimental during development. To test this, we performed a development-only glial EMC4 knockdown and found that the outcomes appear to be dependent on the strength of the knockdown and did not exactly reproduce the adult-stage phenotypes observed with continuous *repo-GAL4* driven knockdown, likely due to partial *GAL80* inhibition limiting the extent of RNAi expression. To confirm the dose-dependent requirement of EMC4, we performed a ubiquitous EMC4 depletion using two drivers with known differences in expression strength: *Tubulin-GAL4* driven knockdown caused complete developmental lethality, while the more moderate *ubiquitin-GAL4* driven knockdown extended female survival and preserved normal adult lifespan in males. When we knocked down other EMC subunits, including those previously found to be structurally required for the entire complex, we observed varied phenotypes, presumably due to differences in RNAi construct targeting efficiency, further supporting a dose-dependent role for EMC. Future experiments will explore whether phenotypic variability arises from differences in EMC subunit expression levels, define the extent of EMC4 reduction required to drive larval defects and adult physiological and behavioral outcomes, and assess the contribution of ER stress.

748T Phosphoregulation of RNA-binding proteins during egg activation

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Egg activation marks the major developmental milestone in which a mature oocyte becomes competent to support embryogenesis. This transition involves conserved signaling events, beginning with a rise in intracellular calcium levels and eventually leading to extensive protein phosphoregulation. However, how these post-translational modifications influence early embryogenesis, including the regulation of maternally deposited mRNAs, remains poorly understood.

To address this gap, I am investigating how RNA-binding protein phosphoregulation coordinates mRNA regulation and translation upon egg activation using *Drosophila melanogaster*, a highly tractable system due to its large and accessible eggs. First, I am assessing the evolutionary conservation and structural features of phosphosites on oocyte RNA-binding proteins, using comparative genomics and structural modeling. Second, I am generating phosphomimetic and non-phosphorylatable alleles of conserved phosphoregulated RNA-binding proteins using CRISPR/Cas9-based genome editing. I will analyze the consequences of these mutations on protein function, global mRNA regulation, and developmental progression. Finally, I plan to manipulate oocyte kinases using genetic and optogenetic tools to determine how kinase activity influences the phosphorylation of RNA-binding proteins during egg activation and subsequent developmental processes.

My findings will uncover how phosphoregulation of RNA-binding proteins during egg activation contributes to the precise post-transcriptional control required for early development. More broadly, they will advance our understanding of how dynamic protein modifications regulate RNA biology, a mechanism that occurs across diverse cell types. Because defects in egg activation can impair fertility—even for couples using assisted reproductive technologies—this research may provide necessary mechanistic insight into a cause of infertility.

749T Understanding development of cell size using multiple sperm morphs in *D. pseudoobscura*

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Sperm length is genetically controlled, and consistent within a single male and a single species, even in the face of environmental variability. Sperm differentiation is thus an excellent model system for investigation of intrinsic cell size control mechanisms. *Drosophila pseudoobscura* (*D. pse*) males produce sperm of three distinct lengths. Each sperm morph grows very reproducibly to a specific length, strongly suggesting that this process is under precise genetic control. Thus, *D. pse* spermatogenesis provides an ideal system to study the molecular mechanisms controlling cell size (sperm length).

D. pse sperm production is continuous; all stages are present within adult testes. Germline and somatic stem cells reside at the apical region of the testis. Each spermatogonium is enclosed by two cyst cells, forming a cyst where germline cells divide and differentiate in synchrony. Each cyst produces either long (300μm), medium (100μm) or short (50μm) sperm. Only long sperm fertilise the egg. The mechanisms underpinning production of distinct sperm lengths remain unexplored.

We previously performed single cell RNAseq on whole *D. pse* testes, which revealed distinct developmental trajectories of short and long sperm morphs from the late spermatogonia to early spermatocyte stage, and revealed several candidate genes which may function in morph development. However, there are limited tools available to experimentally investigate gene function in *D. pseudoobscura*, so we are developing new germline drivers and responders to enable Gal4/UAS RNAi in this species. This includes sperm morph-specific drivers from genes identified as differentially expressed in our scRNAseq dataset. We will use these to understand morph-specific functions of candidate genes.

We have also re-characterised the sperm morphs of several other species in the *obscura* group, finding two distinct morphs in *D. azteca*, *D. affinis*, and *D. subobscura*, and three morphs in the species most closely related to *D. pse* – *D. persimilis* and *D. miranda*. This suggests that the third morph arose after the divergence of the *pseudoobscura* subgroup from the rest of the *obscura* species.

750T The QxxR motif of the RNA helicase Me31B/DDX6 is essential for female fertility and primordial germ cell development

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The QxxR motif is a highly conserved sequence found in DEAD-box RNA helicases, including Me31B (human homolog DDX6), a major family of RNA-dependent ATPases involved in RNA metabolism. In humans, a point mutation in the QxxR motif of DDX6 causes severe developmental delays and intellectual disabilities. To investigate the *in vivo* function of this motif, we generated a *Drosophila* mutant strain (*me31B*^{H333R}) carrying the analogous mutation (H333R; QA**H**R to QA**R**R) using CRISPR gene editing. Phenotypic analysis revealed that this mutation leads to female sterility and various defects in primordial germ cell (PGC) development. To uncover the molecular mechanism underlying these germline phenotypes, we performed transcriptomic (RNA-seq) and proteomic (mass spectrometry) analyses of mutant ovaries, as well as immunoprecipitation–mass spectrometry (IP-MS) to characterize the mutant protein's interactome. These multi-omics approaches identified specific genes and proteins whose expression levels and/or interactions with Me31B were significantly altered by the mutation. Our findings reinforce the essential role of Me31B in germline development and provide new insights into the functional significance of the QxxR motif in DEAD-box RNA helicases.

751T Ubiquitous *Mettl3* Knockdown in *Drosophila melanogaster* Results in Phenotypic Abnormalities During Spermatogenesis

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The most abundant internal mRNA modification is the addition of a methyl group to the 6th position of adenosine known as N^6-methyladenosine (m^{6}A). The m^{6}A modification is crucial for numerous biological processes such as embryogenesis, gonadogenesis and gametogenesis. *Mettl3* is a highly conserved gene which encodes the protein that contains the catalytic domain of the m^{6}A complex. In *Drosophila*, Mettl3 plays a key regulatory role in development. Throughout development, *Mettl3* is highly expressed in gonadal tissue. In the adult testes, Mettl3 is localized to both somatic cyst cells and germline cells. In previous investigations conducted by our lab examining somatic cyst cell specific and germline specific *Mettl3* knockdowns, we found Mettl3 is required for germline viability and normal progression through spermiogenesis. Our current work is focused on simultaneous knockdown of *Mettl3* in somatic cyst cells and germline cells using the Gal4-UAS system. Using a combination of immunostaining and confocal microscopy, we found that ubiquitous knockdowns have a prominent swollen apical tip. The swollen tip phenotype is closely associated with testis muscle cell abnormalities and hub mis-localization. Collectively, these phenotypes appear to culminate in reproductive issues as preliminary data suggests males with ubiquitous knockdown are sterile. We hypothesize that Mettl3 could be interacting with *tsn*, which in part may explain some of the observed phenotypes. Through computation analysis, we found that *tsn* has punitive Mettl3 binding sites. Additionally, using RNA secondary structure predictor software, we found that *tsn* potentially forms secondary hairpin structures which are needed for Mettl3 recognition. Our work has the potential to provide additional insight into the role of Mettl3 in *Drosophila* reproduction. Given the conservation of *Mettl3* and m^{6}A, this work may also provide insight into the role of the modification in other eukaryotic organisms.

752T *Mettl3* is required for germline function during *Drosophila* spermatogenesis

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Modifications to RNA are critical for gene regulation. One of the most prominent modifications added to RNA is N^6-methyladenosine (m^{6}A), which is an addition of a methyl group to the N^6 position. The m^{6}A modification is required for numerous developmental processes within eukaryotic organisms. The highly conserved enzyme that catalyzes this modification is Mettl3. In *Drosophila*, *Mettl3* is highly expressed in somatic cyst cells and germline cells within the testes. Data collected in our lab's previous work suggested a somatic cyst cell specific function, as *Mettl3* knockdown in somatic cyst cells resulted in germline death. In our recent investigation, we examined the germline specific function of *Mettl3* during *Drosophila* spermatogenesis. Our results, using immunostaining in combination with confocal microscopy, indicate that the knockdown of *Mettl3* in the germline results in abnormalities during spermiogenesis, the late-stage process of spermatogenesis. In germline knockdowns, the individualization process of spermatids was disrupted as actin cones bound to spermatids became loosely packed. Additionally, waste bag and mature sperm deficiency were observed in knockdowns, which is also characteristic of abnormal spermiogenesis. These abnormalities appear to have culminated with a noticeable reduction in fertility of the knockdowns relative to controls. Interestingly, *Hsp60B* was misregulated in knockdowns, which could potentially explain some of the observed phenotypes. Together, our results suggest that *Mettl3* is necessary for germline function during *Drosophila* spermatogenesis.

753T Tdrd5l promotes male identity in germline stem cells

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Germline sex determination is regulated by signals from the somatic gonad, and germline autonomous sexual identity regulated by the RNA-binding protein Sex lethal (Sxl). Previously our lab identified *Tudor domain containing protein5-like (Tdrd5l)* as being important for male identity in the germline. Tudor-domain containing proteins are conserved across the animal kingdom for their necessary functions in germline development. Tdrd5l is expressed in the developing germ cells and GSCs in males but is repressed in female GSCs at least in part due to regulation by Sxl. Currently we are working to understand how Tdrd5l promotes male germline identity.

Tdrd5l mutant adult testes exhibit dramatic germline loss which is often seen in mutants for sex determination factors where the sex of the germline does not match the sex of the surrounding soma. One regulator of germline sex determination is the JAK/STAT pathway, which promotes male germline identity but is repressed in females downstream of *Sxl*. Interestingly, Tdrd5l is also important for regulating germline JAK/STAT activity. In *Tdrd5l* mutants, male GSCs showed a reduction of Stat staining indicating a loss of JAK/STAT signaling. To determine how Tdrd5l regulates JAK/STAT signaling, we screened known JAK/STAT repressors for the ability to rescue the *Tdrd5l* phenotype upon loss of function. One hit is the PIAS protein Su(var)2-10. Antibody staining for Su(var)2-10 in *Tdrd5l* mutant testes shows increased Su(var)2-10 expression in the germline. This suggests that Tdrd5l could promote JAK/STAT activity by repressing expression of *su(var)2-10* in the early germline.

Another important signaling pathway active in the GSC niche is the BMP pathway. Our work shows that BMP signaling (pMAD) in GSCs is sexually dimorphic and is substantially higher in female GSCs compared to male GSCs. Loss of *Tdrd5l* in the male germline results in increased pMAD expression in male GSCs suggesting possible feminization of these cells. Further, expression of Tdrd5l in the female germline led to a decrease in GSC pMAD levels comparable to male levels. Lastly, overactivation of JAK/STAT in female GSCs is sufficient to reduce BMP activation, suggesting that JAK/STAT activation in males is what represses pMAD levels in males compared to females. Thus, these show *Sxl* and *Tdrd5l* act autonomously in the germline to regulate key signals from the somatic gonad acting through both the JAK/STAT and BMP pathways to control germline sexual identity.

754T Ecdysone signaling regulates a network of BTB-DBD proteins to control sex-specific gonad development

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In the animal kingdom, most species exhibit sexual dimorphism, or phenotypic differences between the sexes. In *Drosophila*, the key factor controlling sexual dimorphism is Doublesex (Dsx), the founding member of the Doublesex/Mab-3 Related Transcription Factor (DMRT) family that controls sex-specific gonad formation in most animals, including humans. Our lab has shown that Dsx regulates steroid hormone Ecdysone (E) signaling through female-specific Ecdysone Receptor (EcR) expression. E functions to initiate ovary development, while the lack of E signaling is important for the testis to maintain its male fate. Bioinformatic analyses have been used to predict Dsx targets, and many are transcription factors that contain a DNA binding domain (DBD) and a BTB (Broad-complex, Tramtrack, and Bric-a-brac) domain that promotes homo- and heteromeric BTB protein interactions. Here, we focus on three of these factors: *mamo (maternal gene required for meiosis), chinmo (chronologically inappropriate morphogenesis),* and *br (broad),* and how they regulate female vs. male somatic stem cell formation.

We find that *mamo* promotes development of follicle stem cells (FSCs) and follicle cells (FCs) in females, whereas *chinmo* is required for male somatic cyst stem cells (CySCs). Consistent with this, Mamo is expressed in the female somatic gonad, while Chinmo becomes restricted to males. Loss of *mamo* results in the depletion of FSCs and FCs, indicating it is necessary for their establishment and/or maintenance. Conversely, *chinmo* is essential for CySC identity in the testis. Our data indicate that both time- and sex-specific regulation of these genes are controlled by Dsx and E signaling during gonad development. Chinmo and Br act as mutual inhibitors, ensuring that the correct developmental pathway is activated in each sex. During the larval stage, female-specific E signaling activates Br, which represses Chinmo and promotes Mamo expression. Further, either loss of *chinmo* or ectopic E signaling in males causes a sex transformation of the testis, activating Br and Mamo to produce follicle-like cells. Thus, we have identified a key network of BTB-DBD transcription factors that mediate how Dsx and sex-specific E signaling control gonad sexual dimorphism. Given that DMRTs and steroid hormones control sexual development in other animals including humans, characterizing genes critical for sexual identity is of great importance for reproductive health.

755T Increased parental age compromises primordial germ cell migration in *Drosophila melanogaster*

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During embryogenesis, germline precursors called primordial germ cells (PGCs) must migrate to the somatic gonad. While PGC migration is essential for fertility and the continuity of many species, we lack a complete understanding of the genetic and non-genetic factors that influence the process. Using *Drosophila melanogaster*, we've noticed that PGC migration errors increase in tandem with the number of days parents are in embryo collection cages. To test whether this phenomenon resulted from extended time on a yeast-only diet or from increased parental age, we crossed three wild-type strains, aged the adults in standard media vials to specific timepoints, and kept time in embryo collection cages constant. Mis-migrated PGCs were then quantified in embryos generated from 3-, 6-, 9-, 12- and 15-day old age-matched parents. Across all intercrossed wild type conditions, embryos from parents nine days or older showed a significant rise in mis-migrated PGCs. These data demonstrate that even mild increases in parental age compromise PGC migration. We next set out to determine whether increased PGC migration errors are associated with age-dependent decreases in gamete quality, general errors in embryogenesis, or signaling required for PGC migration. First, we measured DNA damage by quantifying γ-H2Av foci in gametes and found no increases in DNA damage even gametes from 28-day old parents. Next, we measured hatch rate as a proxy for general errors in embryogenesis, finding no decreases in embryonic hatch rates with increased parental age. Finally, we assessed whether there were parental age-dependent changes in signaling required for PGC migration, finding a significant decrease in calcium transients in migrating PGCs from older parents. Together, these data suggest increased parental age specifically compromises signaling within migrating PGCs of the next generation, an unexpected phenomenon that links reproductive aging to transgenerational germline development and reveals a need for reproductive developmental biologists to tightly control for parental age.

756T *Barentsz* and *CG18622* Regulate gurken Translation During Oogenesis

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Proper dorsal-ventral (D/V) axis formation in *Drosophila melanogaster* requires accurate localization and translation of *gurken* (*grk*) mRNA. We have cloned two novel mutations that suppress the *grk* translation defect in *spn-B* mutant ovaries. These suppressors map to the exon junction complex protein Barentsz (Btz) and the previously uncharacterized gene *CG18622*.

Prior studies of the role of Btz during oogenesis have largely focused on the regulation of *osk* localization and translation. Given the distinct localization patterns of *grk* and *osk* in the ovary, we were surprised to identify a role for Btz in *grk* translation. We have generated several novel CRISPR-induced alleles of *btz* and are characterizing their effects on *grk* mRNA transport and translation during oogenesis. We have generated recombinant *btz* alleles containing *FRT* sites to induce germline clones. A trimolecular fluorescence complementation (TriFC) assay is also being developed to test for direct Btz–*grk* mRNA binding and to image this interaction *in vivo*. We have created genomic rescue and GFP-Btz constructs to analyze function and localization.

Mutations in *CG18622* were also identified in the *spn-B*^{BU} suppression screen. CG18622 is a small 94 aa peptide with no annotated function. Its role in inhibiting the translational suppression of *grk* mRNA caused by *spn-B*^{BU} was confirmed in several CRISPR-induced alleles, however its mechanism of action is unknown. We are in the process of cloning a GFP-CG18622 transgene to visualize localization of CG18622 within the oocyte. This work will further our understanding of the mechanisms by which D/V axis specification is controlled.

757T *P*-elements co-opt Bruno-1 to ensure transposition in the female germline

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Transposable elements are obligate genetic parasites that rely on host factors to ensure the transcription, translation and localization of their transcripts. While much is known about the negative regulatory machinery that represses TE transcription and eliminates TE derived transcripts, the host factors that positively regulate TEs are largely unknown. P-elements are DNA transposons that recently invaded natural populations of D. melanogaster. Regulation of *P*-element transposition has been studied extensively, and causes a sterility syndrome known as hybrid dysgenesis.

Through mapping natural variation in *P*-element induced hybrid dysgenesis we discovered that bruno-1 is required for dysgenic ovarian atrophy. *bruno-1* encodes an RNA binding protein that is a major translational regulator of key targets throughout oogenesis, including the earliest stages where P-elements transpose. We have discovered that *P*-elements rely on *bruno-1* for their mobilization, as bruno-1 heterozygotes exhibit dramatic reductions in *P*-element excision rates and associated death of germline cells. We have further discovered that Bruno-1's role in *P*-element excision is most likely as a positive regulator of P-element mRNA translation. *P*-element transcripts contain multiple Bruno-1 binding sites, and are often observed in cytoplasmic aggregates in female germaria, and the frequency of which are increased in bruno-1 mutants. Cytoplasmic *P*-element aggregates do not contain me31B, as often occurs with translationally repressed mRNA. We propose that *P*-element transcripts may co-opt Bruno-1 to escape self-aggregation, achieve translation, and ultimately transpose. Our work highlights how transposable elements can harness available host regulatory machinery in germline cells to ensure their selfish propagation.

758T Characterization of test-specific sugar transport and glycolysis genes in *Drosophila melanogaster*

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Twenty-five genes in the *Drosophila melanogaster* genome are annotated as SLC2 type sugar transporters, and five of the sugar transporters appear to be expressed only in the testis. Additionally, several genes encoding enzymes that function in glycolysis have testis-specifically expressed homologs or testis-specific splice variants. The function of these testis-specific genes in spermatogenesis is not clear. During spermatogenesis, germline cells develop into sperm while completely surrounded by two somatic cyst cells. How sugars molecules transit the cyst cells and enter germline cells is not understood. Sugar molecules could be transported out of the cyst cells into the germline cells. Alternativity, pyruvate generated in the cyst cells by glycolysis could be converted to lactate and then transported from cyst cells to the germline cells. The enzyme lactate dehydrogenase could convert lactate back to pyruvate which could be used to generate ATP. This lactate-shuttle is observed in mammalian spermatogenesis. The *Drosophila* germline encodes one testis-specific homolog of lactate dehydrogenase. To assess the role of glycolysis and lactate transport during spermatogenesis, we are using RNAi in the germline and cyst cells of the testis to knockdown function of genes that potentially play a role in the lactate shuttle between cyst and germline cells.

759T Exploring a role for germline Gliotactin during *Drosophila* oogenesis

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In the fruit fly *Drosophila melanogaster,* quality checkpoints during oogenesis prevent female energy investment in low-quality oocytes. For example, either aberrant development or suboptimal nutritional status can activate the mid-oogenesis checkpoint, leading to germ cell degeneration through apoptosis. We previously reported that the axon guidance receptor Frazzled [Fra; Deleted in colorectal cancer (Dcc) in vertebrates] regulates the mid-oogenesis checkpoint. Specifically, germline loss of Fra leads to germline apoptosis at mid-oogenesis; however, how Fra controls oocyte development remains unclear. Intriguingly, Fra activity at mid-oogenesis is independent of its canonical ligand, Netrin. Instead, germ cells require the transcriptional activity of Fra to develop into mature oocytes.

We have identified the septate junction protein Gliotactin (Gli) as a possible Frazzled transcriptional target. Like Fra, Gli is expressed throughout organismal development, but was initially characterized in the embryonic nervous system. In the adult ovary, Gli expression depends on Fra expression: both *fra* ovaries with mutant germlines and ovaries overexpressing Fra have altered levels of Gli transcript. We expect that transcriptional targets of *frazzled* are expressed in the germline and required for progression through mid-oogenesis. Previous reports indicate that Gli is expressed at tricellular junctions in the ovarian soma and in border cell clusters, but its expression in germ cells has not been described. In contrast to *fra* loss of function, Gli knockdown in either the adult ovarian germline or soma does not increase apoptosis in the adult *Drosophila* ovary. Consistent with these data, flies with depleted germline Gli lay a similar number of eggs to control flies. However, Gli knockdown in germ cells, but not in somatic follicle cells, leads to changes in egg chamber morphology at late oogenesis. Specifically, nurse cells in egg chambers lacking germline Gli appear to encroach on oocyte territory after mid-oogenesis. While we continue to investigate the root cause of this phenotype, it appears to be biologically meaningful: eggs laid by females with germline Gli knockdown hatch less frequently than those laid by control females.

We are currently generating tools to further explore the role of Gli in the adult ovary, including alleles for genetic mosaic analysis and a transgenic fly line for rescue experiments. Future work will investigate mechanisms by which germline Gli maintains egg chamber architecture and explore a connection to Fra signaling. Together, these studies will shed light on the cellular mechanisms that preserve oocyte quality.

760T Stochastic process model of meiotic crossover patterning under hypoxic conditions in the presence of experimental mortality

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The rate and distribution of crossing-over along the meiotic chromosome axis has a critical role in both preventing aneuploidy and shaping genome-wide polymorphism. However, classic recombination testcrosses have a substantial missing data problem. Whenever crossing-over in parental meiocytes is inferred from genotypes of offspring, then the loss of information between the F_1 meiotic events of interest and the observable F_2 phenotypic classes introduces both uncertainty and bias into estimated rates and distributions. This loss is particularly drastic under stressful conditions (e.g. hypoxia) for female-limited crossing-over in *Drosophila melanogaster* where 75% of meiotic chromatids segregate to polar body nuclei and up to another 15% are lost to treatment-induced experimental mortality. We address this fundamental limitation by modeling the data-generating process with a stochastic function describing the underlying meiotic events in the F_1 females and then accounting for F_2 experimental mortality. Briefly, this process proposes: 1) double strand breaks occur as a homogeneous Poisson point process along the real half line, 2) a proportion of breaks mature into crossover events in a stationary renewal process with their spacing reflecting the strength of crossover interference, 3) random one-half thinning of this point process accounts for asymmetric female meiosis, 4) fertilization of oocytes by X vs. Y-bearing sperm is mendelian, and 5) the survival of resulting zygotes is modeled as binomial with a rate parameter determined by F_2 phenotypic class. We fit this extended $Cx(Co)^m$ counting process to a series of nested viability models in a 11,085-fly, 6-point testcross [y^1, sc^1, cv^1, v^1, f^1, car^1, $Dp(1;1)sc^{V1}$, y^+] covering the full X chromosome under standard lab conditions. Despite an absolute experimental mortality of 39% (with 33% due to sex-specific viability effects associated with both mutant and wildtype alleles at marker loci) we recovered a total X chromosome genetic map length of 76.8 cM with 80% power to detect 3 cM differences. When this testcross was conducted in airtight chambers with either 21% O_2 (normoxia) or 8% O_2 (hypoxia), marker-associated mortality increased 20% relative to controls and the distribution of F_2 recombinant flies changed substantially, suggesting hypoxia-induced meiotic plasticity. However, applying the extended $Cx(Co)^m$ counting model accounting for sex-specific viability effects revealed these changes only correspond to 1-2 cM difference. This study provides a rigorous null hypothesis statistical testing (NHST) framework for disentangling pervasive marker viability effects from changes in the crossover patterning mechanism in studies that introduce strong genetic and environmental perturbations to meiosis.

761T The niche compensates for catastrophic germ cell loss by altering stem cell cytokinesis

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Tissue homeostasis requires a regulated balance in production of differentiating daughters, or progenitors, from the stem cell population. This balance is controlled by regulatory signals within the specialized stem cell microenvironment or niche. It has been found that in many systems, stem cells respond to progenitor loss by inducing increased rates of stem cell divisions. However, the specific mechanisms by which stem cells and their progenitors respond to tissue atrophy remain elusive. Using the testis as a model system, we used two genetic models to induce progenitor loss and elucidate the subsequent effects on the niche following this stress on the tissue. During homeostasis, germline stem cells (GSCs) engage a delayed cytokinesis program, the timing of which is controlled by niche derived Jak/STAT signaling. Following contractile ring disassembly, GSCs pause cytokinesis progress through formation of a secondary F-actin ring; the ring's persistence is controlled by Jak/STAT signaling and determines the timing of cytokinesis completion and release of daughter cells. We find that induction of germ cell death outside of the niche triggers faster cycling of the GSCs and faster completion of cytokinesis allowing for an expedited release of daughters into the diminished progenitor pool. Importantly, we have discovered that these behavioral changes in the stem cells are driven by modulation of the niche architecture. Following progenitor loss, a compensatory mechanism is enacted to increase niche size through the incorporation of new niche cells. Interestingly, this modulation of the niche is plastic following reprieve of stress from the system. After a rest period of no more progenitor loss, the niche size reduces through loss of niche cells. The enlarged niche, following progenitor loss, results in increased niche signaling to the adjacent stem cells. We find that faster rates of GSC cytokinesis are driven by increased activity of the Jak/STAT pathway. Critically, combining depletion of STAT with induction of progenitor cell death results in loss of the expedited release of daughter cells from the GSCs. Indeed, in this context, GSC cytokinesis fails and daughter cells are no longer released from the niche. Together, this data supports a model whereby under catastrophic progenitor loss, a larger niche is formed, driving a faster release of progenitor cells to replenish the lost tissue.

762T Is the Spindle Orienting Machinery at work in the Female Germline?

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Germline cyst formation in Drosophila requires precisely four rounds of synchronous, incomplete mitotic divisions, generating 16-cell cysts from which a single oocyte is specified. While bag of marbles (bam) initiates cystoblast differentiation (Ohlstein & McKearin, 1997) and cyclin degradation terminates divisions (Lilly, de Cuevas & v Spradling, 2000), the molecular mechanism ensuring exactly four division rounds remains unclear. One possibility is that division is controlled by the canonical mitotic spindle orientation machinery, which includes Mushroom body defect (Mud), Partner of Inscuteable (Pins), and Discs large (Dlg). These proteins work with dynein to generate a localized pulling force that acts on astral microtubules to regulate spindle orientation across systems and cell types. Their function in the germline is unexplored. Our preliminary results indicate that Mud, which is the direct adaptor for dynein, has no obvious function in developing the germline cyst. However, we find that Pins and Dlg do; both factors help to determine the number of cells in each germline cyst. These results suggest that Pins and Dlg, which can interact directly, are working independently of the spindle orienting machinery in germline cyst development. We are now working to understand the mechanism.

763T Juvenile hormone signaling regulates N-Cadherin expression in the male germline stem cell niche

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Hormonal signaling is essential to gametogenesis. As in most insects, in *Drosophila melanogaster* retinoid-like Juvenile hormones (JHs) are essential to female fertility by supporting oogenesis, reproductive diapause, and vitellogenesis. However, the role of JHs in male fertility is less understood. Here, we defined where JH signaling is active in the testes and how it supports male fertility. To this end, we first measured male fertility using a sperm exhaustion assay in Drosophila lacking either a key JH synthesis enzyme or one of two classes of JH degradation enzymes and found that both JH synthesis and degradation are required for male fertility. These data suggest that either a precise JH titer or spatiotemporal distribution of JH signaling is necessary for male fertility. To distinguish between these two possibilities, we investigated where JH signaling is active using a fluorescent JH reporter, finding that JH signaling is active in the germline stem cell (GSC) niche, or hub, of male larval, pupal and adult testes. To investigate how JH signaling supports GSC niche function, we determined how hub identity and GSC homeostasis was affected in mutant larvae lacking the JH transcription factors, Methoprene tolerant (Met) and germ cell expressed (gce). We found that N-Cadherin (CadN), a marker of the testis GSC niche, was absent in these *Met, gce* mutant testes, suggesting either CadN is a target of JH signaling or niche cell identity was compromised. To assess hub identity, we knocked down Met or gce and assessed the expression of multiple niche markers. We found that only CadN expression was reduced, suggesting CadN is a target of JH signaling. Further supporting this conclusion, we found that JH degradation enzyme mutant larvae have increased CadN mRNA expression levels relative to wildtype controls. Consistently, modENCODE ChIP data indicates there are several JH transcription factor binding sites along the Drosophila *CadN*. Together, these data not only suggest that CadN expression is regulated by JH but also JH signaling may support male fertility in part by maintaining CadN levels in male GSC niches.

764T STIL is specifically expressed and required in the *Drosophila* female germline

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Female germ cells go through a dramatic differentiation process to give rise to a viable oocyte. Numerous RNAs and proteins are required during this process, however, the transcriptional control of these programs are poorly understood. The putative transcription factor, *stand still* (*stil*), is required for female germline development in *Drosophila*. The *stil* locus is bound by and transcriptionally responsive to another essential germ cell-specific transcription factor, OVO. Therefore, we sought to better understand the relationship between STIL and OVO that drives female germ cell differentiation. We have found that endogenously tagged STIL localizes to germ cell nuclei in all stages of oogenesis, just like OVO. New *stil* loss-of-function alleles lead to germ cell death in ovaries, ranging from exclusively “germ pockets” containing mitotically dividing germ cells to a complete loss of germ cells. Furthermore, *stil* RNAi ovaries exhibit delayed oocyte selection and meiotic arrest. RNA-seq on *stil* RNAi ovaries and somatic cells ectopically expressing *stil* generated a list of STIL-regulated genes composed largely of mitotic and meiotic regulatory pathways. Surprisingly, immunoprecipitation followed by mass spectrometry revealed that STIL is not in complex with OVO, and instead the most significant protein to pull down with STIL was the uncharacterized CG9346, which shares some homology to a human splicing factor. Altogether, these results suggest that STIL is required for regulating the mitosis-to-meiosis transition in ovaries and that it may do so by transcript regulation. We will be exploring this further by conducting RNA immunoprecipitation followed by sequencing to see if STIL interacts directly with RNA, and which RNAs, to further allow us to understand the role of STIL in female germ cell development.

765T Over-expression of *Eip75B* in ovarian somatic cells disrupts follicular morphogenesis and egg chamber development

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Nuclear receptors link nutritional signals to cellular responses necessary for development, reproduction, and viability. In *Drosophila*, two nuclear receptors, Ecdysone Receptor (EcR) and *Ecdysone-induced protein 75B* (*Eip75B*), are transcribed in response to the steroid hormone ecdysone and genetically interact to regulate developmental transitions and reproduction. Characterized by a unique N-terminal sequence, *Eip75B* has three protein isoforms. Depending on the cellular context, *Eip75B* functions as either a transcriptional target of EcR or a repressor of EcR-responsive genes. Yet while both are expressed and necessary for proper oogenesis, their molecular relationship in ovarian cells is unclear. Previous studies found that loss of *Eip75B* arrested egg chamber development during mid-oogenesis, and isoform-specific over-expression resulted in egg chamber apoptosis. However, it is unclear which cells control egg chamber viability, or how *Eip75B* controls apoptosis. To address this question, we created transgenes that over-express *Eip75B-A* or *Eip75B-B* that can be driven in either the germline or the soma. Over-expression of either isoform in all somatic cells results in developmental lethality. When over-expression was restricted to ovarian follicle cells and the brain, we detected a block in mid-stage oogenesis and an increase in follicle cell apoptosis, recapitulating prior studies. Moreover, we found that over-expression of *Eip75B-A* or *Eip75B-B* resulted in broad mis-regulation of follicle cell identity prior to apoptosis. Our current studies seek to over-express *Eip75B* in specific follicle cell populations to find which subtypes are responsible for egg chamber developmental progression. Given that follicle cell identity is also regulated by EcR, we are also currently testing the interaction of these proteins in follicle cell development. Together, the data suggests that *Eip75B* is sufficient to regulate egg chamber apoptosis in response to environmental factors.

766T OVO target genes are dependent on OVO and an unknown cofactor for full expression

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The *Drosophila* female germline-specific transcription factor OVO has recently been shown to transcriptionally regulate numerous well characterized maternal-effect genes that are required for embryonic development. OVO binds at open transcriptional start sites genome-wide, and this binding positively influences gene expression. However, it is still unclear exactly the mechanism through which OVO regulates transcription. We hypothesize that OVO may be modulating chromatin accessibility to promote transcription. To test this model, we utilized two OVO germline target genes, *bicoid* (*bcd*) and *oskar* (*osk*), and placed the OVO bound genomic fragment (OVO enhancer fragment) upstream of a basal promoter and H2A-3xV5 reporter gene. We also made a version of the same OVO enhancer fragments but deleted all the significant OVO DNA binding motifs within. These reporters were placed in a well-established heterochromatic and euchromatic regions on the X chromosome and found that both the *bcd* and *osk* reporters were able to drive expression of the H2A-3xV5 specifically in germ cells of the ovary, which is where OVO is expressed. In contrast, the enhancer fragments without the OVO DNA binding motifs lacked reliable germline expression. This result indicates that OVO binding is required for prolonged enhancer activity. We wanted to test if OVO binding alone was sufficient in activating germline-specific promoters within somatic cells. We saw no expression of the reporter when only the OVO binding site was present, but when another strong transcription factor binding site was also present, there was strong germline expression, suggesting that OVO alone is not sufficient to activate transcription, and it requires a cofactor. We are currently conducting experiments to test OVO's transcriptional regulation in a non-native context via ChIP, ATAC-seq, RNA-seq and Micro-C. Moving forward, we are also interested in determining what proteins are in complex with OVO at these regions to better understand how OVO directs female germ cell specific transcription.

767F Effects of environmental agrotoxins on oviposition habitat choices

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Agricultural toxins like herbicides can spread to other habitats through wind, water, or non-agricultural direct application, where all types of organisms can be exposed. Glyphosate-based herbicides (GBH), such as Roundup, are the most commonly used pesticide, in the US and internationally. We examine the effects of glyphosate and GBH on *Drosophila melanogaster* as a model organism to establish extent and mechanisms of toxicity to animals. We previously established high levels of mortality in larvae, as well as behavioral and reproductive effects. In current studies, we examine oviposition habitat choice of flies not previously exposed to GBH to determine whether they are able to identify and avoid GBH contaminated oviposition sites to promote offspring survival. Newly eclosed adults were kept in mixed sex groups with yeasted medium for 7 days. We moved 30 females into each oviposition chamber with a 4-quadrant petri dish containing medium with varying concentrations of Roundup Super Concentrate for 3 hours followed by counting eggs in each quadrant. We were surprised to find that females laid most eggs in the highest concentration quadrant available (Carballo & Lambert), but colleagues found the expected opposite effect (Rizzo & Johnson). Since then we have endeavored to determine why females choose an oviposition habitat that was highly lethal to their offspring, focusing on the hypothesis that females respond to GBH-induced reduction of bacterial populations rather than to GBH itself. We have introduced tetracycline or LPS with and without GBH to manipulate perceived bacterial load. The antibiotic tetracycline with or without GBH increases oviposition, while preliminary results suggest that LPS, which mimics the presence of *E. coli* bacteria, reduces oviposition, consistent with our hypothesis. In addition, we are attempting to clarify the discrepancy between results from the two labs by using different types of medium (non-nutrient agar, molasses/cornmeal medium) and comparing 2 vs. 4 concurrent treatments. Non-nutrient agar induced the same amount of oviposition as GBH-free molasses/cornmeal medium, both less than GBH contaminated medium. Preliminary results indicate that, in our hands, females lay more eggs in GBH contaminated medium regardless of whether they have 2 (control, GBH 10 g/L) or 4 choices (control + 3 concentrations of GBH up to 20 g/L). Overall these results support the hypothesis that GBH contaminated medium attracts fecund females by killing bacteria, however the inter-lab discrepancies remain unexplained. This may indicate that flies are unable to discriminate directly between contaminated and uncontaminated oviposition habitats, leaving resulting larvae at high risk. To the extent that these results can be generalized to other insects, GBH may have dramatic effects on insect populations in agricultural and nearby environments.

768F Mechanisms of Glyphosate-Based Herbicide Toxicity: Mortality, reproduction and behavior

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While people use herbicides to kill plants, extensive evidence indicates that they can also be toxic to animals. Glyphosate-Based herbicides (GBH), like Roundup, are the most commonly used pesticide, used around households, in public spaces, and in agriculture before planting, during growth on Roundup Ready crops, and as a pre-harvest desiccant on seed crops like grains and legumes. Our lab group uses *Drosophila* as a model system to examine GBH toxicity to animals, and has identified effects on survival of adults and especially larvae, ovary and testes size and reproductive output, and multiple behaviors including feeding, activity and oviposition. More recently, we are exploring several non-exclusive hypotheses about mechanisms of GBH toxicity, including the role of genetics, hormones, the nervous system and characteristics of the microbiome of the substrate. Using the *Drosophila* Genetic Reference Panel, we are exploring quantitative genetic architecture to identify genes that impact GBH susceptibility. We have determined that lines vary greatly in susceptibility, and begun to explore individual genes involved including some related to the internal microbiome. We used precocene and methoprene to inhibit and mimic the activity of Juvenile Hormone (JH) on ovary and testes size in exposed and unexposed flies. GBH exposure decreases the size of ovaries and testes, as does precocene. However, precocene does not affect ovary or testes size in exposed flies, suggesting that GBH might also disrupt the JH pathway. Preliminary results suggest that methoprene increases ovary and testes size, and we are exploring it's interaction with GBH exposure. Both GBH and a new formulation of Roundup that is not glyphosate-based impact geotaxis, a corollary of neuromuscular health. This affect correlates with locomotor behavior (activity), suggesting a role of neurotoxicity in causing the effect of GBH on activity. We have mixed results regarding the effect of nutritional state on activity level using protein content as a measure of nutritional state and by altering protein content using dietary treatments. We suspected that the antimicrobial activity of GBH might affect nutrient availability, and account for it's effects on activity, but that appears to not be the case. However, we do have evidence that habitat choice for oviposition is influenced by the microbiome of the habitat and the effect of GBH on it. We have identified a role of genetics, hormones, neural systems, and microbiomes in mediating toxic effects of GBH on various aspects of survival, reproduction and behavior. These results provide evidence that herbicides affect many aspects of insect biology in ways that could dramatically affect population size, particularly near agricultural areas. This would interfere with ecosystem services provided by insects, including pollination, required for about 75% of the crop species consumed by humans.

769F How an RNA binding protein cascade controls the switch from cell proliferation to differentiation

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The switch from cell proliferation to cell differentiation must be tightly regulated in the adult stem cell lineages that maintain and repair tissues. Insufficient proliferation can impair tissue maintenance, while failure to stop proliferation can lead to cancer. Using Drosophila spermatogenesis as a model adult stem cell lineage, we are discovering that the switch from proliferation to differentiation is controlled by a regulatory cascade of RNA binding proteins. Drosophila spermatogonia go through a fixed number of transit amplifying divisions before differentiating into spermatocytes. Action of the Bag-of-marbles (Bam) protein and its RNA-binding structural partners is required for spermatogonia to stop proliferating and differentiate into spermatocytes. Previous work in the lab showed that the number of transit amplifying divisions is set by the time it takes Bam protein to reach a critical threshold, and that the key role of Bam for the switch is to repress expression of the RNA binding protein Held-Out-Wings (HOW). Previous studies showed that HOW, and its mammalian homolog Quaking, can alternatively splice target transcripts in immune, muscle, and neural cells (Neumann et al., 2022). We propose a model in which HOW, in male germ line stem cells and early spermatogonia, causes alternative splicing of target transcripts, leading to expression of protein isoforms that drive proliferation and/or block differentiation. When accumulation of Bam protein in mid-to-late transit amplifying spermatogonia represses HOW expression, these proliferation transcript isoforms are lost and/or new isoforms that drive spermatocyte differentiation become expressed. Through a combination of biochemical and bioinformatic approaches, I am identifying potential targets of HOW to test for function in regulating spermatogonial proliferation and spermatocyte differentiation.

770F Advantages of Combining Stage-Enriched Bulk and Single-Cell RNA-Seq to Improve Detection of Post-Meiotic Gene Expression in Drosophila Spermatogenesis

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Over the past decades, single-cell RNA sequencing (scRNA-seq) has become an important tool for transcriptomic studies, enabling unprecedented resolution of cellular heterogeneity. However, technical issues such as dropout events and shallow coverage can limit the detection of lowly expressed or rare transcripts, particularly in late spermatogenesis stages. Drosophila spermatogenesis, characterized by a continuous progression from stem cells to highly specialized post-meiotic cells, exemplifies this challenge. In this study, we demonstrate that stage-enriched bulk RNA-seq of isolated post-meiotic cells detects a higher number of post-meiotically expressed genes compared with recent single-cell and single-nucleus RNA-seq datasets. Direct comparison between these approaches reveals that, while bulk RNA-seq lacks single-cell resolution, it provides superior sensitivity for capturing global gene expression in rare or differentiated populations such as post-meiotic cells. We show that integrating stage-enriched bulk RNA-seq with single-cell data is a more accurate and comprehensive view of gene expression dynamics throughout spermatogenesis. This combined approach mitigates the inherent biases of each individual method, enhancing detection power without losing cell-type specificity, in post-meiosis and highlights the complementary strengths of both strategies in uncovering the full complexity of spermatogenic transcriptional programs.

771F Sec23 and Sec24 form a heterodimer involved in COPII vesicle trafficking that is critical for *Drosophila* oogenesis

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The process of cell fate determination is a major question in developmental biology, and the current understanding of mechanisms by which it occurs is very limited. In *Drosophila melanogaster*, oogenesis begins with the division of germline stem cells, eventually leading to the formation of a 16-cell cyst. From here, one is selected to become the future egg, while the other 15 become supporting nurse cells. An EMS mutagenesis genetic screen in *Drosophila* identified several mutations in genes that lead to defects in oocyte determination, ultimately leading to a loss of eggs and fertility. Sec24CD, which forms the inner part of the vesicle coat involved in COPII anterograde trafficking, was identified from this screen. Since this was the first known role for COPII trafficking in oogenesis, we were interested in investigating whether other COPII components were also involved in oogenesis. Sec23 interacts with Sec24 to create the inner coat of COPII vesicles. To study and analyze the role of Sec23 in oocyte determination, Sec23 CRISPR mutants were studied; disruption via CRISPR resulted in rudimentary ovaries lacking recognizable egg chambers, demonstrating that Sec23 is required early in oogenesis. However, given the dramatic phenotype observed, investigation of Sec23 in oocyte identity could not be assessed. RNAi knockdown of Sec23 was performed using a variety of germline-specific Gal4 drivers to visualize the effect of Sec23 on oocyte formation. A variety of mutant phenotypes resulted, indicating Sec23 is necessary during oocyte development. Investigating the role of Sec23 and COPII vesicle trafficking allows for a better understanding of the molecular mechanisms that control oocyte fate determination in *Drosophila*. Additionally, current efforts pursuing the identification of COPII vesicle cargo through TurboID analysis will provide insight into what proteins are necessary for oocyte fate determination.

772F Altering Misshapen phosphorylation sites in the *Drosophila* egg chamber

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Intercellular bridges are an essential structure found in developing germ cells. These cell-cell connections allow for sharing of cytoplasmic material and coordination of behavior; defects in intercellular bridges can lead to infertility. Some of the largest intercellular bridges connect the germline cells in the developing egg chamber. Each egg chamber contains a cluster of 16 germline cells (1 oocyte and 15 supporting nurse cells), which are connected by 15 ring canals. Ring canals are formed following remodeling of the midbody after germline cell division. They mature to form a multilayered structure, which allows them to expand approximately 10-fold in diameter during oogenesis. Defects in ring canal formation, stability, or expansion can lead to nurse cell multinucleation, incomplete nurse cell dumping, and infertility. Currently, we have only a partial understanding of the pathways and proteins that promote ring canal expansion and maintain stability. The Misshapen (Msn) kinase localizes to germline ring canals, and it has been shown to regulate ring canal size and stability. Despite its critical role in the germline, the mechanisms that regulate Msn localization and activity are not known. In the intestine, the Tao kinase has been shown to phosphorylate and activate Msn. Therefore, the goal of this work is to determine whether the same phosphorylation site affects Msn localization and/or activity in the germline. Future experiments could further investigate the role of Tao and other potential regulators of Msn.

773F Elucidating the roles of inorganic ions during egg activation in *Drosophila melanogaster*

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Nearly all animals' eggs undergo an oocyte-to-embryo transition, called egg activation. This is a critical period when an arrested oocyte resumes its biological progression to initiate embryonic development. A rise in intracellular Ca^{2+} levels is a universal signal for egg activation. In Drosophila, mechanosensitive Trpm channels in the oocyte membrane mediated Ca^{2+} influx when the oocyte leaves the ovary during ovulation. In some animals, Mg^{2+} or Sr^{2+} can mimic some effects of Ca^{2+} in egg activation. We investigate how extracellular Mg^{2+}, Sr^{2+}, or Zn^{2+} affect egg activation in Drosophila. Our preliminary results show that Mg^{2+} and not Sr^{2+} can allow activation of Drosophila oocytes in the absence of Ca^{2+}; in contrast, increased Zn^{2+} inhibits Drosophila egg activation. This result is consistent with our previous finding that Zn^{2+} levels drop upon activation. It also resonates with studies of mammalian oocytes that report an inhibitory role for zinc, which is expelled from oocytes via "zinc sparks" during their activation. Our findings advance our understanding of fundamental mechanisms that activate eggs for embryogenesis and establish strong parallels between those in Drosophila and other animals.

774F Fueling Fertility: Probing the Role of *INDY2* in *Drosophila* Testes Function

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Male infertility accounts for nearly half of all infertility cases worldwide, yet the underlying molecular mechanisms remain poorly understood. Members of the human Solute Carrier (SLC) family, particularly SLC13A (Na^+-coupled anion transporters) and SLC5A1 (Na^+-glucose cotransporter, SGLT1), play critical roles in metabolic regulation and male reproductive physiology. Previous studies demonstrated that SLC13A1-deficient mice exhibit reduced fertility, whereas pharmacological activation of SLC5A1 enhances sperm quality, underscoring a conserved connection between membrane transport, cellular metabolism, and fertility. In *Drosophila melanogaster*, two orthologues of SLC13 exist- *Indy* (I am not dead yet*)* and *Indy2*. While *Indy* has been extensively characterized for its role in citrate transport, metabolism, and lifespan regulation, *Indy2* remains largely uncharacterized. *Indy2* is a protein-coding gene located on the third chromosome encoding a 592-amino-acid transmembrane protein. Sequence alignment revealed 29.61% and 13.14% homology with human SLC13A1 and SLC5A1, respectively. Conserved domain analysis (NCBI CDD) identified *Indy2* as a member of the CitT superfamily, predicted to function as a citrate transporter with 11 transmembrane helices and intracellular N-terminus and extracellular C-terminus orientation. Transcriptomic data from FlyAtlas2 indicate that *Indy2* is exclusively expressed in adult testes. Consistently, our preliminary data confirmed that *Indy2* transcripts and protein localize to the testis membrane. Functional assays revealed that *Indy2* mutants display significantly reduced fecundity and fertility, evident by decreased egg laying, pupal eclosion, and adult progeny counts, suggesting its essential role in reproduction. Reciprocal crosses between control and mutant males/females are underway to determine whether the fertility defect is male-specific. Cytoskeletal staining using phalloidin (F-actin) and DAPI revealed disrupted actin filaments in *Indy2* mutants, indicating compromised sperm structure and motility. Given that mitochondria generate ATP required for F-actin polymerization, we are investigating potential alterations in mitochondrial structure and function. Since CitT-family transporters regulate TCA intermediates and ATP metabolism, *Indy2* loss of function may impair testicular energy metabolism, thereby affecting sperm integrity. Collectively, our findings identify *Indy2* as a novel, testis-specific citrate transporter essential for sperm structure and male fertility in *Drosophila*, offering new insights into conserved metabolic mechanisms underlying male infertility.

775F Functional unknomics through structure-informed homology detection in *Drosophila*

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Nearly a fifth of the annotated protein-coding genes in well-studied model organisms, including humans, have no known molecular function. The absence of putative function for many of these proteins can partly be attributed to the failure of homology detection at the sequence level, either due to rapid protein divergence or the potential emergence of de novo genes. In Drosophila melanogaster, 498 protein-coding genes are annotated in the Pfam database, which contains 151 domains of unknown function (DUFs). Of these, over 200 protein-coding genes are either highly enriched or restricted to brain and testis tissues, based on the modENCODE tissue profiling data; yet, their functional roles remain unknown. Here, we utilize AlphaFold protein structure predictions and remote structural homology detection to assess the similarity of DUFs to previously characterized protein domains and conduct functional studies to elucidate the putative functional roles of DUFs. First, to evaluate the accuracy of AlphaFold predictions, we examined Drosophila proteins with experimentally solved structures in the Protein Data Bank that were not part of AlphaFold's training dataset. We found that, on average, AlphaFold predictions are more than 70% accurate using the TM-score metric for assessing topological similarity. We then used Foldseek to detect structural homology for Drosophila DUFs and identified remote homology for 236 proteins containing 64 DUFs in the Swiss-Prot and PDB databases with a Foldseek similarity e-value < 0.05. Our findings from the Foldseek searches were independently validated using additional distant-homology detection methods that incorporate protein language models. Nearly 40% of DUF proteins for which we could assign a putative function based on domain similarity are highly expressed in the testes. As fertility-related phenotypes are relatively easy to identify from RNAi knockdown and CRISPR knockout resources available in Drosophila, we conducted a loss-of-function screen for 10 proteins containing DUF4777 and DUF4780, which we have identified as related to winged-helix domain and the RNA-recognition motif domain (RRM), respectively. Of these, we identified CG9641, which contains a DUF4780/RRM-domain as a novel male sterility gene. We are currently seeking a functional understanding of the roles of CG9641 during Drosophila spermatogenesis. Overall, our study demonstrates the effectiveness of structure-guided homology detection in investigating the putative functions of previously uncharacterized proteins. Additionally, our computational pipeline can be readily scaled to characterize DUFs in annotations from other model organisms, including vertebrates.

776F Genetic Interaction between Tao and Misshapen in the Germline

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Intercellular bridges are structures that enable the transport of materials between adjacent cells. They are found within developing eggs and sperm across the animal kingdom, but some of the largest intercellular bridges are found in the developing egg chamber. Within the egg chamber, these structures, known as ring canals, allow the transfer of cytoplasmic material from nurse cells to the developing oocyte. The formation and maintenance of these ring canals is a highly regulated process that ensures proper oocyte growth and development. Therefore, discovering the proteins and pathways that regulate ring canal stability and expansion is essential. Recent studies have shown that the Ste20-like kinase Misshapen (Msn) localizes to the ring canals during oogenesis and regulates their size and stability. However, the upstream mechanism by which Msn is activated remains unknown. In other Drosophila tissues, including the midgut and border cells, Msn is phosphorylated by Tao, a serine/threonine kinase belonging to the Ste20 family of MAP4Ks. Therefore, the current study hypothesizes that Tao similarly activates Msn in the germline, regulating ring canal expansion or stability. To test this hypothesis, germline expression of Tao and Msn will be altered. Future work will focus on dissecting how this kinase pair regulates the structure and stability of the ring canals during oogenesis.

777F Investigating the Role for RNA Binding Protein Staufen in rDNA Magnification

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The germline exists to transgenerationally transmit genetic information, yet inherently unstable genomic regions, such as tandem repeats like ribosomal DNA (rDNA), complicate this function due to their tendency to be spontaneously lost through intrachromatid recombination. Thus, the germline mechanisms must combat repeat instability to ensure germline stability across generations, especially at essential repetitive regions like rDNA. rDNA magnification is a mechanism that recovers lost rDNA copies through the asymmetric division of male germline stem cells (GSCs) in *Drosophila.* During asymmetric GSC division, a process called unequal sister chromatid exchange (USCE) creates increased rDNA copy number at a single sister chromatid during replication, and that chromatid is then biasedly segregated to the daughter that remains a GSC instead of the differentiating cell. However, the mechanisms that generate these unequal chromatids and achieve biased segregation of USCE products in GSCs remain unknown. Here we show that the protein Staufen (Stau), an RNA binding protein that directs cell fates in other asymmetric divisions, restricts rDNA magnification activity in GSCs. We are now testing the possibility Stau regulation of rDNA magnification functions through regulating the asymmetric properties of USCE.

Though Stau has well characterized roles in other cell types, including the female germline, its activity in the male germline remains unclear. Through an ongoing screen of possible rDNA magnification repressors, we identified that Stau inhibition in GSCs sporadically induces rDNA magnification. Using immunofluorescence, we discovered that Stau protein is asymmetrically localized within interphase GSCs, with more Stau foci localized at the hub adjacent side. Furthermore, we also see that Stau foci are more abundant in GSCs compared to GBs. These findings suggest that Stau may have a role in influencing the partitioning of cellular components during asymmetric GSC division, which may regulate GSC-specific USCE activity. We are actively investigating if Stau is asymmetrically inherited during GSC division and determining if Stau regulates the production and segregation of unequal rDNA products during USCE. By understanding Stau's function within the context of rDNA magnification in asymmetric division we will have more insight into how asymmetrically divided factors direct the biased inheritance of genomic content during lineage asymmetry.

778F Using CRIMIC tools to identify novel germline-enriched genes in *Drosophila* females

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In addition to serving as a source of DNA for the next generation, mature oocytes also provide many essential determinants necessary to support embryonic life post-fertilization. For example, many maternal mRNAs are transcribed in early germ cells, from which oocytes differentiate and mature. Single cell RNA sequencing data (scRNA-seq) identified many genes expressed in germ cells; however, it is unclear whether germ cell-enriched mRNAs contribute specifically to oocyte development, or are instead enriched in the oocyte as support for the developing embryo. Moreover, many of the genes enriched in germ cells remain incompletely characterized at the functional level. To begin to better understand the function of germ cell-enriched genes, we mined scRNA-seq data for germ cell-enriched genes and assessed their expression *in situ* using available CRISPR-mediated integration cassette (CRIMIC) gene lines, in which *Gal4* is inserted into the endogenous gene locus. Ovary samples were co-labeled with anti-Hu li tai shao and anti-LaminC antibodies to distinguish individual ovarian cell populations and a fluorescent reporter to visualize expression of the gene of interest. Genes were classified corresponding to their expression in somatic, germline, or stem cell populations. Among these, we identified *CG17597*, orthologous to human *Sterol Carrier Protein 2* (*SCP2*), as a novel germ cell-enriched gene. Intriguingly, despite mRNA enrichment in mitotic germ cells, we also found that many CRIMIC reporters reflected germ cell-enriched expression only in post-mitotic germ cells. Given the similarity across independent CRIMIC insertions, we hypothesize that the CRIMIC vector design does not support Gal4 expression in germline stem cells or undifferentiated germ cells. To test this hypothesis, we redesigned the CRIMIC vector with a P10-based 3' untranslated region and are currently comparing expression to the original lines. By crossing these new flies with a UASp-based reporter strain, we expect to see more uniform reporter expression throughout the germarium. If successful, this approach will allow us to better assess the transcriptional output of undifferentiated germ cells and generate new tools for the germline community.

779F Establishing the role of nuclear pore proteins in maintaining transcriptional quiescence in the early germline

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Germ cells are the specialized cells that give rise to gametes, ensuring the continuity of life across generations. These cells are distinguished by the presence of germ granules, assemblies of maternally deposited RNAs and proteins that play a crucial role in germ cell development and function. The primordial germ cells (PGCs) are the first cells to form during Drosophila embryogenesis. PGCs inherit the germ granules, which confer maternal control over the cells and promote PGC-specific developmental programs. One of these programs establishes transcriptional quiescence in the nascent germline, delaying the transition to zygotic control until after gastrulation. Two germ granule components critical for this quiescence are polar granule component (pgc), which inhibits carboxy-terminal domain of RNAPII (CTD) Ser 2 phosphorylation (pSer 2) required for transcriptional elongation, and Nanos (nanos), which is essential for translational repression of factors crucial for zygotic expression. Loss of function of pgc and/or nanos results in premature zygotic transcription, leading to loss of PGC identity and subsequent PGC apoptosis. However, other non-germ-granule mechanisms that help ensure this early transcriptional quiescence are not yet fully understood. Previous studies have shown that the Nuclear Pore Complex (NPC) contains two nucleoporins (NUPs) – Megator and NUP98 – that are depleted in PGCs as compared to somatic nuclei. Megator and NUP98 are a part of the NPC nuclear basket, and have been previously shown to play a role in active transcription, suggesting a possible link to transcriptional quiescence. We have shown that Megator expression is depleted in PGCs prior to the onset of zygotic transcription, then slowly rises post zygotic genome activation. This pattern is consistent with a potential regulatory role in the early germline. Previous studies have shown that Megator helps provide an open chromatin environment in the Drosophila genome. Knockdown of Megator by RNAi leads to premature accumulation of pSer 2 in PGCs, suggesting that Megator may be crucial for regulation of genes that maintain transcriptional quiescence in PGCs. Future studies include investigating the role of Nup98 in delaying the MZT of PGCs and investigating mechanisms driving Megator and Nup98 depletion in PGCs, hypothesizing that germline-specific factors such as pgc and/or nanos actively suppress their expression or stability. This approach will uncover novel germ cell specific mechanisms, providing insights into the fundamental mechanisms governing germline establishment with implications for broader fields such as reproductive biology and stem cell research.

780F Beyond metamorphosis: Characterizing the function of *crooked legs* in ovarian development

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In the *Drosophila* ovary, a network of intracellular and extracellular factors regulate the transition from germ cell to viable oocyte. Among these, the steroid hormone ecdysone is necessary in both the germline and soma for proper oocyte development. Although the signaling pathway has long been implicated in oogenesis, the transcriptional targets of ecdysone, and their putative roles in ovarian cell function, remain largely uncharacterized. Our previous studies using single cell RNA-sequencing revealed a zinc-finger transcription factor, *crooked legs* (*crol*), as a putative ecdysone transcriptional target in the germline. Previous studies characterized *crol* as an orchestrator of metamorphosis and a necessary coordinator of imaginal disc development. However, its role in ovarian development remains uncharacterized. Here, we show *crol* is endogenously expressed in both germline and somatic cells of the ovary, with highest levels in early undifferentiated somatic cells in the germarium. To first characterize the role of *crol* in the soma, we depleted its expression in various follicle cell populations using RNAi-based methods. We find that *crol* deficiency in early follicle cells has a significant impact on the progression of germ cell cysts, leading to an increase in germline cyst collisions. This preliminary data highlights a potential non-cell autonomous role for *crol* in maintaining proper encapsulation and progression of developing germ cells. We are currently investigating whether *crol* also autonomously regulates germ cell proliferation and/or differentiation, and whether the expression of *crol* is dependent on ecdysone signaling. Taken together, this work identifies novel roles of a previously uncharacterized protein in the proper development of female germ cells.

781F Investigating the role of Maf-S in the Drosophila ovary

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Stem cell self-renewal must be tightly regulated to maintain tissue homeostasis. The steroid hormone ecdysone directs female germline stem cell (GSC) self-renewal through the coordination of a transcriptional signaling cascade. Ecdysone elicits this response by binding to a heterodimeric complex Ecdysone Receptor (EcR) and Ultraspiracle (Usp). Using single-cell RNA sequencing, we recently identified potential transcriptional targets ofEcR signaling that may regulate GSC function. One potential candidate of interest is Maf-S, a transcription factor from the MAF family. In mammals, the transcriptional regulator Maf-S regulates cell fate and gene expression. However, the role of Maf-S in the ovary remains uncharacterized. We find that Maf-S is expressed in late germ cells and somatic cells. We are currently investigating the function of Maf-S in ovarian cells through gain- and loss-of-function analyses, including two new germline-compatible tools that can over-express Maf-S specifically in germ cells. Understanding the function of Maf-S in the ovary will better our understanding on how steroid hormones influence stem cell fate.

782F JAK/STAT Signaling and Downstream Targets in Soma-Germline Communication During *Drosophila* Spermatid Individualization

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Spermiogenesis in *Drosophila melanogaster* provides a powerful model for investigating the molecular mechanisms underlying sperm maturation and soma-germline communication. A critical terminal step of spermiogenesis is individualization, during which actin-based individualization complexes (ICs) migrate along interconnected spermatid bundles to remove cytoplasmic bridges and produce individual sperm. Individualization requires JAK/STAT signaling in somatic cyst cells; however, the downstream mechanisms regulating this process remain incompletely defined. A previous RNA-Seq study identified 336 genes that were significantly downregulated upon loss of JAK/STAT signaling in somatic cyst cells. To elucidate the potential roles of these candidates in regulating spermatid individualization and soma-germline interactions, we performed gene ontology (GO) analyses using DAVID and PANTHER and integrated these results with STAT92E binding-site proximity data. This approach highlighted overrepresented processes related to cytoskeleton organization, membrane remodeling, and soma-germline communication, and prioritized candidates most likely to act downstream of JAK/STAT during spermatid individualization.

783F Mapping the Synaptonemal Complex Proteome to Understand Meiotic Recombination Regulation

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During meiosis, homologous chromosomes pair, recombine, and segregate to ensure the faithful transmission of genetic material to haploid gametes. Errors in this process can result in aneuploidy, a leading cause of infertility and miscarriage. The synaptonemal complex (SC), a highly conserved protein structure that forms between homologous chromosomes, facilitates crossover formation and is essential for accurate chromosome segregation. Understanding how the SC promotes proper crossover formation is critical, as SC errors can disrupt chromosome segregation. Despite extensive genetic and cytological characterization, the molecular interactions that regulate SC maintenance remain poorly understood.

The SC acts as a dynamic platform with two main functions: facilitating meiotic protein movement and repairing programmed double-strand breaks, as crossovers and gene conversions. To better understand the role the structure of the SC plays in its function, we are taking advantage of a set of partial loss-of-function SC mutants that display defects in SC assembly, premature disassembly, and altered crossover patterning. Using an SC protein endogenously tagged with TurboID, a promiscuous biotin ligase, we will identify protein interactions that are lost or enriched in these mutants. This approach enables in vivo labeling of proteins that are in close proximity to the SC. Biotinylated proteins will be purified from ovaries and identified by mass spectrometry, providing an unbiased view of proteins that physically associate with the SC. This work will provide insights into the molecular mechanisms of how SC structure and function influence recombination outcomes to ensure accurate meiotic chromosome segregation.

784F Differential regulation of cytokinetic proteins during midbody remodeling to form ring canals in Drosophila germ cells

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Intercellular bridges called ring canals are formed by incomplete cytokinesis in dividing germ cells throughout the animal kingdom and in certain somatic cells. In these cases, cell division proceeds as usual to the final abscission stage, focusing our attention on how midbody-induced abscission is blocked. With live imaging, we found a “new” highly conserved midbody remodeling step where midbody core proteins rapidly transform from a large spherical organelle in the fully constricted cleavage furrow to a membrane-associated ring that becomes the ring canal. USP8 inhibition of the ESCRTIII abscission complex discovered in the Huynh lab follows this midbody core-to-ring transformation. To understand how the midbody transition is regulated, we are studying the midbody protein Pav (MKLP1), homologs of which are present in all studied ring canals, and Sticky (Citron Kinase). We are testing if a particular Pav phosphorylation status is needed to promote midbody core remodeling by generating non-phosphorylatable (4T-A) or phosphomimetic (4T-D) mutations in the region containing the N-terminal Cdc2/Cdk1 phosphorylation sites. These mutant forms will be tested by MARCM clonal analysis. In an RNAi screen, we found that reduced expression of the *sticky* gene caused abnormal ring canals, suggesting that Sticky plays a role in blocking abscission. We are investigating which domains of Sticky are involved using a structural-function analysis of protein domains by generating a GFP-tagged targeted deletion constructs. In parallel, we are studying the importance of Sticky’s interaction with its binding partner Rho1 GTPase with CRISPR editing of two predicted Rho1 binding sites. We have also screened a library of endogenously tagged Rho GAPs and GEFs from the Bellaiche lab for localization to ring canals and the effects of perturbing their expression with gem line RNAi. Our study will uncover a cooperative regulatory mechanism by which MKLP1, Citron Kinase, and Rho1 GTPase mediate the dynamic remodeling of cytokinetic structures into specialized RCs, providing new insight into how intercellular bridges are established and maintained during development.

785F Cadherin Dynamics in Ring Canal Stability During Oogenesis

Lawrence Pierce, Lindsay Lewellyn Butler University

Intercellular bridges are essential structures that connect developing germ cells. The germline intercellular bridges, or ring canals, in the developing egg chamber provide an excellent model to study these structures. The germline ring canals connect the supporting nurse cells to the developing oocyte. During egg chamber development, ring canals expand approximately 10-fold in diameter; this expansion is essential for proper egg formation. While many proteins that regulate ring canal size and/or stability have been identified, there is still much to learn. Cadherins are cell-cell adhesion proteins that are localized near ring canals and may play a role in stabilizing or anchoring these structures. Mutations that disrupt cadherin levels or localization cause defects in ring canal stability, but the normal behavior and regulation of cadherins in the germline has not been described. Analysis of live and fixed samples has allowed us to describe differences in germline cadherin localization and behavior; future work will expand this analysis and test the role for additional cadherin regulators in maintaining ring canal stability and promoting expansion.

786F EcR in ovarian germ cells promotes EGFR signaling in overlying somatic cells

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Oocytes are carefully packaged into ovarian follicles, each containing a maturing germ cell surrounded by a layer of somatic cells that secrete protective eggshell and chorion proteins. This conserved arrangement is essential for proper oocyte development and reproductive success. Continuous coordination and bi-directional signaling from somatic cells to germ cells is necessary for proper oocyte packaging. In *Drosophila*, although germ cell packaging was presumed to be largely controlled by somatic follicle cells enveloping passive germ cells, recent studies suggest that germ cells themselves produce motor forces that drive somatic encapsulation. Here, in support of this hypothesis, we present data suggesting that cyst encapsulation is dependent Ecdysone Receptor, a steroid hormone receptor known to control multiple aspects of oogenesis. Using tools to deplete *EcR* levels or block transcriptional activation specifically in the germline, we show that germline-autonomous *EcR* is necessary for the timing of cyst encapsulation. In the absence of *EcR*, germ cell encapsulation is slowed, resulting in increased incidence of cyst collision events in the germarium. *EcR* facilitates germ cell cyst encapsulation by specifically promoting EGFR signaling in overlying somatic cells, leading to proper escort cell shape, follicle cell proliferation, and suppression of pre-stalk cell gene expression. Overall, these data suggest that in addition to its well-characterized roles in somatic follicle cells, *EcR* is necessary in the germline to promote ovarian follicle assembly and development.

787F Post-Mating Fly Cell Atlas (PM-FCA) Reveals Global Transcriptional Remodeling During Reproductive Processes in Both Sexes

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Reproduction is an energetically costly process that remodels the entire body to support fetal growth while maintaining maternal health. These remodeling events occur at physiological, behavioral, and even mental levels and are orchestrated by intricate molecular and cellular mechanisms. Reproduction-induced responses have been studied in specific tissues involved in the endocrine, digestive, immune, and reproductive systems. However, a comprehensive understanding of reproduction-induced changes throughout the entire organism at cellular resolution, as well as the intercellular crosstalk underlying these effects, has not yet been achieved.

Here we generated post-mating Fly Cell Atlas (PM-FCA) using whole-organism single-nucleus RNA sequencing to address this critical gap. This atlas profiles single-nucleus transcriptomes from the heads and bodies of post-mating females at 1 day and 3 days, post-mating males, as well as their virgin counterparts. It characterizes over 200 distinct cell types from more than 334,000 nuclei, enabling us to reveal reproduction-induced transcriptional responses at the cell-type level. Intriguingly, cells from the gut, reproductive system, fat body, and muscle were identified as primarily altered in both short-term and long-term female responses, as well as in male responses after mating. In both sexes and at both post-mating time points in females, body cell types exhibited transcriptional patterns and magnitudes distinct from those of neurons in the head. We also conducted gene regulatory network (GRN) analyses to identify key regulators in the primarily affected cell types. This pioneering whole-organism post-mating atlas serves as a valuable resource to facilitate the discovery of global transcriptional responses to reproduction in all cell types of both sexes, as well as the key signals, such as hormones, orchestrating whole-body changes.

788S Investigating Sperm Quality Control Mechanisms in *Drosophila melanogaster*

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Sperm development in *Drosophila melanogaster* is a highly organized process essential for male fertility. Previous work from our lab has shown Bone Morphogenetic Protein (BMP) signaling from somatic cyst cells to germ cells as a key pathway during late spermatogenesis. BMP ligands such as Glass bottom boat (Gbb) activate receptors like Saxophone (Sax) in germ cells to promote elongation and differentiation of spermatids. When *Sax* is disrupted, sperm bundles become disorganized and mitochondria develop abnormal morphology, producing the sperm scattering phenotype. Although BMP signaling through*Sax*is required to maintain spermatid structure, it remains unclear how defects in mitochondrial or meiotic defects cause sperm scattering and lead to cyst disorganization. Bulk RNA-sequencing comparing control and *Sax* RNAi testes identified several downregulated genes involved in mitochondrial activity, spermiogenesis, and a combination of both pathways. These candidates are being tested through RNAi screening and genetic rescue experiments to determine whether they function downstream of *Sax* signaling and contribute to sperm scattering. Live imaging of *Sax* RNAi testes suggests that sperm scattering may also result from separation between nuclei and axonemes at the basal body region. Centrosomes that are normally anchored to the nuclei start to drift away as elongation progresses. This separation causes the spermatid heads and tails to lose alignment, and the cyst begins to lose its structure. The defect becomes clear at the canoe and leaf stages, when many nuclei shift toward the apical side and the cyst starts to break apart. Mitochondria in these cysts also appear enlarged and lose their normal shape. These observations suggest that scattering begins when centrosome anchoring fails, disrupting the link between nuclei, mitochondria, and axonemes. To test this idea, partial-null clones are being generated within individual cysts to create a mix of mutant and normal spermatids. Tracking these cysts with live imaging will show whether defective cells are held back, removed, or allowed to mature. Together, my project seeks to identify how sperm structure is maintained during reproductive checkpoints and how defects in this process contribute to infertility.

789S Interrogating the function of Vasa in *Drosophila* germ cell migration

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Germ granules are ribonucleoprotein condensates essential for the specification of the primordial germ cells (PGCs) in the *Drosophila* embryo. These granules contain numerous proteins, including the conserved RNA helicase Vasa, and mRNAs encoding proteins crucial for suppressing somatic or promoting germ cell fate. Germ granules are inherited by the PGCs and persist through germ cell migration and gonad coalescence; however, the function of individual germ granule proteins during these stages has remained elusive. For example, mutations or RNAi knockdown of *vasa* impede PGC formation, thereby preventing analysis of its function later in embryogenesis. Using the auxin-inducible degron system to deplete Vasa in PGCs, we found that it is indeed required during germ cell migration. We are currently elucidating how Vasa ensures continued PGC development, hypothesizing that it regulates the translation of actin-modulating factors. To identify its mRNA targets, we are using RNA co-immunoprecipitation to capture interactions across the embryonic stages of germ cell migration. The result of this work will provide insight into the specific role of Vasa in PGC development, which is an RNA-binding protein present in innumerable metazoan germlines.

790S Detection of DNA damage in *Drosophila melanogaster* embryos lacking yolk proteins

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During oogenesis, Drosophila oocytes import nutrients that will be critical for the development of the eventual embryo. Yolk proteins, and their import via *yolkless*, are essential for the developing embryo's viability. Female flies lacking *yolkless* are sterile, but in rare cases a subpopulation of their embryos initiate development. By DAPI staining, we found that these embryos exhibit signs of DNA damage, displaying nuclear fallout and anaphase bridges prior to cellularization, in embryos heterozygous and homozygous for *yolkless* respectively. Because so few *yolkless* embryos develop, we then examined DNA damage in the embryos of females mutant in all three yolk proteins simultaneously ('*yp1-3')*. Females of this genotype are sub-fertile, with embryos that can develop to cellularization. Consistent with our *yolkless* results, our preliminary data on *yp1-3* embryos showed nuclear fallout in these embryos, and signs of extensive DNA damage (visualized with γH2Av) in the mutants' embryos, relative to their *yw* controls (which also showed some DNA damage, but less). Among the *yp1-3* cohort of developing embryos, in addition to nuclear fallout, we observed preliminary evidence for asynchronous nuclear divisions and reorganization of yolk nuclei in the center. Ongoing work aims to further investigate asynchronous division in *yp1-3* deficient embryos and characterize the consequence of embryos observed to have mild and extensive DNA damage.

791S Role of Groucho in Drosophila spermatogenesis

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Defects in signaling between the germline and soma during spermatogenesis can lead to an arrest in sperm development and infertility. While many questions remain regarding the mechanisms regulating spermatogenesis, numerous signaling pathways have been implicated in germline-soma communication. Previous studies have shown that Notch signaling is active in the supporting somatic cyst cells surrounding 4- to 16-cell spermatogonial cysts. While early cyst cells express Traffic Jam (Tj), Eya begins to be expressed in somatic cyst cells surrounding 4-cell spermatogonia and both are expressed in somatic cyst cells through the 16-cell spermatogonial cyst stage, in a stage termed the transition zone. By the time somatic cysts cells surround spermatocytes, Traffic jam is no longer expressed, and only Eya persists. We have found that increased and decreased levels of Notch signaling negatively impact spermatogenesis. We observed that increased Notch signaling in somatic cyst cells results in sustained Tj expression, preventing the transition to Eya-only late cyst cells, halting spermatogenesis and causing sterility. Decreased Notch signaling affects the timing of germline differentiation. In order to better understand the downstream effectors of Notch signaling, we performed RNA-seq on testes with increased and decreased Notch activity. We identified *groucho* (*gro*), the homolog of mammalian Transducin-like Enhancer of Split, and two *Enhancer of split* (*E(spl*)) *complex* genes, *E(spl)mBeta* and *E(spl)m3,* as downstream targets of Notch in the testis. Previous work has shown Gro inhibits activation of Notch targets by binding to the transcription factor, Suppressor of Hairless (Su(H)), while also cooperating with E(spl) proteins to repress transcription. In addition, its activity is negatively regulated by Epidermal Growth Factor Receptor (EGFR) via phosphorylation by Mitogen Activated Protein Kinase (MAPK). As earlier research found that loss of EGFR signaling in testes halts germ cell development and induces cell death, which can be rescued by activated Notch overexpression, we propose that Gro may function as a point of integration for Notch and EGFR signaling pathways. To understand the role of Gro in spermatogenesis, we are examining the effects of increasing or decreasing Gro activity and levels on spermatogenesis. In addition, we are using genetic interaction analysis to explore how Gro function relates to the Notch and EGFR signaling pathways in spermatogenesis.

792S Discovering meiosis-mediating genes using RNA interference and cytological analysis

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Meiosis is a unique process for sexually reproducing mechanisms to create four genetically diverse haploid daughter cells– one oocyte and three polar bodies in females. Some of the most important aspects of the process are the genetic recombination that happens in prophase and the separation of chromosomes into daughter cells. Failure to recombine often looks like persistent breaks in the DNA which never get fixed. Failure in separation, known as nondisjunction, results in aneuploidy. Despite many studies being done, there is a need to identify novel genes of interest and describe their impact though not all are conserved. To do so, our project took genes thought to be implicated in meiosis and tested them to record their impact on meiosis and fertility. These genes come from two data sets; one which encompasses *Drosophila* ovary-enriched genes (FlyAtlas 2) and one with orthologs of human genes that have mutations in mothers with elevated aneuploidy. Each gene went through two tests to identify defects at early or later stages of meiosis. In the course of the project, about 350 RNAi lines were tested. From these screens, positive results were defined as sterility or heightened nondisjunction of over 2%. These positive results were further prioritized based on predicted function and analyzed with cytology for their physiological impact. Specifically, we looked at the formation of the synaptonemal complex (SC) and double strand breaks (DBSBs). The SC is a highly conserved protein complex which facilitates stabilization of paired chromosomes and promotes the breaking of the strands and reforming into crossovers. After cytological analysis, the positive results fell into one of three categories. The first category was for those knockdowns which result in a complete loss of the SC and also DSBs. The rest of the positive results were either DSB impacting genes or SC impacting genes. *Rad9* and *Rab40* have both shown break repair defects and DBSBs persist throughout early development and fail to make a viable oocyte. *HUWE1* and *CG12259* have been found to cause maternal age effects as the ovaries deteriorate over a short period of time. These knockdowns showed fully formed and healthy ovaries in young females but loss of germ cells in older females. In the future, we look forward to testing the last of the genes on our lists and further characterizing the physiological impact of these genes.

793S Characterization of the Mechanism of PhLP3 Function during Spermatogenesis

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Prior research in the Mierisch lab has demonstrated that Phosducin-like protein 3 (PhLP3) is necessary for proper sperm development.*PhLP3* mutation results in scattered spermatid nuclei and a failure to completely elongate, leading to an arrest in spermatogenesis and male infertility. In other species, PhLP3 has been demonstrated to interact with the Chaperonin containing TCP-1 (CCT) complex to regulate the folding of actin and tubulin. This suggested that PhLP3 may regulate the cytoskeleton to promote proper sperm development. In addition, PhLP3 exhibits redox activity, suggesting this activity may also be important for its function during spermatogenesis. To analyze the localization of PhLP3 relative to cytoskeletal structures. *Drosophila* Schneider 2 (S2) cells are being used as a model in an in vitro system. Immunostaining for PhLP3, actin, and tubulin suggests PhLP3 may colocalize with tubulin. To confirm this localization pattern, we have generated a Myc-tagged PhLP3 for expression in S2 cells. At the same time, we will analyze PhLP3 localization with actin and tubulin via immunostaining of whole testes. While previous studies have demonstrated that PhLP3 has redox activity, it is unclear if this activity is related to its ability to regulate the cytoskeleton or spermatogenesis. To test the importance of redox activity for PhLP3 during spermatogenesis, we are using CRISPR to generate mutations in cysteines within and around the PhLP3 active site and will examine spermatogenesis in the resulting mutants. From these studies, we will determine if PhLP3 functions as a redox-regulated cytoskeletal co-chaperone. Understanding of how PhLP3 redox activity affects cytoskeletal organization will provide new insights into how the thioredoxin-linked mechanism regulates spermatogenesis and fertility in *Drosophila melanogaster.*

794S SPC105R's Structure and Function in Meiotic Kinetochores

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Errors in meiosis I lead to chromosome nondisjunction and aneuploidy, one of the main causes of infertility and genetic disorders. To ensure proper chromosome segregation, kinetochores regulate biorientation of homologs and co-orientation of sister centromeres. Within the kinetochore, the KMN complex (MIS12 complex, KNL1/SPC105R, and the NDC80 complex) acts as an interface between kinetochores and microtubules. SPC105R, the *Drosophila* orthologue of the human gene KNL1, is essential for sister chromatid cohesion and chromosome biorientation.

We hypothesize that the C-terminal domain of SPC105R has three key functions: (1) the KMN1 protein within the MIS12 complex recruits SPC105R through a physical interaction with its C-terminal region, (2) the SPC105R C-terminal subsequently recruits the NDC80 complex in the outer kinetochore to establish end-on microtubule attachments, and (3) the C-terminal domain is required for proper cohesion of homologs to ensure accurate biorientation and chromosome segregation. Using two novel C-terminal mutations, we aim to define which regions are necessary for SPC105R's roles in maintaining kinetochore structure and function during meiosis I. One mutant is sterile and has biorientation defects yet cohesion is intact. These findings may be applied to KNL1's conserved role and its potential contribution to aneuploidy in human oocytes.

We also investigate how kinetochore proteins depend on one another for kinetochore assembly. Current kinetochore assembly models assume a "linear" recruitment where each protein builds off the last. However, previous research shows a more complex codependency within the kinetochore complex. For example, localization of the inner protein SPC25 is reduced in the absence of the outer protein NDC80. Additional experiments will test interactions between other KT proteins, such as NUF2, MIS12, and SPC25 to clarify kinetochore organization.

Finally, we test a method to address limitations in RNAi depletion. Instead, we use a protein degradation system as an alternative to test the proteins' roles in meiosis. Determining which proteins preserve kinetochore stability, specifically the role of SPC105R, will provide a foundation for future co-orientation and biorientation studies in *Drosophila*. These findings may also advance research on the meiotic mechanisms that contribute to infertility and aneuploidy in humans.

795S Exploring B chromosome drag in *Drosophila melanogaster* males

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B chromosomes are non-essential chromosomes that are often derived from the essential set of chromosomes. These B chromosomes can exhibit drive (>50% transmission frequency) to be maintained in a population despite their non-essential nature. In alignment with this, the B chromosomes in a *matrimony* heterozygous mutant stock (referred to as *mtrm*^{126}*/TM3)* of *Drosophila melanogaster* are subject to drive through females. Conversely, paternal B chromosome transmission in the *mtrm*^{126}*/TM3* stock was found to result in drag, where B chromosomes were transmitted at a lower than Mendelian frequency (<50%). Studies on drive suggest that suppressors of drive, such as drag, often appear in systems to balance out the negative effects of drive, but little is known about the mechanism of drag. In this study, we explore the mechanism of B chromosome drag through the paternal lineage in *D. melanogaster*. We first aim to test the hypothesis that paternal drag is due to a detriment to the male germline, which would manifest as lower overall fertility. Counter to our initial hypothesis, preliminary sperm exhaustion assays revealed higher fertility of *mtrm*^{126}*/TM3* males compared to wild-type males. These sperm exhaustion assays also revealed two populations of *mtrm*^{126}*/TM3* males: when experimental males aged to around 11-16 days, the fertility of some males dropped faster than others (28.67+/- 3.21 total progeny for "low fertility males" vs. 90.8 +/- 10.26 total progeny for "high fertility males", average +/- standard deviation). Interestingly, the progeny of these low fertility males inherited a higher number of B chromosomes compared to the progeny of the high fertility males. This suggests that B chromosome transmission may have a relationship with paternal fertility. Future areas of exploration will focus on identifying what genetic factors result in this early high fertility and late onset infertility and the cellular mechanism of paternal B chromosome drag.

796S Regulation of Spermatogenesis by the Transcription Factor Ribbon

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Ribbon (rib) is a BTB family transcription factor that functions as both a transcriptional activator and repressor. Previous research has demonstrated that rib regulates the coalescence of somatic cells to form the embryonic gonad and can function cooperatively with the BTB transcription factor Longitudinals lacking (Lola) to regulate embryonic gonad development. In Drosophila melanogaster males, we have found that increased levels of rib can negatively impact testis development. When rib is overexpressed in the somatic cyst cells throughout development, testis are significantly reduced in size and fail to produce mature sperm, resulting in sterility. When rib overexpression is limited to adulthood, testes appear stringy and thin with early germ cell overproliferation. In order to identify the downstream targets of rib transcription factor activity, RNA-sequencing was performed on control and rib overexpression testes leading to the identification of talin and shot as rib downstream targets, suggesting that rib may regulate expression of cytoskeletal proteins in somatic cyst cells to promote proper germline differentiation. Current studies are also exploring the relationship of rib to other BTB proteins during spermatogenesis.

797S Disentangling the thermal sensitivity across reproductive stages in *Drosophila*

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Extreme temperatures, including those that are now more frequent with global warming, can compromise fitness before reaching an organism's lethal temperature. Indeed, heat-induced sterility has emerged as a major cause of population declines in many ectotherm species. In *Drosophilid* flies, heat-induced sterility is widespread across species and may influence population viability and shape geographic distributions. Although many studies attribute heat-induced sterility primarily to male infertility from impaired spermatogenesis after heat exposure, females can also become temporarily or permanently sterile following heat stress. The precise mechanisms causing female heat-induced sterility remain less studied but may involve thermal damage to unfertilized eggs, stored sperm, developing embryos, or/and overall physiological condition.

Here, aiming to disentangle the effects acting at different reproductive stages, we conducted parallel experiments across multiple *Drosophila* species and tested these potential mechanisms by exposing female flies to heat stress either before or after mating and subsequently measuring their reproductive output over a week following exposure. Our preliminary results show that both egg number and hatch rate declined significantly in females exposed to high temperatures, although many recovered within a few days. Females heat-stressed before mating, when eggs were unfertilized and sperm not yet stored, showed minimal effects, whereas those stressed after mating suffered greater reproductive loss. The recovery of reproductive output also suggests that sterility was not caused by permanent sperm depletion. Together, these results suggest that fertilized embryos are potentially more heat-sensitive than early-stage eggs or stored sperm, emphasizing the importance of understanding how thermal stress acts across reproductive stages to better predict population resilience and evolutionary responses.

798S Investigating the reproductive role of male-derived yolk proteins in Drosophila

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Yolk proteins (Yps) are traditionally characterized as female-derived molecules that are essential for embryogenesis in *Drosophila melanogaster*. However, Majewska et al., 2014 showed that somatic cyst cells in the male testes express *Yp1-3* during late spermatogenesis and release Yp-containing vesicles into the seminal vesicle lumen, where these proteins bind to spermatozoa during post-testicular maturation. Using sex-specific isotope labeling and mass spectrometry, McCullough et al., 2022 proteomically identified male-derived Yps on sperm in the reproductive tract of mated females. Together, these findings raise the possibility that male-derived Yps contribute to sperm reproductive success. Using genetic tools, we are investigating the importance of male-produced Yps on fertility and sperm function. With automated image analysis tools such as Quantifly (Waithe et al., 2015), we are quantifying fecundity, fertility, and hatch rate of in matings with Yp-deficient males (Tanaka et al., 2022) compared to controls. With the goal of understanding the reproductive roles of male-derived Yps, we will assess aspects of sperm function in the absence of Yps, and will use microscopy (with GFP-tagged Yps) and biochemistry to examine how Yps interact with sperm.

799S Characterization of Combover membraneless organelles in *Drosophila* spermatogenesis

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Spermatogenesis is essential for sexual reproduction and perpetuation of species. Mammals and flies share common stages of germ cell differentiation and development into mature sperm, including a syncytial stage of early spermatids that must be resolved. In *Drosophila*, after mitosis and meiosis, early spermatids elongate at the elongation complexes (ECs), the distal part of the axonemes, where membrane remodeling occurs. Upon elongation, spermatids individualize through the action of individualization complexes, 64 actin cones that form near the spermatid heads and move distally, removing cytoplasm and organelles and ensuring each axoneme is properly enclosed by its own plasma membrane.

Our lab has shown that mutations in *combover* (*cmb*), a direct Rok kinase substrate, cause fertility defects in *Drosophila* males. *cmb* encodes two main protein isoforms, with the B isoform being fully contained within the A isoform. Both Cmb isoforms form puncta in syncytial spermatocytes and localize to ECs and growing axonemes in spermatids. Intriguingly, Cmb is an intrinsically disordered protein, and proteins with intrinsically disordered regions often form membraneless organelles (MLOs) that can organize multiprotein complexes and induce membrane curvature. We hypothesize that Cmb forms MLOs that are functionally relevant in spermatogenesis steps involving both membrane and cytoskeleton components.

Colocalization studies in testes show close association of Cmb puncta, but not overlap, with the early endosome marker Rab5, while there is not association with the late endosome marker Rab7 nor lysosomal marker Lamp1, suggesting Cmb forms distinct structures. We will present data suggesting that Cmb forms MLOs in transfected cells in an isoform specific manner. Live imaging confocal microscopy further supports Cmb phase separation as we can detect fusion and fission of puncta, typical of dynamic MLOs, altogether, suggesting that Cmb forms MLOs that are functionally relevant for *Drosophila* spermatogenesis.

800S Does the Drosophila Y chromosome affect sex determination?

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In 1916, Bridges showed that the Y chromosome in Drosophila melanogaster does not determine sex, as XX and XXY are female, while XY and XO are male. Over the next century, our understanding of the role of the Y chromosome has been mainly limited to its contribution to male fertility. Notably, though, more recent research has found that the Y chromosome harbors cryptic variation affecting global gene expression. However, to our knowledge no one has tested whether the Y chromosome has any effect on sex determination even if it is clearly not the primary determiner.

In D. melanogaster, the switch gene Sex-lethal (Sxl) determines sex: in females, Sxl expression is activated in early embryos, while in males it remains inactive. Five known X-linked signal elements (XSEs) are 'counted' by flies to determine how many X chromosomes they carry, and these XSEs act as positive transcriptional regulators of Sxl. Other autosomal and maternally expressed genes also regulate Sxl, demonstrating that the sex determination signal is polygenic. Furthermore, some of the known Sxl regulators have only modest effects on expression and there is extensive natural variation for the penetrance of mutations affecting Sxl. These results suggest that additional quantitative factors may affect Drosophila sex determination.

We have devised a scheme to test for potential sex determination regulation of the Y chromosome by introducing an extra Y chromosome in females (XXY) and males (XYY), and then comparing their viability (XX vs XXY; XY vs XYY) in sensitized genetic backgrounds that partially disrupt the sex determination pathway. Our preliminary results suggest that an extra Y is moderately deleterious to females in some backgrounds. We are currently performing additional tests in males and also considering approaches to identify specific regions of the Y that may affect sex determination.

801S Determining how Ecdysone signaling influences sexual development

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In many animals, males and females exhibit sexual dimorphism, or physical differences between the sexes. In fruit flies, the gene *doublesex* (*dsx*) helps control these differences, especially in the development of gonads. Interestingly, homologs of Dsx are now known to control sex-specific gonad development most or all animals, including humans. We have shown that Dsx controls Ecdysone (E) signaling, paralleling the human steroid hormones Estrogen and Testosterone, that help promote development of the gonads and other tissues. In the gonads, Dsx regulates the Ecdysone Receptor (EcR) so that it is more active in females. While E is known to be critical for ovary formation, the specific genes that E regulates, and their functions remain unclear. To address this, I am using genetic tools to identify potential E targets in the gonads that are essential for proper ovary development.

E also rises and falls at key times in a fruit fly's life cycle and triggers changes like molting and metamorphosis. Pulses of E during larval stages are hypothesized to influence ovary development by triggering formation and differentiation of specific cell types, but the timing of these peaks in the gonads is unknown. To test this, I am using an E activity reporter to visualize hormone dynamics and determine which peaks correlate with formation of specific gonad cell types. Overall, this work will reveal how hormone signaling drives sex-specific gonad formation. Because flies and humans share conserved sex determination genes and hormone signaling systems, these findings will provide broader insights into reproductive development across species.

802S Regulation of Spermatogenesis by Hindsight (Hnt) Areeba Musharraf, Allyson Terrell, and Jennifer Jemc Mierisch

Areeba Musharraf, Jennifer Mierisch Biology, Loyola University Chicago

Studies of gametotogenesis in *Drosophila melanogaster* provide insight into the genetic mechanisms underlying stem cell regulation, cytokinesis, meiosis, and gamete maturation. Many genes implicated in gametogenesis in flies are well conserved across species, including humans, suggesting they may perform similar roles in regulating gamete production and fertility. Previous studies exploring the genetic regulation of oogenesis in *Drosophila* identified Hindsight (Hnt)/Pebbled (Peb), the homolog of the Ras responsive element binding protein 1 (RREB1), as a regulator of somatic follicle cell development that acts downstream Notch signaling. However, a role for Hnt in the testes has not been examined. Immunostaining for Hnt reveals expression in germ cells during the late spermatogonia and early spermatocyte stages and late somatic cyst cells. To explore the function of Hnt in these cells we are performing cell-specific RNAi knockdown in the germline and somatic cyst cells using the GAL4/UAS system. Preliminary results of *hnt* knockdown in the somatic cyst cells show the presence of more late somatic cyst cells and more late spermatogonia and early spermatocytes. Ongoing analysis will help us better understand the role of Hnt during spermatogenesis and how homologous proteins may function similarly in other species to regulate sperm production.

803S Evolution in action: How rapidly-evolving sex chromosome conflict may induce speciation

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Selfish meiotic drive systems (SMDs) can distort progeny sex-ratio (SR) and/or induce sterility. Although widespread in nature, the molecular mechanisms of SMDs, and how they are silenced to restore Mendelian segregation, remain largely mysterious. Importantly, their rapid evolution means that classic model organisms may not be suited to reveal their fundamental features, breadth and impact. Instead, we used non-model Drosophila species, including *D. simulans*, to uncover critical roles for hpRNA class endo-siRNAs in suppressing incipient sex chromosome conflicts. Molecular genetic studies reveal de novo meiotic drive loci encoded by the X chromosome, including members of the simclade-specific Dox family, which are suppressed by members of the Nmy class of hpRNAs. Long read genomic data from wild fly lines reveals extraordinary polymorphism and copy number variation in Dox genes across individuals. We interpret this to reflect an ongoing arms race between SMD distortion and suppression by small RNAs. We currently seek to understand the mechanistic basis of sex ratio distortion and sterility induction by Dox family members. A potential clue is the recent origin of Dox family genes from protamine, which helps to condense the paternal genome in sperm. We are using molecular and genetic tests to evaluate how Dox family genes might impair DNA packaging. Overall, we hypothesize that rapidly-evolving sex chromosome conflicts may be agents of speciation.

804S Examining the role of *Chigno* in epithelial expansion within the adult *Drosophila* testis stem cell niche

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Stem cells play a critical role in organ and tissue maintenance. *Drosophila* testes are a paradigmatic system for elucidating mechanisms controlling stem cell behavior. This system harbors two stem cell types: sperm-producing germline stem cells (GSCs) and cyst stem cells (CySCs) that regulate GSC self-renewal while producing cyst cells that promote differentiation of GSC daughters into sperm. These stem cell types are docked to a cluster of quiescent hub epithelial cells that reside at the apex of each testis coil. The hub cells and CySCs are both derived from somatic gonadal precursor (SGP) cells that arise from the embryonic mesoderm. Prior work has also shown that adult CySCs must continuously maintain male stem cell identity to prevent conversion into follicle stem cells (FSCs) that produce ovarian follicular epithelia. We are studying the role of the uncharacterized gene, *CG11180/Childless Gambino* (*Chigno*), in regulating CySC behavior. We previously showed that inhibition of *Chigno* in the CySC lineage leads to expansion of undifferentiated germ cells at the testis apex. Here we describe data showing that *Chigno* RNAi knockdown in the CySC lineage also causes epithelial expansion at the testis apex in a subset of testes. We hypothesize that this occurs for the following reasons: (i) *Chigno* normally functions to repress conversion of CySCs into hub epithelial cells, (ii) *Chigno* represses conversion of CySCs into female follicle stem cells (FSCs), or (iii) *Chigno* acts to represses hub cell division. We will present data indicating that *Chigno* is not involved in controlling hub cell division and assessing whether epithelial expansion occurs via loss of male identity or conversion of CySCs to a hub cell fate. By studying mechanisms controlling fate conversion between mesenchymal and epithelial stem cell types, this work has implications for understanding formation of secondary tumors from metastatic cancer cells. As Chigno is homologous to the human tumor suppressor, PINX1, understanding Chigno's function in *Drosophila* testis stem cells may have broader implications for cancer treatment in people.

805S Sperm combing enables proteomic analysis of sperm types in *Drosophila pseudoobscura*

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How cell size is regulated is a fundamental question in developmental biology. While cell size control has been extensively studied in dividing cells, how non-dividing cells differentiate to a specific size is less understood. Sperm cells are an ideal model to study this as their length is tightly regulated within a species and within individuals. *Drosophila pseudoobscura* (*D. pse*) is a unique system for investigating this as males produce three distinct sperm morphs, long (300µm), medium (100µm) and short (50µm) within the same testis. Approximately 50% of the sperm are long, 25% medium and 25% short. Only long sperm fertilise eggs; medium and short sperm have non-fertilising roles in protecting the long sperm against the harsh environment of the female reproductive tract. The differential proteomic composition underlying these distinct cell morphologies and functions has not been investigated. Similarly, the mechanism that ensures each male spermatid bundle differentiates into one of these three distinct lengths remains unknown.

To investigate the protein composition of *D. pse* sperm morphs we developed a novel "sperm combing" method to separate long sperm from short and medium sperm. This enabled label-free LC-MS proteomics on purified samples of long sperm, short/medium sperm, as well as mixed (unsorted) sperm. Comparative analysis between these samples showed low variance within replicates and high variance between morphs, confirming effective separation. Across all samples we identified over 500 proteins, including a core proteome shared by all sperm types and numerous differentially enriched proteins. Surprisingly, short and medium sperm had the most differentially enriched proteins in comparison to long sperm.

We are using HCR-FISH and cross-validation with our scRNAseq data to investigate the expression patterns of genes associated with differential sperm proteomes. We are also mapping the testis anatomy with HCR-FISH using markers for specific cell types and stages to better understand the organisation of spermatogenesis.

Amongst the proteins enriched in long sperm were sperm tail proteins. Amongst the proteins enriched in short and medium sperm were a class of proteins orthologous to accessory gland proteins in *Drosophila melanogaster*. While long sperm are enriched for sperm tail components, we speculate that short and medium sperm might have higher affinity than long sperm for somatically produced secreted proteins in the ejaculate.

806S Assessing the effects of DILP8 overexpression on Drosophila Female Reproduction

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Mature follicle number is tightly regulated in Drosophila ovaries to protect oocyte health, but how females count mature follicles to coordinate ovulation and oogenesis remains unclear. Previous studies showed that Drosophila insulin-like peptide 8 (DILP8) expression peaks in late-stage mature follicles, and that depletion of dilp8 leads to virgin egg-laying defects and mature follicle accumulation, suggesting that DILP8 may act as a mature follicle sensor that triggers ovulation. This study aimed to test whether overexpressing DILP8 could enhance egg laying in virgin females, and if this overexpression could be used to rescue the defects we see in our dilp8 mutant females. Contrary to our hypothesis, we show that overexpression of DILP8 in late-stage mature folliclesusing the Gal4-UAS and LexA-LexAop binary expression systems results in a reduction in virgin egg laying, while mated egg laying numbers remained normal. Similarly, ectopic overexpression of DILP8 in earlier egg chambers, stages 10-14, resulted in a virgin, but not mated, egg laying defect. Additionally, overexpression using a biologically inactive form of DILP8 resulted in a virgin, but not mated, egg laying defect, suggesting that overexpression itself, rather than DILP8 activity, is affecting aspects of egg laying under the virgin condition. Currently, we are assessing what might be contributing to these defects by quantifying the number of mature follicles in our DILP8 overexpression flies. We are also testing whether ectopic DILP8 can rescue the virgin egg-laying defects in dilp8 mutant females.

807S A dedicated endonuclease drives mitochondrial genome destruction in developing sperm

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Maternal inheritance of mitochondrial DNA (mtDNA) is nearly universal among eukaryotes, yet the mechanisms that prevent paternal mtDNA from being inherited have remained obscure. In both *Drosophila* and humans, paternal mtDNA is actively eliminated during spermatogenesis, producing mature sperm with mitochondria that lack their genomes. Given the circular topology of mtDNA, I hypothesized that its removal requires a dedicated endonuclease. To test this, I systematically screened all Drosophila endonucleases for mitochondrial matrix localization using a split-GFP assay. I then assessed mtDNA elimination in mutant flies of the top candidates. Here, I identify Hotaru, a previously uncharacterized, testis-specific GIY–YIG endonuclease, as a central player of paternal mtDNA elimination. Hotaru localizes to the mitochondrial matrix and is expressed in elongated spermatids immediately prior to individualization, coinciding with the developmental window in which mtDNA elimination occurs. Loss of *hotaru* results in mature sperm that retain abundant mtDNA. Catalytic-dead mutations in Hotaru abolish both its nuclease activity *in vitro* and mtDNA elimination *in vivo*, demonstrating that its enzymatic activity is essential for mtDNA elimination. Using long-read Nanopore sequencing, we further show that Hotaru cleaves mtDNA within the control region. Together, these findings identify the first dedicated nuclease responsible for paternal mtDNA elimination in animals. This work establishes a mechanistic foundation for understanding paternal mtDNA elimination and suggests that analogous nuclease-driven pathways may operate broadly across animals.

808S *bore da* and *prynhawn da*, a duplicate gene pair with diverging functions in spermatogenesis

Helen White-Cooper[1], Fiona Messer[1], April Talbot[1], Christopher Von Ruhland[1], Saurabh Chaudhary[1], Tomos Snell[1], Georgina Menzies[1], Karen Doggett[2], Jianqiao Jiang[2] [1]Cardiff University, [2]Zoology, Oxford University

Spermatogenesis is a dynamic system for studying both conserved biological processes and rapid evolutionary innovation. We investigated a pair of testis-specifically expressed genes, *bore da* (*boda, CG43077*) and *prynhawn da* (*pryn, CG31752*). Contrary to previous classification of *boda* as a *de novo* gene, phylogenetic and synteny analyses reveal that it originated via segmental duplication of *pryn*. *pryn* is present throughout *Brachycera*, while *boda* originated in the *melanogaster* species group. Comparative evolutionary analyses indicate that *pryn* is under strong purifying selection, while *boda* experiences relaxed and diversifying selection, consistent with neofunctionalization. Structural modelling and molecular dynamics simulations, coupled with yeast-2-hybrid interactions suggest that Boda can bind the tMAC subunit Aly, whereas Pryn shows weaker interaction potential. Consistent with this, Boda protein is nuclear and chromatin associated while Pryn localises to cytoplasmic puncta. Functional assays demonstrate that *pryn* is essential for male fertility, ensuring proper sperm head organization. The basal body insertion into the nucleus is displaced rostrally, so that the axoneme overlaps laterally with the caudal region of the nucleus in *pryn* mutant sperm. In contrast, *boda* mutants are fertile, with only mild defects in sperm production and seminal vesicle size. These findings highlight how gene duplication can drive functional divergence and highlight the potential for integration of novel components into established regulatory networks during spermatogenesis.

809T Regulation of Yki by Rho Gap: filling in the GAPs of intestinal homeostasis

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Efficient replacement of damaged cells is essential the growth and patterning of developing tissues and for maintaining tissue integrity. In the metazoan gut, this regenerative demand is met by intestinal stem cells (ISCs), which undergo asymmetric division to produce one self-renewing ISC and one differentiating daughter cell that becomes either an absorptive enterocyte (EC) or a secretory cell. This balance is critical for sustaining tissue homeostasis throughout the adult lifespan - however, the mechanisms and specific factors which maintain and control this process are largely unknown. Identifying key factors of intestinal regeneration, and elucidating how they contribute to these mechanisms, is critical for bettering understanding of tissue homeostasis and localized stem differentiation. We identified Rho GTPase-activating protein 15B (RhoGAP15B), the *Drosophila* homolog of mammalian ARAP1/2/3, as a contributing factor to gut homeostasis, and noncanonical interactor of Yorkie (Yki). Yki (orthologous to mammalian Yap), is a transcriptional coactivator of the Hippo signaling pathway, which plays a crucial role in controlling tissue growth and development. RhoGAP15B contains three primary functional domains: a GAP domain that typically terminates cell signaling events; two PH domains that in ARAP3 restrict integrin-based focal adhesions to the basement membrane – loss of which leads to dysplasia; and two PPxY motifs, which allow physical binding to Yki. We hypothesize RhoGAP15B regulates proliferation and inhibits cellular damage response in ISCs and ECs. In ISCs, RhoGAP15B promotes proliferative quiescence and proper cell fate. Its loss disrupts basement membrane adhesion, expands the ISC pool, and impairs differentiation. Overexpression of Yki inhibitors prevents overproliferation driven by *RhoGAP15B*-kd in ECs, but not RhoGAP15B depletion in ISCs. These observations support a model in which RhoGAP15B regulates adult gut renewal by modulating multiple signaling pathways in a cell type-specific manner through its diverse domains. We plan to explore this model using both Crispr/Cas9 RhoGAP15B null flies, and the GAL4-UAS system to overexpress RhoGAP15B and versions of it containing domain mutations driven to ISCs, ECs, and both. This will allow us to elucidate RhoGAP15B's role in intestinal homeostasis to further understand molecular and mechanosensitive stimuli that drive localized stem cell differentiation.

810T Effect of chronic, unpredictable mild stress on intestinal stem cell homeostasis

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The link between psychological stress and immunosuppression, endocrine alterations and altered metabolism have all been well established and could explain the connection between stress and disease. By comparison, studies exploring the effects that chronic psychological stress may have on the regenerative potential of adult stem cells are comparatively lacking. *Drosophila* has been previously used to investigate how diverse stressors affect stem cells, but mostly focusing on acute, intense and localized stressors. This may be partly explained by the scarcity of experimental paradigms to model chronic psychological stress in flies. Some research groups have recently described the use of chronic unpredictable mild stress (CUMS) paradigms, which in flies evoke behavioral and neurophysiological changes usually associated to stress-induced depression in mammals. Here we implemented a modified version of a CUMS protocol, in which flies are exposed to a pseudo-randomized daily sequence of thermal shocks, ethanol intoxications and sleep interruptions. We found that adult females exposed to our modified CUMS protocol displayed significant behavioral changes, including an altered response to perturbations, exploratory behaviors in an open-arena assay, and losing their ability to develop functional tolerance to ethanol, which may reflect a learning impairment. We also characterized the effect that our modified CUMS protocol had on intestinal stem cell (ISC) homeostasis. Interestingly, we find that our CUMS protocol phenocopied several effects caused by the localized over-expression of Unpaired (Upd), a cytokine known to activate the STAT pathway and mediate the regenerative response of ISCs to chemical injury and infection, as well as aging, which is known to induce an inflammation-like dysplastic phenotype in the gut. Furthermore, the combination of our CUMS protocol and either Upd over-expression or aging rarely showed a significant enhancement of the observed phenotypes, revealing a double dominant epistasis between treatments, which in turn suggests that they likely operate via congruent genetic pathways. We therefore propose that *Drosophila* ISCs can be used as a powerful experimental system to further explore conserved pathways connecting mild and unpredictable chronic stress to stem cell biology, and thus probe alternative links between psychological stress and disease across species.

811T Characterizing non-linear effects of single vs. dual genetic manipulations in *Drosophila melanogaster* intestinal stem cells

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Many organs depend on adult stem cells (ASCs) for homeostatic tissue turnover, as well as to recover from injury and disease. Like their embryonic counterparts, ASCs can divide asymmetrically, giving rise to a new stem cell and a sister cell that differentiates into specific cell types to replace those cells lost from a tissue. An ASC decision to divide or not, and to self-renew or differentiate, results from the coordinated activity of several so-called master regulator genes (MRGs), which collectively control the expression and/or activity of hundreds of targets in response to intrinsic and extrinsic signals. Recent work from our lab identified and validated a series of targets for two of such MRGs (Escargot and STAT). Here we address an important subsequent question: do these MRG targets act primarily independently from each other, or do they engage in significant genetic interactions with one another? To address this question, we began a systematic exploration of single vs. dual-manipulation experiments, using the inducible esg-Gal4ts system to control the expression of different combinations of UAS responder constructs in intestinal stem cells (ISCs) and their immediate progeny. More specifically, we are exploring all possible combinations of over-expressing or knocking-down three MRG targets identified in our previous work: the nucleoskeletal protein Klaroid (Koi), the cytoskeletal adaptor protein CAP and the metabolic transporter Indy. Using fluorescence microscopy of the posterior midgut, lifespan and paraquat tolerance assays, we sought to determine whether dual genetic manipulations produce linear or synergistic effects compared to the corresponding single manipulations. Here we present preliminary evidence demonstrating that dual genetic manipulations, perhaps not surprisingly, led to complex phenotypes that deviate from the simple sum of single effects. Moreover, we have observed intriguing epistasis patterns, in which one of the two manipulated targets appears to be epistatic to the other with respect to some phenotypes, but hypostatic for others. While not particularly surprising from an experimental genetics perspective, our findings nonetheless emphasize the need to incorporate a more careful and systematic exploration of non-linear genetic interactions among MRG targets. These functional intermediates are often preferred as potential therapeutic targets due to their narrower molecular and biological scopes; however, they may still engage in complex interactions with other relevant genes and pathways, which could in turn lead to devastating therapeutic outcomes that are not evident until later phases in the search for new therapies.

812T Investigating the role of SUMO precursor protein

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Small ubiquitin-like modifier (SUMO) proteins are post-translational modifiers of a vast number of proteins and therefore regulate almost every aspect of cellular function. Vertebrates and plants have several different SUMO genes whereas yeast, nematodes, and flies have only one. In all cases SUMO is expressed as a precursor protein with a C-terminal tail that is clipped off before the mature protein is conjugated to target proteins. Although the length and sequence of the C-terminal tail can vary, its presence is evolutionarily conserved in all eukaryotes that have been sequenced so far. In *D. melanogaster*, the C-terminal tail of SUMO consists of just two amino acids, A and P. To investigate the role of the SUMO precursor protein, we used the CRISPR-Cas9 system to delete the six base pairs encoding the C-terminal A and P from the *Sumo* gene. To our surprise, the resulting $Sumo^{\Delta AP}$ flies are homozygous viable and fertile and display no obvious visible or behavioral abnormalities. We are currently testing their ability to tolerate irradiation and other forms of stress. We are also generating FRT $Sumo^{\Delta AP}$ flies to use for FLP-mediated clonal analysis.

813T Cell fate analysis by current and past enhancer expression during regeneration

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Tissue regeneration is a complex process that involves the recruitment of surviving cells to replace what has been damaged. Drosophila can regenerate a number of tissues, including the larval imaginal disc, which are the larval precursors of adult structures, such as the eye and wings. Regeneration in imaginal discs occurs through the formation of a blastema, or a zone of highly proliferative and relatively dedifferentiated cells that are the cellular source of the regenerated tissue. An important question for the field of regenerative biology is to what degree does regenerating tissue recapitulate normal development versus undergo a distinct set of patterning events. Regenerative repatterning may require cells to take on new cell fates that are distinct from normal development. The mechanisms that regulate regenerative repatterning remain largely unknown. To investigate the order of repatterning events, we aim to compare cell-type specific patterning and lineages between developing and regenerating tissues. There are many publicly available enhancer-driven LexA tools that have the potential to provide insights into how distinct developmental cell lineages are altered during Gal4-based genetic manipulations. To this end, we combined a LexAOp driven fluorescence reporter, a LexAOp driven FLP recombinase and FRT based lineage tracing fluorescence reporter so that we could rapidly determine real-time and past expression of enhancer-drive LexA lines with a single cross. As this system is based on and analogous to G-TRACE and is called LexA technique for real-time and clonal expression (L-TRACE). The approach allows for characterization of current and past expression of LexA lines while allowing for independent genetic manipulations with the GAL4/UAS system. Here we present our ongoing work using L-TRACE to characterize enhancers expressed during wing imaginal disc development and following Gal4-based manipulations that induce both cell fate changes and regeneration.

814T Detailing the functions of Cytokine/JAK/STAT signaling during *Drosophila* midgut regeneration

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Understanding cytokine/JAK/STAT signaling is crucial, as it plays central roles in immune and inflammatory responses, regeneration, wound healing, and human diseases such as auto-immune disorders, inflammatory bowel diseases (IBD), and cancers. While the human inflammatory response is intricate, involving numerous cell types and cytokines, the *Drosophila* system offers a simpler model, with only three cytokines, Unpaired 1, 2, 3 and a single gene encoding their receptor, Domeless (Dome). It also has only one Janus Kinase (JAK; Hopscotch (hop)), and one known STAT transcription factor, STAT92E. Previous studies showed that the JAK-STAT pathway has important functions in the *Drosophila* midgut regeneration, including cell proliferation and differentiation. However, it is still unknown what specific roles cytokine signaling plays in the different gut cell types. To understand how Upd cytokines regulate gene expression during stress-induced gut regeneration, we mapped the dynamics of JAK–STAT pathway activation across midgut cell types. Using multiplexed FISH to profile transcriptional expression and CRISPR/Cas9-mediated protein tagging to track pathway components, we found that upd1 and upd2 are expressed in intestinal stem cells (ISCs) and enteroblasts (EBs), whereas upd3 is restricted to enterocytes (ECs). All major midgut cell types express the core JAK–STAT components Dome, Hop, and STAT92E. By integrating CUT&Tag mapping of STAT92E binding sites with FISH, we show that JAK–STAT activation in ECs promotes epithelial damage responses by inducing the EGFR ligand Keren (Krn). These results reveal cell-type-specific Upd–JAK–STAT interactions that coordinate regenerative signaling in the Drosophila gut. By identifying STAT92E target genes and mapping their functions in specific cell types, we will construct a molecular framework for cytokine-driven regeneration. These insights are highly relevant to human intestinal inflammation and cancer, where chronic cytokine signaling can drive early tumorigenesis. The results will not only deepen our understanding of fundamental cell signaling principles but also reveal conserved therapeutic targets to prevent or reverse tissue degeneration and inflammation-induced cancers.

815T Investigation of genes with blastema-specific expression during regeneration

Celeste Fryling, Cigdem Sarikartal, Josh M Pazin, Keisha M Roldán Báez, Akriti Saha, Rebeccah L. Messcher, Melanie I. Worley Biology, University of Virginia

For the field of regenerative biology, there remain many unanswered questions, including when an organism regenerates, to what extent is regeneration accomplished via activating the same molecular mechanisms required for normal development versus regeneration-specific processes. To address this question, we use the model organism Drosophila melanogaster, which provides a wide availability of powerful genetic tools to support dissection of gene function in vivo, including the ability to screen gene interactions and quantify them through regenerative growth assays. Specifically, we study regeneration in imaginal discs, the larval precursor to adult structures such as legs and wings. In the larvae, imaginal discs are capable of robust regeneration with characterized stages, including the formation of a localized zone of proliferation and enhanced plasticity, called a blastema. Through the use of single-cell analysis we were able to identify several previously uncharacterized genes that are upregulated and expressed uniquely within regions of the blastema. We are screening through these candidate genes for ones that have unique temporal and spatial expression patterns over the course of regeneration. Current work aims to determine which molecular pathways regulate the transcriptional activation of these candidate genes and to determine how reduction or loss of function of these genes impacts regenerative growth and repatterning. Our future work aims to elucidate the mechanisms by which these candidate genes are functioning to regulate distinct phases or processes of regeneration.

816T Matrix Metalloprotease 1 promotes cell fate change for epithelial-to-epithelial transition during regeneration after radiation damage in Drosophila

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Ionizing radiation (IR) is used to treat cancer, but therapeutic failure occurs when surviving cancer cells change fate and regenerate tumors through acquired stem cell-like properties. While transcriptional mechanisms underlying cell fate plasticity have been characterized, the cellular processes enabling cell movement during tissue regeneration remain unclear. We reported previously that hinge cells of the Drosophila larval wing disc convert to pouch fate and translocate to help regenerate the pouch that suffers from more IR-induced apoptosis. We report here that IR increases the expression of ECM-associated proteins in the hinge, including secreted proteases and cell adhesion modulators. Functional validation using RNA interference revealed that secreted Matrix Metalloprotease 1 (Mmp1) and the related secreted protease homolog Scarface (Scaf) are required in hinge cells for IR-induced cell fate conversion and translocation. IR, we found, induces Mmp1 and Scaf transcripts in hinge cells via cell-autonomous JNK signaling. Overexpression of Mmp1 specifically in the hinge was sufficient to elevate Mmp1 protein levels throughout the disc and induced cell fate change of both hinge and non-hinge cells but only in the context of irradiation. Confocal imaging in a time course demonstrated that cells undergoing fate conversion remain within the epithelial layer with little evidence for delamination or epithelial-mesenchymal transition (EMT). We propose ECM proteolysis as a critical mechanism enabling cellular reorganization during tissue regeneration. Mmp enzymes are important for cancer biology because of their role in ECM remodeling, extracellular signaling, and EMT. Our findings demonstrate for the first time that Mmp1 is necessary and sufficient for one epithelial cell type to switch to another epithelial cell type after radiation damage. These results provide a mechanistic basis for radiation therapy-induced cell fate plasticity.

817T A Novel lncRNA-Mediated Regulatory Axis of Lysine-Specific Demethylase 1 Controls Germline Stem Cell Differentiation during *Drosophila* Oogenesis

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Cellular differentiation is precisely regulated to support tissue development and homeostasis. Lysine-specific demethylase 1 (Lsd1), an epigenetic eraser, maintains plastic chromatin states to promote cell proliferation, whereas aberrant Lsd1 expression contributes to tumorigenesis. In cancer cells, interactions between long non-coding RNAs (lncRNAs) and Lsd1 have been shown to influence tumorigenic gene expression; however, the physiological roles of lncRNA-mediated modulations of Lsd1 remain elusive. Our recent identification of *Lsd1-Interacting Non-coding RNAs* (*LINRs*) from *Drosophila* ovaries provides a unique opportunity to elucidate how *LINRs* and Lsd1 function coordinately to impact cell differentiation *in vivo*. We discovered that *LINR-1* and *LINR-2* regulate oogenesis, at least in part, by regulating germline stem cell (GSC) expansion. In germ cells, the presence of *LINRs* is required for proper targeting of the Lsd1 complex to specific gene loci, thereby fine-tuning the GSC's responsiveness to niche signals. Moreover, *LINR-2* appears to also affect GSC expansion in a non-cell-autonomous manner, functioning within escort cells to ensure a well-defined and restricted GSC niche. Together, the cell-specific roles of *LINR-1* and *LINR-2* in controlling GSC differentiation uncover a novel and intricate lncRNA-mediated regulatory axis of Lsd1 that underlies programmed stem differentiation *in vivo*.

818T Tissue-intrinsic signaling affects formation of the testis niche.

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Stem cells play an important role in tissue maintenance and self-renewal. A cellular microenvironment, the niche, is required to maintain stem cells. Understanding how a niche forms and functions is key to stem cell biology yet is challenging as most niches are inaccessible to imaging during their initial development. The *Drosophila* testis niche provides an optimal model to study niche formation, as our lab developed a live imaging platform to enable *in vivo* visualization of niche development. This niche forms at the anterior of the embryonic gonad, a spherical arrangement of germ cells encysted by somatic cells. Directly opposite the niche at the gonad posterior are the male specific gonadal precursor cells (msSGPs) that coalesce with the gonad prior to niche formation. We have ablated these msSGPs using two different genetic means and found that the niche has disrupted morphology, suggesting that msSGPs are required to shape an anterior niche. Indeed, without msSGPs, we find the niche is less spherical, has higher surface area, and its cellular constituents have higher internuclear distance, while niche volume and cell number remain unaffected. Thus, niche shape, rather than size, is disrupted. Additionally, niche cells must migrate to the gonad anterior and arrest after contacting other niche cells at that location. Without msSGPs, these cells persist in migration and intersperse among anterior niche cells, suggesting a mechanism for disruption in niche shape. Niche architecture is critical to niche function, and we find that without msSGPs, the niche fails to regulate asymmetric cell division of its surrounding GSCs. Given the requirement for msSGPs in shaping the niche and the specific position of msSGPs at the posterior, we hypothesize a signaling cue originating in these cells serves to compact and localize the niche at the gonad anterior. We have turned to published scRNA sequencing data to identify candidate signals (Mahadevaraju et al., 2022). Our data confirms that Nord, a secreted protein that modulates other diffusible signals, is expressed in msSGPs and plays a role in niche shaping. Our ongoing work will define the mechanisms by which msSGPs, and msSGP expression of *nord*, enable proper assembly of the testis niche. Concepts in stem cell niche biology have repeatedly translated from the *Drosophila* testis to other systems, and our work unveils a novel mechanism by which a non-niche gonadal cell population regulates niche morphogenesis.

819T *Lactobacillus brevis* that secretes ornithine can prophylactically promote regeneration processes in *Drosophila*

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This study examines the effects of microbiome on activation of host regeneration processes. As an injury model, we utilized the *Drosophila* limb, which does not normally regenerate. We find that supplementing *Lactobacillus brevis* ATCC 367 promotes activation of regeneration processes in the amputated limb, as characterized by change in wound healing, enhanced tissue survival, and eventually partial regrowth of the limb. To investigate how *L. brevis* influences host regeneration, we combined genome-scale metabolic modeling with experimental measurements, and found that L. brevis secretes the amino acid ornithine. Flies fed with *L. brevis* indeed show higher extract levels of ornithine and altered levels of enzymes that metabolize ornithine. Directly administering ornithine to the flies promotes regeneration processes in the limb. In summary, this study presents evidence that supplying a bacteria that secretes ornithine can promote regeneration processes in a host organ that does not normally regenerate. Further, *L. brevis* supplementation can be performed prior to injury, opening up the possibility of prophylactically improving response to injury.

820T An Xrp1-mediated damage response pathway is essential for gut regeneration

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Ribosomes are the machinery that synthesize all proteins of living cells. Ribosome deficiency has been many human diseases, from neurodegeneration to cancer. However, the impact of Ribosome deficiency-induced protein metabolic disorders on tissue regeneration remains poorly understood. We recently found that the transcription of over 90% of the ribosome genes is consistently reduced in the fly gut under various pathological conditions, including infection. Yet, the mechanism driving this global repression remains unknown. Through a genetic screen, we identified Xrp1, the Drosophila ortholog of human C/EBP homologous protein (CHOP), as the key factor responsible for this repression. We found that various stresses (e.g. infection, toxins) trigger the production of reactive oxygen species (ROS), which cause RNA damage that leads to ribosomal collisions in gut enterocytes (ECs). These stalled ribosomes activate the GCN2–pEIF2a signaling pathway, which in turn induces Xrp1 expression. Xrp1 then suppresses global ribosome gene transcription. This represents a previously unrecognized mechanism, as earlier studies have viewed Xrp1 primarily as a downstream effector of ribosome deficiencies. Our findings suggest a feedback loop between Xrp1 and ribosome biogenesis to maintain protein homeostasis during gut regeneration. Beyond repressing ribosome production, Xrp1 upregulation in ECs also induces the cytokine Upd3, which promotes intestinal stem cell (ISC) proliferation through activation of the JAK-STAT and EGFR-MAPK pathways. In addition, our data suggest that Xrp1 upregulates detoxification genes, such as the GstD family, to counteract the harmful effects of gut infection. Interestingly, this detoxification function appears to operate independently of the classical Keap1/Nrf2 pathway. In summary, our study identifies Xrp1 as a central regulator of the gut damage response and regeneration. By limiting ribosome biogenesis in damaged ECs, Xrp1 helps conserve energy and ensures that ISCs can sustain regenerative capacity.

821F Necrosis Induces DNA Damage to Drive Regenerative Proliferation

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Necrosis is a form of lytic cell death that can occur in every multicellular organism, resulting in complex and poorly understood tissue outcomes, making understanding this phenomenon a significant challenge. Therefore, the overarching goal of my research is to understand the impact necrosis has on neighboring tissue. Our lab has advanced research into necrosis by developing a powerful genetic manipulation system called Duration And Location (DUAL) Control to study cell death and regeneration.

We found that necrotic cell death leads to caspase activation, normally indicative of apoptotic cell death, surrounding the wound. However, they do not behave like normal apoptotic cells, fail to undergo cell death, and appear to be essential for successful regeneration. Given their non-apoptotic tendencies, we have termed these cells “Necrosis-induced caspase-positive cells” (NiCP). My initial data demonstrates two things. First, the initiator caspase Dronc mediates the regenerative response following necrosis. Secondly, the transcription factor p53 is indispensable for both the formation and function of NiCP. Although Dronc and p53 are highly conserved, their involvement in tissue recovery following necrosis in the wing disc has not been previously described.

To investigate the genetic networks surrounding Dronc and p53 following necrosis, I developed a protocol to specifically label and isolate NiCP containing tissue surrounding the wound. RNA extraction and bulk RNA sequencing of these cells at two regenerative time points comprehensively profiles gene expression changes at both the formational and functional stages of NiCP. GO term analysis revealed upregulation of genes involved in DNA damage sensing and repair and cell cycle regulation. The sequencing data and genetic manipulation to suppress DNA damage provides evidence that necrosis induces DNA damage to support regeneration. Indeed, differential gene expression analysis uncovers the upregulation of Caspase Activated DNAse, which is a member of the DNA fragmentation factor–related family. Functional testing reveals that manipulating CADs, as well as inhibitors of CADs (ICADs), changes the proliferative response to necrotic wounds. Thus, our data suggest that Dronc, CADs, and P53 cooperate in promoting regenerative outcomes.

822F Cell Proliferation and Neurogenesis in the Injured Adult *Drosophila* Brain

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Neurological damage from traumatic brain injury (TBI), Alzheimer’s, and Parkinson’s, results in inflammation. In the adult Drosophila brain, a penetrating traumatic brain injury (PTBI) can trigger not only an innate immunity-mediated inflammatory response, but also neurogenesis; yet the relationship between inflammation and adult neurogenesis remains unclear. To investigate the relationship between activation of the innate immune system and neurogenesis post-injury, we used the UAS/Gal4 system to ubiquitously knock down Relish (Rel), a transcription factor and downstream component of the IMD innate immunity pathway. A thin needle was used to create a penetrating injury through the mushroom body in the adult central brain. The brains were subsequently dissected, fixed, stained and imaged using confocal microscopy to visualize proliferating cells over a 72-hour post-injury window. Proliferating cell counts were indexed to total numbers of glia in both control and *Rel-RNAi* knockdown brains using Imaris software. We found that cell proliferation varies greatly over a 72-hour period post-injury. Initially, there is a spike in glial proliferation, followed by a later surge in neuronal proliferation, suggesting that there is a biphasic response to injury in the brain. Additionally, the *Rel-RNAi* knockdown brains exhibited significantly less glial proliferation than the control brains post-injury leading us to hypothesize that partially inhibiting the inflammatory response may lead to increased neurogenic potential in adult Drosophila brains post-injury.

823F Recognition of pathogenic bacteria by intestinal progenitors promotes adult *Drosophila* midgut regeneration via PGRP-MKK3-p38 signalling

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When enteropathogenic bacteria breach the intestinal epithelium, they are recognized by epithelial and immune cells that elicit an intestinal regenerative response. However, less is known about whether and how intestinal progenitors directly detect invading pathogenic bacteria and couple this to their proliferation. Here we show that adult *Drosophila* midgut progenitors recognise peptidoglycan from pathogenic bacteria through the peptidoglycan recognition proteins, PGRP-LC and PGRP-LE, and translate this to ISC proliferation by stimulating MKK3-p38 signalling. Moreover, we find that PGRP-LC/LE-MKK3-p38 signalling in progenitors regulates p38 activation throughout the midgut epithelium after infection, indicating that progenitors can influence the regenerative microenvironment in a non-cell autonomous manner. Whilst it was previously thought that ISC proliferation in both mammals and flies is driven solely by damage-induced signals after infection, our work reveals that progenitors can directly recognise pathogenic bacteria and mount a strong parallel regenerative response that spreads throughout the midgut epithelium. Increased ISC proliferation after bacterial recognition may also serve as a strategy to repopulate the epithelium with uninfected cells.

824F Niche BMP signals control testis aging and GSC competition via Hairless, Imp, and Chinmo

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Aging is accompanied by progressive tissue decline and shifts in stem-cell clonality, yet the niche signals that drive these changes are not well defined. In the *Drosophila* testis, we identify a regulatory axis in which age-related attenuation of niche BMP signals and their effectors in germline stem cells (GSCs) upregulate the co-transcriptional suppressor Hairless (H). Acting independently of canonical Notch signaling, H represses the RNA-binding protein Imp in aged GSCs. Reduced Imp lowers the transcription factor Chinmo, a key regulator of testis aging and GSC competition, thereby de-repressing secretion of the heparan sulfate proteoglycan Perlecan (Pcan). Pcan accumulates in the testicular lumen, remodels the niche, and promotes GSC loss while enriching for remaining GSCs with low Imp/Chinmo or high H. Restoring BMP signals in the niche, overexpressing Imp, or depleting H in GSCs reverses testis aging phenotypes Moreover, GSC clones with reduced Imp or elevated H are hypercompetitive, displacing wild-type neighbors and monopolizing the niche. Together, these findings define a BMP ⊣ Hairless ⊣ Imp → Chinmo pathway that governs testicular niche aging and reveal how “winning” GSCs co-opt aging mechanisms to dominate the niche.

825F Mechanistic insights into dedifferentiation in the Drosophila testis post-irradiation

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Not only are stem cells integral for development, but maintaining a healthy stem cell population throughout life is also critical for tissue and organ function. While asymmetric division is the predominant mode of adult stem cell renewal, reversion of differentiated cells back into stem cells, via a process known as dedifferentiation, becomes an increasingly important source of stem cell renewal with age or during times of stress. Dedifferentiation has been studied in many species and tissues, and several pathways have been implicated, but the mechanism of dedifferentiation is still not fully understood. First described in the Drosophila testis after the removal and recovery of the stem cell maintenance factor Stat92E, dedifferentiation has since then been studied in the context of aging or starvation as well as with genetic manipulation of differentiation factors. Recently, we found that dedifferentiation can also be studied in the context of irradiation in the Drosophila testis niche. This niche is constituted of quiescent hub cells which provide signals to maintain two populations of stem cells: somatic cyst stem cells and germline stem cells (GSCs). After a single dose of 75 Gy of irradiation, most GSCs are lost but then fully recover over the following few days. Using live imaging to follow cell dynamics in recovering testes, we have found that dedifferentiation of spermatogonia back to GSCs occurs at a high frequency under these conditions. We have also used a Bam-GFP reporter, which is normally expressed only in differentiated spermatogonia, to transiently mark GSCs that arise by dedifferentiation. To investigate the molecular mechanisms of dedifferentiation using our irradiation assay, we plan to take a candidate gene approach, focusing first on the Jun N-Terminal Kinase (JNK) and Bone Morphogenic Protein (BMP) pathways as both have been previously implicated in spermatogonial dedifferentiation in the Drosophila testis. We are using the Gal4/UAS system to conditionally deplete these pathways from germ cells or cyst lineage cells and assessing the ability of GSCs to recover from irradiation via confocal microscopy and live imaging.

826F Context-Dependent Roles of Wnt6 Signaling in Regulating Hematopoietic Differentiation and Immune Response in Drosophila

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Drosophila hematopoiesis is a critical developmental process centered in the lymph gland, where hematopoietic progenitors differentiate into three main hemocyte lineages: plasmatocytes (macrophage-like cells), crystal cells (involved in melanization), and lamellocytes (involved in encapsulation immunity). Upon immune challenge, such as parasitic wasp (*Leptopilina boulardi*) infection, these progenitors rapidly differentiate into effector hemocytes, demonstrating the dynamic plasticity of the *Drosophila* hematopoietic system. While pathways such as Notch, Hedgehog, and JAK/STAT are well-established regulators of hematopoiesis, the role of Wnt signaling, particularly Wnt6, remains poorly defined. Our lab recently showed that Wnt6 regulates cell cycle progression and progenitor maintenance in the lymph gland; however, its role in directing lineage-specific differentiation during homeostasis and immune stress remains unexplored.

To address this gap, we modulated Wnt6 signaling in lymph gland progenitors using the *domeMESO-GAL4* driver. Wnt6 loss-of-function (LOF, *Wnt6-RNAi*) increased differentiation of both crystal cells ($Hnt^+/PPO2^+$) and plasmatocytes ($P1^+$). Notably, LOF plasmatocytes displayed a more mature phenotype, characterized by higher P1 intensity, compared with wild-type conditions. Under naive conditions, no lamellocyte differentiation was observed. Conversely, Wnt6 gain-of-function (GOF, UAS-Wnt6) significantly expanded the progenitor population (domeMESO>GFP^+), suggesting a role in progenitor maintenance.

We then examined the immune response to *L. boulardi* infection in circulating hemocytes. Neither Wnt6 LOF (*Wnt6-RNAi, Pan-RNAi*) nor Wnt6 GOF (*UAS-Wnt6*) altered the overall frequency of lamellocyte differentiation. Downstream Wnt activation, via β-catenin GOF (Axin-RNAi, UAS-Arm^{S10}), markedly reduced circulating lamellocytes post-infection. Furthermore, Wnt6 and Arm^{S10} GOF increased progenitor release into circulation. This novel finding suggests that progenitor mobilization can occur independently of complete differentiation under immune stress.

Together, our results reveal context-dependent roles of Wnt6 signaling and its downstream effectors in regulating hemocyte lineage commitment, hematopoietic plasticity, and immune adaptation. Future studies will examine β-catenin GOF lymph glands following wasp infection to determine whether reduced lamellocyte production results from impaired differentiation or restricted release from the lymph gland.

827F Paraneoplastic renal dysfunction in fly cancer models driven by inflammatory activation of stem cells

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Tumors can induce systemic disturbances in distant organs, leading to physiological changes that enhance host morbidity. In Drosophila cancer models, tumors have been known for decades to cause hypervolemic “bloating” of the abdominal cavity. Here we use allograft and transgenic tumors to show that hosts display fluid retention associated with autonomously defective secretory capacity of fly renal tubules, which function analogous to those of the human kidney. Excretion from these organs is blocked by abnormal cells that originate from inappropriate activation of normally quiescent renal stem cells (RSCs). Blockage is initiated by IL-6-like oncokines that perturb renal water-transporting cells and trigger a damage response in RSCs that proceeds pathologically. Thus, a chronic inflammatory state produced by the tumor causes paraneoplastic fluid dysregulation by altering cellular homeostasis of host renal units.

828F Uncovering the role of Wnt signaling pathway in regulating germline stem cell maintenance and progenitor cell differentiation in the *Drosophila* testis

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Adult stem cells are regulated by signals from their microenvironment to self-renew or generate progenitor cells throughout life. The Wnt signaling pathway, a key niche cue, remains incompletely understood in terms of its cellular mechanisms governing stem cell maintenance and differentiation. The *Drosophila* adult testis provides an ideal model, as it contains a well-defined and conserved niche of hub cells that support early germline development during spermatogenesis. Hub cells regulate two stem cell populations, germline stem cells (GSCs) and somatic cyst stem cells (CySCs), which divide asymmetrically to produce spermatogonia (SGs) and early somatic cyst cells. The early somatic lineage forms an additional microenvironment for proliferative SGs transitioning to differentiated spermatocytes. Using GFP reporters driven by Wnt genes, we found that *Wnt4* and *Wnt6* are highly expressed in hub cells, CySCs, and early somatic cells, whereas *Wingless* is restricted to CySCs and the first few tiers of somatic cells. Wntless, the transmembrane protein required for Wnt secretion, accumulates in somatic cyst cells and hub cells, indicating that these cells both express and produce Wnt ligands. To assess Wnt activity, we co-expressed two Wnt-responsive reporters, *Frizzled3-RFP* and *Naked-LacZ*, with the germline differentiation marker *Bam*. *Naked* is detected in hub cells, GSCs, and Bam-negative SGs, while both *Naked* and *Frizzled3* are expressed in mid-to-late cyst cells associated with Bam-positive SGs. Using Imaris to plot intensity profiles relative to distance from the hub, we identified a shift of Naked expression from early germline to mid-to-late cyst cells that closely correlates with the onset of Bam. Our phenotypic screening of germline- or somatic-specific perturbations of Wnt activity revealed two disease models: (1) significant GSC loss ($p<0.0001$) upon early germline expression of constitutively active armadillo/β-catenin or either constitutively active or negative shaggy/GSK3β; and (2) reduced 4- and 8-cell SG cysts ($p<0.0001$), increased 16-cell SGs ($p=0.0024$), and delayed Bam accumulation upon both somatic expression of RNAi targeting armadillo/β-catenin or constitutively active shaggy/GSK3β. These results suggest that Wnt signaling plays two stage-specific roles: an autonomous requirement for GSC maintenance and a non-autonomous function in regulating germline differentiation. Steady Wnt expression in early somatic cells likely coordinates these roles across the stem cell and SG differentiation niches, providing a tractable model for dissecting niche–stem cell interactions relevant to adult and cancer stem cell biology. Ongoing work will compare transcriptional profiles of the two models to identify Wnt downstream target genes and potential crosstalk with other pathways specified for governing GSCs and SGs development.

829F Chondroitin sulfate regulates proliferation of *Drosophila* intestinal stem cells

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The basement membrane (BM) plays critical roles in stem cell maintenance and activity control. In *Drosophila* midgut, our results show that chondroitin sulfate (CS), a major component of the BM, is required for proper regulation of intestinal stem cells (ISCs). We also found that CS is essential for maintaining barrier integrity to prevent leakage of the midgut epithelium. Two critical proteins, Chondroitin sulfate synthase (Chsy) and Chondroitin polymerizing factor (Chpf), form a heterodimer complex responsible for polymerizing CS. Loss of *Chsy* resulted in elevated levels of ISC proliferation during homeostasis, leading to midgut hyperplasia. Regeneration assays demonstrated that *Chsy* mutant ISCs fail to properly downregulate mitotic activity at the end of regeneration. *Chpf* mutants showed increased ISC division during midgut homeostasis and regeneration, similar to *Chsy* mutants. As *Chpf* expression is induced by tissue damage during regeneration, our findings suggest that Chpf functions with Chsy to facilitate CS remodeling and stimulate tissue repair. We propose that Chsy and Chpf play an important role in the completion of BM repair during the midgut regeneration process.

830F Age-dependent ectopic SREBP activation promotes niche-to-stem cell conversion and loss of male germline stem cell niche homeostasis

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The capacity of stem cells to self-renew or differentiate has been attributed to distinct metabolic states. In the Drosophila testis stem cell niche, both germline stem cells (GSCs) and somatic cyst stem cells (CySCs) employ strategies to control lipid levels to promote stem cell maintenance. When lipid catabolism is impaired, neutral lipids accumulate in lipid droplets (LDs) promoting stem cell exit from the niche through differentiation, suggesting a role for lipid metabolism in the control of stem cell maintenance. In addition, impaired lipid catabolism has been observed in organs and tissues with age. Hence, we sought to investigate the precise mechanisms involved in age-related loss of stem cell niche homeostasis triggered by the dysregulation of lipid metabolism.

In the testis, both niche and stem cell number decrease with age. We found that LDs accumulated with age in somatic “hub” cells, the main organizing and support structure in the testis niche. Downregulation of the lipogenesis factor sterol regulatory element binding protein (SREBP) suppressed LD accumulation in hub cells with age and rescued the age-dependent decline in hub maintenance. Ectopic activation of SREBP in young animals was sufficient to drive lipogenesis and promote hub cell loss through their conversion into CySCs. Furthermore, we show that SREBP activation can repress *escargot*, a member of the Snail family of transcription factors and a master regulator of hub cell identity. Although some mechanisms have been shown to promote hub to stem cell conversion, this is the first time that a lipid metabolism gene has been implicated in such a phenomenon. This evidence supports a model where SREBP activity is kept low in the hubs of young animals to maintain *escargot* levels and prevent hub cells to be lost through conversion into CySCs. Our findings highlight a critical role for lipid homeostasis in stem cell maintenance, providing a framework for investigating the impact of aging and metabolic diseases on stem cell function and tissue homeostasis.

831F Non-canonical Wnt signaling reorients the mitotic spindle in adult stem cells to amplify the stem cell population

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Somatic stem cells must switch between division modes to ensure the proper generation of stem cells versus differentiated cells during tissue repair. We have previously identified that the positioning of the mitotic spindle in adult *Drosophila* intestinal stem cells (ISCs) drives cell fate decisions of daughter cells (Hu *et al*. 2019). ISCs with planar spindles divide symmetrically to generate two ISCs while ISCs with oblique spindles divide asymmetrically to generate one ISC and one differentiated cell. The regulation of spindle orientation, how spindle orientation ultimately affects cell fate, and whether these processes are conserved in cell fate determination within mammalian tissue remains unclear. Here, we demonstrate that non-canonical Wnt signaling is activated during adaptive tissue resizing to orient planar spindles and increase ISC numbers. We find that Wnt pathway proteins localize to the basolateral cortex in dividing ISCs, suggesting that non-canonical Wnt signaling may serve as a cortical cue to recruit the mitotic spindle. Interestingly, we find evidence supporting a conserved role of spindle orientation in regulating cell fate in adult mouse tissue. Stem cells in the mouse airway exhibit planar spindles following injury, but switch to an oblique spindle after longer periods of recovery. This switch reflects an observed increase of differentiated cells, suggesting that, similar as in flies, oblique spindles in airway stem cells promote asymmetric divisions.

832F Neural stem cell response to neuron loss in developing brain

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Neural tissues often fail to self-repair after injury or disease. Although neurogenesis continues throughout adulthood in some animals, it is typically restricted to specific regions and by neuron types. To better understand the molecular mechanisms that limit regenerative capacity of neural tissues, we are developing a drug-based approach to conditionally ablate mushroom body neurons in the developing *Drosophila* brain. Neural stem cells (neuroblasts) in *Drosophila* respond to extrinsic cues such as nutrient availability, but whether they can sense and respond to injury remains unclear. The mushroom body, the center of *Drosophila* olfactory learning and memory, is composed of Kenyon cells (KCs), which are neurons sequentially generated by mushroom body neuroblasts (MBNBs) through temporally regulated transcription factor cascades. MBNBs may respond to neurons loss in a similar way as regeneration mechanisms observed in other *Drosophila* tissues. For example, it is known that damage to imaginal discs can produce a substantial delay in pupariation to accommodate extra cell divisions and regenerative repatterning. In contrast, the hindgut is able to regenerate by accelerating the mitotic cycle, by decreasing G1 length, to ensure development within time constraints. Therefore, we hypothesize that MBNBs respond to KC loss by modulating their proliferative dynamics, either by accelerating the cell cycle or by delaying developmental progression, to replace lost neurons. Future experiments will elucidate how distinct subtypes of KCs are produced depending on which compensatory mechanisms are employed. This work will reveal how neural stem cells detect and respond to damage cues and define the intrinsic and extrinsic factors that limit the regenerative capacity of the nervous system.

833S Hormone Receptor 4 is Required in Adult Female Neurons to Regulate Germline Stem Cell Maintenance

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Inter-organ communication is vital for the maintenance and proliferation of adult stem cells, allowing for the continual growth and health of an organism. The adult *Drosophila melanogaster* ovary serves as a valuable model for studying stem cell maintenance, as this energy-intensive process is highly sensitive to physiological cues. Nuclear receptors (NR) are an evolutionarily conserved superfamily of ligand-binding transcription factors that play a key role in oogenesis. However, how the specific tissues and mechanisms that required NRs to regulate ovarian stem cell lineages are not fully understood. We previously showed that the NR Hormone receptor 4 (Hr4) regulates oogenesis in female *Drosophila*. Somatic *Hr4* knockdown using *tubulin-Gal4* increased GSC loss, reduced GSC proliferation, enhanced early germline cyst death, slowed follicle growth, and led to vitellogenic follicle degeneration. *Hr4* knockdown in specifically muscle tissue resulted in GSC loss and reduced follicle growth (PMID: PMC7970067). In adult females, *Hr4* transcripts and Hr4 protein are detected in the head, suggesting that Hr4 may be required in brain cell populations for oogenesis. To investigate this, we knocked down *Hr4* using the pan-neuronal *nSyb-Gal4* driver in adult female flies. Pan-neuronal knockdown of *Hr4* decreased GSC maintenance and we are determining whether Hr4 activity is required in neurons for GSC proliferation, survival of germline cysts, and egg chamber growth. Furthermore, we are similarly testing whether Hr4 activity is required in the glia for these aspects of oogenesis. In the future, we will utilize a modified DamID (NanoDam) to identify the specific Hr4 targets in neurons and glia that mediate communication from the brain to the ovary. Our findings will highlight how nuclear receptor activity in distinct cell types facilitates differential effects to control peripheral tissue homeostasis.

834S Sterile injury leads to expenditure and replenishment of crystal cells

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Immune cell development from hematopoietic stem and progenitor precursors involves complex and interconnected signaling from multi-tissue niches. Despite this complexity, the central pathways and mechanisms of the Drosophila immune system are conserved in the innate immune system of many vertebrates. Our goal is to examine the effects of sterile injury on different types of Drosophila blood cells and assess whether damage-associated innate immune signaling can stimulate hematopoiesis.

There are 3 major hemocyte types in Drosophila: plasmatocytes (PLs), which release cytokines and engulf pathogens; crystal cells (CCs), which rupture to release anti-microbial reactive oxygen species and enzymes that produce clot-promoting melanin to catalyze wound healing; and lamellocytes (LMs), which encapsulate parasitoid wasp embryos. It has been shown that LM induction occurs because of epidermal puncture, which activates the Toll-, JAK-STAT-, and JNK-mediated inflammatory signaling pathways. However, we do not yet understand how injury affects PL and CC populations.

Larval hemocytes can circulate freely within the hemolymph or home to epidermal-muscular pockets known as sessile pools. Hematopoiesis takes place in the sessile pools. Moreover, blood cells can shift between sessile and circulating niches. We find that sterile injury of third instar Drosophila larvae causes a temporary drop in the number of sessile CCs, which are presumably mobilized and expended during clotting. However, sessile CCs recover by 24 hours post-injury, likely due to replenishment. We also observed an increase in the total number of circulating hemocytes by 24 hours post injury, presumably due to PL expansion. We are currently investigating whether these changes in blood cell populations following injury are due to a shift in how cells are partitioned between circulating and sessile niches, or if injury-induced hematopoiesis is taking place. Since inflammatory signaling through the Toll, JAK-STAT, and JNK pathways is highly conserved in vertebrates, this project has the potential to further elucidate the consequences of injury-induced inflammation on blood cell development in different models.

835S Investigating the Neuron-Glia Response to Injury in Embryonic *Drosophila* Central Nervous System

Lilly Stiles, Hemlata Mistry Biochemistry, Widener University

When the central nervous system (CNS) is injured, neurons and glial cells respond rapidly and in a highly coordinated way to begin repairing the damage. Several genes that act with the Notch signaling pathway, including *kon tik*i (*kon*), *prospero* (*pros*), *islet antigen 2 (ia 2*), and *Drosophila insulin like peptide 6* (*dilp 6*), have been linked to injury responses in the larval CNS (Hidalgo et al., 2021, eLife). This project investigates how these Notch-related genes respond to CNS injury in late-stage embryos. To distinguish procedural and injury-induced effects, embryos were divided into two groups: mock-injured embryos that were handled but not injured, and embryos that were injured using a fine glass needle to cause trauma to the CNS. cDNA was generated from RNA harvested from each group. Additionally, control cDNA was utilized to validate and optimize each primer for specificity and amplification efficiency. Quantitative PCR is being used to measure relative expression changes between mock and injury samples. These findings can help clarify how developmental timing and Notch pathway activity influence glial gene regulation after injury. This work helps reveal conserved pathways that support nervous system repair and may ultimately inform strategies for spinal cord regeneration in vertebrates.

836S The *Drosophila* TET homolog is a key regulator of intestinal stem cell establishment

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Developmental programs must be precisely coordinated to ensure adult tissue integrity and organismal longevity. Disruption of these processes often leads to irreversible defects in tissue homeostasis. The *Drosophila* midgut provides a powerful model to study stem cell specification during organ remodeling, as its cellular lineages and regulatory pathways are highly conserved with mammals. During metamorphosis, some adult midgut progenitors (AMPs) give rise to the adult intestinal stem cells (ISCs), while other AMPs differentiate into functional gut cell types. However, the mechanisms that govern which and how certain AMPs adopt the stem cell fate remain poorly understood.

Here, we identify the *Drosophila* TET homolog (dTET) as a key regulator of ISC establishment during gut metamorphosis. Loss of dTET results in a region-specific depletion of adult ISCs in the anterior midgut, leading to compromised stress resilience and reduced lifespan. Interestingly, we find that dTET expression transitions from broad distribution in larval AMPs and niche cells to being progressively restricted to ISCs and enteroblasts in the developing and adult gut. Lineage-tracing and ISC-specific marker analyses reveal that dTET-high AMPs in late pupae commit to the adult ISC fate. Temporal knockdown experiments further reveal that dTET is dispensable during larval stages but required during the first 48 hours of metamorphosis, pinpointing a critical window for ISC fate specification.

These findings uncover a previously unrecognized role for dTET in adult stem cell establishment, suggesting a developmental mechanism for long-term tissue maintenance. Given the evolutionary conservation of TET proteins, this work provides new insight into how TET proteins coordinate stem cell identity and tissue longevity across species.

837S Diet Regulates Muscle Stem Cell Pool and Muscle Growth in *Drosophila*

Lucia Lopez Ortega, Jalen Nguyen, Ayushi Bhadra, Abigail Sowers, Kumar Vishal San José State University

Skeletal muscles are continually used throughout the life cycle of an organism and require constant remodeling and/or repair. This is achieved through specialized cells called muscle stem cells (MuSC)/satellite cells. Following a developmental signal or muscle injury, MuSCs exit quiescence, undergo proliferation (symmetric and asymmetric division), and finally differentiate to contribute to neonatal muscle growth and homeostasis. Mammalian studies have highlighted the role of diet in the regulation of MuSc proliferation and muscle growth and repair. However, the mechanism through which this regulation takes place is largely unknown. This study leverages the genetic advantages of the Drosophila melanogaster model system to dissect MuSC function. Drosophila adult muscle progenitors (AMPs) are functionally similar to mammalian MuSCs with conserved signaling pathways, less genetic redundancy and a simpler microenvironment. We are using amino acid free diet or genetically block the diet sensing pathway (Pi3K/Akt/Tor signaling) to dissect how diet regulates MuSC population and function during development. Our preliminary results show that the loss of PI3K/Akt/Tor signaling in MuSC drastically reduces the MuSC pool in the wing disc. Importantly, this reduction is due to cell cycle stalling and reduced proliferation. Furthermore, blocking Akt/Tor signaling results in a significant reduction in muscle size during the early pupal stages. Finally, Drosophila fed on amino acid free diet also results in significantly fewer MuScs and impaired muscle growth. Our next step is to identify downstream molecules that interact with Akt/Tor to amplify MuSC pool and muscle size. Given the fundamental similarities between Drosophila and mammalian MuSCs the proposed study will help us yield insights relevant to understanding how diet influences the mammalian MuSC activation/proliferation to promote neonatal muscle growth and repair during aging and muscle disease.

838S The role of Lipophorin Receptor 1 in regenerating *Drosophila* wing imaginal discs

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Regeneration is a complex process requiring the activation of many genes and pathways to restore lost tissue and its patterning. Using the *Drosophila* imaginal wing discs, our lab has previously found that regeneration often fails to completely restore tissue both in terms of tissue mass and patterning. One intriguing difference is that regenerated wings discs develop into adult wings with smaller cells than their non-damaged counterparts. Lipophorin Receptor proteins are Low Density Lipoprotein Receptor family transmembrane proteins responsible for uptake of neutral lipids and signaling molecules. In a genetic screen for regeneration genes, we found that heterozygosity for the gene *Lipophorin Receptor 1* (*LpR1*) resulted in enhanced regeneration and adult wings with cells larger than their undamaged counterparts. By contrast, mutations in *Lipophorin Receptor 2 (LpR2)* caused large adult wing cells independent of tissue damage but did not lead to improved regeneration. The cell size phenotype of *LpR1* and *LpR2* mutants offers potential insight into how cell size is controlled during regeneration. In addition to lipid uptake, *LpR1* also regulates Toll signaling through the uptake of the Toll signaling inhibitor Necrotic. Toll signaling is not currently known to have a role in the regenerating disc. Through studying *LpR1, we* seek to understand how Toll signaling and lipid uptake constrain regeneration and modulate the size of cells in the imaginal wing disc following tissue ablation.

839S How does a Follicle Stem Cell in the *Drosophila* ovary become a Follicle Cell?

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In the anterior half of the germarium of a *Drosophila* ovary, germline cystoblasts mature into 16-cell spherical region 2a cysts while passing between quiescent, enveloping somatic Escort Cells (ECs). After the cyst flattens to form a lens shape spanning the germarium width in region 2b, it is surrounded by proliferating somatic Follicle Cells (FCs) which are required for egg chamber development and are continually produced by Follicle Stem Cells (FSCs), somatic stem cells that are located in the middle of the germarium. Lineage tracing studies show that about 16 FSCs are positioned between the ECs and FCs and are arranged principally in two rings, with cell bodies along the basement membrane. Cell bodies of Layer 1 FSCs lie immediately anterior to a spatial landmark—the anterior border of strong Fas3 membrane protein expression—while Layer 2 FSCs reside about one cell diameter further anterior. FSCs in Layer 1 directly give rise to FCs, whereas Layer 2 FSCs can differentiate into ECs. However, FSCs can move between layers, and individual FSC lineages may generate both ECs and FCs and all FSCs share the same functional potential. Importantly, FSC division and differentiation are distinct processes that can be regulated independently. The choice of an individual FSC—whether to divide, differentiate, or change A/P position—appears to be stochastic but is strongly influenced by signaling pathway activity. The JAK-STAT pathway promotes FSC differentiation into FCs, encourages posterior movement into Layer 1, and suppresses EC production, while Wnt pathway activity has the converse consequences. JAK-STAT activity increases to the posterior of FSCs as Wnt pathway declines, so stochastic A/P movements would be reinforced by positive feedback. A/P movements might in theory be due to somatic cells crawling along the basement membrane or associating more or less strongly with other somatic cells or with germline cysts. We have been testing whether the likelihood of an FSC becoming a FC is altered by increasing or decreasing the activities of various adhesion molecules, including DE-cadherin (expressed in somatic and germline cells) and some homophilic adhesion molecules expressed only in somatic cells (Fas2, Fas3, Nrg).

840S Investigating function of CG11180/Chigno in somatic cyst stem cells using bulk RNA-sequencing

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Stem cell dysregulation can lead to loss of tissue homeostasis and various disease states including cancer or infertility. Exploring mechanisms that regulate stem cell behavior is, therefore, of critical importance. The testis stem cell niche in *Drosophila melanogaster* offers an excellent model system to study these mechanisms. Our research focuses on understanding the role of the uncharacterized gene, *CG11180/Childless Gambino* (*Chigno*), in controlling stem cell behavior within this niche. Specifically, we are examining its function in somatic cyst stem cells (CySCs) that regulate maintenance and differentiation of sperm-producing germline stem cells (GSCs). Our prior work has shown that knockdown of *Chigno* in the CySC lineage causes expansion of stem cell populations and differentiation defects leading to reduced fertility. To better characterize the role *Chigno* plays in CySC regulation, we performed bulk RNA-seq analysis. To focus our analysis on the testis stem cell niche, we dissected the apical tip of testes from adult males after RNAi knockdown of *Chigno* in the CySC lineage. Through comparison with control testes, we find that expression of genes associated with *Chigno* function is significantly impacted. Initial results exploring regulatory pathways that modulate stem cell behavior, also indicate that genes associated with stem cell self-renewal are upregulated while those associated with differentiation are downregulated. GO and pathway analyses have uncovered a variety of potential pathways that may shed further light on Chigno's role in controlling testes stem cell behavior. Subsequent experimentation through RNAi and other methods will explore these potential pathways. As *Chigno* is homologous to the mammalian tumor suppressor, PinX1, which is also expressed in the reproductive system, these studies may have important implications for human cancer and infertility.

841S Lipin-mediated regulation of neural stem cell behavior

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Tissue-specific stem cells are the building blocks during development and fuel the regenerative capacity of our body. Stem cell function is regulated by intrinsic, local, and systemic stimuli to adjust regeneration to tissue demands (PMID: 20144784, 21109189). Distinct metabolic states influence stem cell behavior, yet the molecular mechanisms that integrate cellular and organismal metabolism with stem cell fate decisions and tissue homeostasis remain poorly understood (PMID: 32185173, 34042969).

Lipids play a crucial role in cell and tissue homeostasis by providing a primary energy source for cells, serving as building blocks for membranes and organelles, and acting as signaling molecules. Imbalances in lipid homeostasis can result in lipotoxicity, cell death, and age-related diseases like cardiovascular disease, insulin resistance, diabetes, neurodegeneration, and cancer (PMID: 34547235). Lipins are phosphatidic acid phosphatases (PAPs), necessary for the biosynthesis of triacylglycerides (TAGs), the foundational component of lipid droplets (PMID: 18941140). Lipin proteins can also translocate to the nucleus and act as a transcriptional regulator to stimulate the expression of fatty acid oxidation (FAO) genes and repress the expression of genes involved in lipogenesis (PMID: 19369868). Thus, Lipins function at a crossroads between lipid anabolism and catabolism and constitute a primary candidate to address how lipid homeostasis affects stem cell behavior.

Our preliminary data indicate that depletion of the single *Drosophila* homologue of Lipin, *dLpin*, in neural stem cells, known as neuroblasts (NBs), results in mislocalization of the polarity protein Miranda and an increase in Deadpan (Dpn)+ NBs. We hypothesize that depletion of *dLpin* impacts the composition of the plasma membrane, which disrupts proper localization of factors that direct asymmetric division. Lipidomics and in-vivo lipid species sensor analysis support our hypothesis that phospholipid pools in the brain are affected by *dLpin* depletion in NBs. Although we observe an increase in Dpn+ NBs in the central brain region upon *dLpin* depletion, a concomitant increase in the brain area was not observed. Depletion of *dLpin* leads to changes in phosphoinositide pools, which in turn impacts Insulin signalling, limiting tissue growth. Our studies will shed light on the mechanisms linking lipid metabolism, stem cell behavior, and brain development and homeostasis, providing an important framework for developing lipid-based therapeutic interventions for regenerative medicine.

842S Screening for Novel Genes in Regeneration

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To better understand the genetic regulation of regeneration, we conducted a screen in *Drosophila* larvae knocking down candidate genes during regeneration. While adult *Drosophila* are incapable of regenerating damaged appendages, some imaginal discs of *Drosophila* larvae have been proven to regenerate if the injury is sustained early in development. Imaginal discs are larval precursors to many important adult structures. Early experiments used physical damage and tissue transplantation to study regeneration in imaginal discs, but this process is labor intensive and technically challenging. A major advance has been the development of genetic systems that enable the ablation of a specific portion of the wing disc at a specific time in development which is followed by regeneration driven by cells from the damage-adjacent tissue. Single-cell transcriptomic studies have identified genes expressed in regenerating discs (Worley et. al., 2022, PMID 35820420) which represent a list of candidates for genes that regulate regeneration. The DUAL Control genetic ablation system (Harris et. al. 2020, PMID 32490812) enables genetic ablation as well as the overexpression or knockdown of genes in the regeneration blastema. We have been conducting a screen of our candidates using the DUAL Control system. In our findings, we uncovered several genes that modify the regenerative ability of larvae which have not previously been described in the context of regeneration.

843S Piezo-Mediated Basement Membrane Repair in the Adult Gut

Bryant Humphries, Aubrie Stricker, Andrea Page-McCaw Cell and Developmental Biology, Vanderbilt University

Basement membranes are highly conserved, sheet-like extracellular matrices that provide critical structural support to almost every tissue type in the human body. They can be damaged by acute and chronic conditions and must be repaired to maintain proper function and preserve overall health of the organism. Little is known about this repair process, however. Recent work from our lab uncovered an exciting new matrix repair paradigm in the Drosophila midgut, where specialized enteroblasts termed Matrix Menders act as a local source of matrix components to repair the underlying basement membrane. Additionally, the mechanosensitive cation channel Piezo was found to be required for the activation of Matrix Menders and subsequent basement membrane repair. However, the mechanism by which Piezo initiates the basement membrane repair remains unknown. My current works aims to shed light on this mechanism by determining where Piezo is acting – both cellularly and subcellularly – and by elucidating the downstream pathway(s) affected by Piezo activation. While global Piezo knockout abrogates the repair process, it remains unknown where Piezo protein localizes, and where it is required for basement membrane repair. Using endogenously labeled Piezo-GFP, I have shown that Piezo protein localizes to a subset of intestinal stem cells, consistent with transcriptional reporter data. Additionally, my data suggests that Piezo localizes specifically to the basal side of these cells, which is the side in contact with the basement membrane. As an orthogonal approach, I am currently using cell type-specific Piezo knockdown and Piezo rescue to determine the cell type in which it acts. I am also working to characterize calcium dynamics in intestinal stem cells following Piezo activation using a live imaging approach. Additionally, I plan to identify the transcriptional regulator(s) driving collagen IV transcription in Matrix Menders following basement membrane damage. Overall, this work aims to provide a mechanistic understanding of this novel basement membrane repair process.

844S Identification of CG11180/Chigno interacting proteins functioning in the testis stem cell niche

Carolyn F Payne, Christy Vilcas, Greg Byun, Oliver Kerscher, Matthew Wawersik Biology, William & Mary

The uncharacterized gene, CG11180/Childless Gambino (Chigno), is strongly expressed at the apex of *Drosophila* testes. Specifically, Chigno is observed in germline and cyst stem cells as well as their immediate progeny. Prior experiments have shown that somatic Chigno RNAi knockdown causes cyst stem cell expansion at the expense of cyst production; leading to germ cell differentiation defects and reduced fertility. The likely human homolog of Chigno, PINX1, is a tumor suppressor that is localized to the nucleolus where it functions in telomere regulation and ribosome maturation. While we find that Chingo is also localized to the nucleolus, the molecular function of Chigno remains unknown. To better understand Chigno function, a yeast-two hybrid screen was conducted to identify Chigno binding partners. Through this screen, 14 potential binding proteins were identified and a subset with nuclear or nucleolar localization were subsequently tested for functional roles in the adult testis. Here we present data on identified interactors and the impact of RNAi knockdown on cyst stem cell behavior. Through these studies aimed at elucidating function of Chigno in fly testes, we hope to better understand mechanisms by which Chigno homologs regulate cellular behavior in complex tissues with links to human disease.

845S Investigating the mechanisms of dedifferentiation in the stem cell niche

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Stem cell maintenance relies on coordinated differentiation and self-renewal. Misregulation of this balance can contribute to aging, tissue degeneration, and cancer. In the *Drosophila* testis, germline stem cells (GSCs) surround a hub of post-mitotic cells that create a complex signaling environment for GSC maintenance. GSCs typically divide asymmetrically to produce one self-renewing stem cell that remains at the hub, and one differentiating cell that is displaced from the niche. However, asymmetric division alone cannot maintain a stable GSC population, as GSCs leave the niche at a rate of approximately one cell per day. Dedifferentiation, a process by which differentiating cells revert to a stem cell state, helps replenish lost GSCs. Dedifferentiation is important for stem cell maintenance and tissue regeneration in many species, though the underlying mechanisms are poorly understood. Using bulk RNA sequencing, we identified genes that are differentially expressed during dedifferentiation in the *Drosophila* testis. We found that knockdown of *me31b, RabX2, and CCHa2* inhibits dedifferentiation in the testis. We are also investigating the involvement of somatic support cells in dedifferentiation. Using long-term live imaging, we found that somatic support cells, which couple GSCs and their differentiating daughter cells, accompany dedifferentiating germ cells toward the niche, suggesting a potential role for these cells in dedifferentiation. Our work contributes to the understanding of stem cell maintenance and highlights potential genes and mechanisms that regulate cell dedifferentiation.

846T Not just “winging it”: an updated practical guide to *Drosophila* husbandry and genetics

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Resources like *Fly Pushing* (2004, Ralph Greenspan), *Drosophila: A Laboratory Manual* (2004, M. Ashburn, K. Golic, & S. Hawley), and a more recently distributed *Drosophila* laboratory manual (2019, M. Markstein) have been integral to the fly community, particularly to biologists entering the field of *Drosophila* genetics. Numerous contributions from hundreds of scientists over the years have led to tremendous expansions of the genetic tools and resources available to scientists. Periodic updates to our collective body of resource material are essential, both to help the *Drosophila* research community stay up-to-date on available genetic tools and to aid in the training of new biologists or scientists hoping to incorporate *Drosophila* into their existing research programs. Here, we present a digitally accessible guide to *Drosophila* husbandry and genetics. Our guide builds on the seminal resources provided in years past to cover essential topics around *Drosophila* husbandry, maintenance of larger collections, and setting up experimental crosses. In addition, we provide facile, practical explanations for both established and emerging genetic methods such as recombination, transgenesis, clonal/mosaic analysis, and more. We aim to make this collection publicly available as a training resource for research labs or for instructional settings that implement the *Drosophila* model for students. Finally, creating a digital version of this collection facilitates periodic updates as our research toolkit continues to expand in years to come.

847T *Drosophila* to model nanoparticle-based drug delivery to the eye

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Age-related macular degeneration (AMD) and diabetic retinopathy are the leading causes of vision impairment in the United States. Both diseases cause degeneration of the posterior eye and require the delivery of therapeutics to slow disease progression. The most common method of therapeutic delivery are intravitreal injections. However, these highly invasive injections must bypass several biological barriers of the eye before the drugs reach the retina and pose a serious risk for inflammation or infection. Since the prevalence of AMD and diabetic retinopathy is also steadily increasing, we urgently need to develop alternatives for therapeutic delivery.

Here, we use the *Drosophila* eye as a model to develop a nanoparticle-based drug delivery system. As proof of concept, our goal is to restore vision in blind *histidine decarboxylase* mutants that lack the key enzyme to synthesize histamine, the neurotransmitter of fly photoreceptors. Since like in humans, vitamin A (all-*trans*-retinol) is absorbed in the gut and transported to the eye for the formation of visual pigments, we use all-*trans* retinol as a targeting molecule to direct histamine-carrying nanoparticles to the eye. To this end, we engineered star-shaped gold nanoparticles that carry the targeting molecule all-*trans* retinol and the drug histamine. To release the drug at the eye level, we take advantage of gold nanoparticles intrinsic light sensitive properties. Since light readily passes through the eye, we will expose nanoparticle-fed flies to specific light wavelengths to trigger drug release in the eye and rescue vision. In our preliminary experiments, we found that nanoparticle stability was enhanced by the addition of thiol methoxy-polyethylene glycol (mPEG-SH). Moreover, the incorporation of a Schneider’s Medium-based protein corona around the nanoparticle surface increased both the drug and targeting molecule payloads. Nanoparticle feeding to histidine decarboxylase flies was sufficient to restore vision in a light-dependent manner as confirmed by behavioral phototactic assays. These promising preliminary data suggests that all-*trans* retinol enabled the targeted delivery of drug-loaded nanoparticles to the fly eye.

Taken together, the major goal of this study is to engineer a nanoparticle-based drug delivery system as a novel therapeutic approach to treat eye diseases.

848T Genetic analyses enabled by the Fourth Chromosome Resource Project connect previously unstudied genes to mutant phenotypes

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To date the Fourth Chromosome Resource Project (FCRP) has deposited roughly 815 stocks for the genetic analysis of its 79 protein coding genes. Here we provide an update on each stock set and illustrate new nervous system phenotypes uncovered by each. We employed T2A.GAL4 and eGFP strains to better understand *maverick* (*mav*), a paradoxical gene that is both conserved in mammals but completely dispensable for development to adulthood. The data suggest an important adult function with extensive transcription in the adult brain and reveals that mutants display a significantly reduced lifespan. We employed UAS fly and human cDNAs for four sets of pairwise analyses in the eye disc and adult eye. For two human disease genes, eye phenotypes constitute a potential path for determining their mechanism of action. We employed new truncation mutations to generate MARCM clones in the larval eye disc for *eyeless* (*ey*) and *twin of eyeless* (*toy*) that resulted in antenna duplications. In the brain, *ey* clones yielded a diachronic phenotype with the larval brain containing an adult stage mushroom body. In the adult brain, clones induced at second instar encompassed the entire glial blood brain barrier providing a platform for analysis of permeability. Taken together, the ease with which phenotypes were elicited from both previously unstudied and well-known genes suggests that a considerable amount of interesting biology has been hiding in plain sight on the fourth chromosome.

849T A Method to Discriminate Two *white* Alleles

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The *white* gene has played an important role in the evolution of *Drosophila* molecular genetics. The majority of transgenic *Drosophila* strains were made utilizing a modified *white* gene as the plasmid selectable transgenic marker while injected into a *white* (*w-)* mutant strain during P-element transformation or Site-Specific integration. Two widely used white mutants, w^{1} and w^{1118}, have distinctly different mutations consisting of the insertion of a *Doc* transposable element or a large deletion, respectively. Recently, it has been shown that w^{1} and w^{1118} alleles have different rates of retinal degeneration with w^{1118} having a stronger. Here, I demonstrate a set of PCR analyses that can distinguish the *white* mutant alleles w^{1} and w^{1118}.

850T Targeted Enhancer Silencing and Sex Determination Disruption via RD-CRISPRi.

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Precise modulation of cis-regulatory modules (CRMs) is essential for dissecting enhancer function and understanding developmental gene regulation. We developed and systematically applied RD-CRISPRi, a CRISPR interference strategy using dead Cas9 (dCas9) fused to minimal repressor domains (~9 amino acids, RDs), to silence enhancers in *Drosophila melanogaster*. This system operates with both standard gRNAs and truncated gRNAs (tgRNAs), enabling flexible targeting without inducing double-strand breaks (DSBs). Targeting eye- and wing-specific CRMs of the *wingless (wg)*locus revealed differential penetrance and spatial specificity, with complete repression of eye CRMs and variable effects in wing CRMs. RD-CRISPRi targeting of the wing enhancer of *apterous (ap)* and the T1-specific CRM of *Sex combs reduced (Scr)* produced robust loss-of-function phenotypes, confirming enhancer-specific transcriptional inhibition. Analysis of the L2 vein CRM of *knirps (kni)* demonstrated spatial precision, with central gRNA sites overlapping transcription factor binding motifs yielding the strongest repression. Extending RD-CRISPRi to sex determination loci, targeting *Sex-lethal (Sxl)* and *transformer (tra)* disrupted female viability and sexual differentiation in a generation-dependent manner. Finally, we engineered a split sterility gene drive by inserting a cassette into the *prosalpha2* locus containing two distinct components: (i) a gRNA that induces a DSB for homing-based copying of the drive cassette, and (ii) tgRNAs that do not induce DSBs but efficiently repress transcription of *Sxl*. Cas9-RD was supplied from a separate construct under an Actin promoter. Population cage trials achieved rapid population suppression. These findings establish RD-CRISPRi as a versatile platform for enhancer interrogation and demonstrate its potential for population control strategies through precision gene drive design.

851T Large-scale *in-silico* discovery of protein-protein interactions in *Drosophila*

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Recent advances in AI-driven protein structure modeling now enable *in-silico* prediction of protein-protein interactions (PPIs), facilitating large-scale, high-throughput screens. Despite this progress, *in-silico* screens are computationally prohibitive, requiring expensive processors (e.g. GPUs) and long timescales (e.g. months). Here, we describe an optimized *in-silico* PPI screening pipeline using AlphaFold-Multimer at a high-performance computing (HPC) data center, which dramatically reduces computation time, expense, and manual intervention. A key component of these optimizations is tailoring computational resources based on protein size. We have used this pipeline to perform 36 *Drosophila* proteome-wide “one-to-many” screens, as well as “many-to-many” screens testing hundreds of secreted and transmembrane proteins for interaction. In total, we have screened over 500k *Drosophila* PPIs, and we will present biological insights from these screens, including novel hormone-receptor candidates and interacting partners for uncharacterized smORF peptides. We are expanding our screens to include other biomolecular interactions (e.g. metabolite-protein) using AlphaFold3 and Boltz-2. Our optimized pipeline can be easily adapted for other HPC data centers, PPIs, and species, enabling more scientists to discover *in-silico* protein-protein interactions at scale.

852F Screening split-GAL4 combinations for expression outside the nervous system

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The split-GAL4 system enables cell-specific transgene expression and has primarily been used to target neuronal populations in the fly brain. The Bloomington *Drosophila* Stock Center currently distributes a collection of ~4,400 split-GAL4 hemidriver pairs with characterized expression patterns in the central nervous system (CNS). However, expression in other tissues has not been examined in a systematic way. Here, we describe ongoing efforts to identify and characterize lines from this collection that show expression outside the CNS. Using whole-animal live imaging, we are surveying reporter expression of these hemidriver combinations across tissues in larvae and adults. Our preliminary results show diverse and reproducible patterns in several other tissues, including the gut, muscle and epidermis, in addition to the expected patterns in the brain. Overall, this work adds value to an established split-GAL4 collection and identifies lines that may be useful for non-neuronal, tissue-specific studies of development, physiology and disease.

853F Genome wide elucidation of cis-regulatory elements and gene regulation in fly aging and pro-longevity

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Aging is a complex biological process marked by progressive functional decline, with gene regulation playing a crucial role in mediating age-related changes across tissues. In this study, we perform a genome-wide elucidation of cis-regulatory elements (CREs) and gene regulation mechanisms associated with aging and pro-longevity in Drosophila melanogaster. With single-nuclear and multi-omics approaches, we mapped cis-regulatory regions across the fly genome, identifying enhancers, promoters, and other regulatory elements that modulate gene expression in aging and longevity-promoting contexts by low temperature. We integrated chromatin accessibility and transcriptome data from young to aged flies to build a comprehensive landscape of CRE activity and transcription factor binding dynamics across different tissues and cell types. By characterizing regulatory networks and transcription factor motifs that control age-related and pro-longevity gene expression, we uncovered specific regulatory mechanisms that drive tissue resilience and lifespan extension. With combined aging features revealed on chromatin and transcripts level, we construct a more informative and biological aging and pro-longevity map.

854F Genetically encoded voltage sensors for probing membrane potential

Sam Zheng Bloomington Drosophila Stock Center, Indiana University

Voltage-sensitive indicators are important optical tools for the study of cell membrane potential, which is vital for the function of all cell types. The Bloomington Drosophila Stock Center (BDSC) distributes a large number of genetically encoded voltage indicators (GEVIs) for imaging neuronal activity with single-cell resolution, tracing activities of a large population or in multiple brain areas during behavior. Multiple indicators with variable spectral characteristics can be combined with other optogenetic and chemogenetic tools for control and visualization of neuronal activity. Newly developed voltage indicators such as Voltron combine the genetically encoded sensor and photostable synthetic dye for long in vivo imaging. While the majority of voltage sensors are developed for application in the neurons and muscles, a few GEVIs are especially useful to probe the membrane dynamics of non-excitable cells such as those lining the epithelium. With the careful consideration of brightness, linearity, speed and cytotoxicity, these tools can be used to measure various signals (subthreshold and suprathreshold events) in many cell types and tissues.

855F Exploiting sex-specific myosin genes to selectively eliminate flight in *Aedes aegypti*

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The yellow fever mosquito *Aedes aegypti* is a competent vector for arboviruses such as dengue, chikungunya, and Zika. Ecological concerns with current insecticidal measures and insecticide resistance in endemic regions necessitate new and sustainable modes of population suppression; among these are species-specific genetic biocontrol strategies. Previous work with this species has shown that genes required for flight represent viable genetic targets for the suppression of *Aedes aegypti*. In *Drosophila melanogaster*, as in most arthropods, only one gene encodes the class II myosin heavy chain (MHC) in the flight muscle. *Aedes aegypti* represents an interesting exception, as it contains multiple MHC paralogs with sex-specific patterns of expression. The myosin gene *myo-sex* is required for flight in males and tightly linked to the dominant male-determining factor *Nix*. A paralog of *myo-sex* named *myo-fem* is located on an autosome, but expressed specifically in females; this gene is required for female flight but dispensable in males. Recent efforts to identify specific contributions of *myo-sex* to male physiology led to the creation of an in-frame deletion which produces a flightless phenotype in heterozygous individuals. This deletion does not affect sequences associated with the actin-binding or nucleotide-binding domains of the translated peptide, and instead appears within the *Src-homology 3* (SH3) domain, a highly conserved region encoding a structure of unknown function in *Drosophila*. Due to the isolation of this mutation from binding interfaces on the MHC head, we hypothesize that this mutant encodes a *dominant negative* Myo-sex product. A synthetic *myo-sex* transgene with the same mutation controlled by its native promoter was introduced into wild-type *Aedes aegypti*, resulting in a male-specific dominant flightless phenotype with near-100% penetrance. CRISPR-Cas9 mutations to the female myosin, *myo-fem,* were screened to identify another in-frame deletion near a homologous site to the original *myo-sex* mutation. To date, this mutant has exclusively produced flying males and flightless females, which suggests that we have produced a female-specific dominant negative allele in addition to the previous male-specific allele. Our results encourage further investigation of an understudied region of the MHC coding sequence, and the resulting sex-specific flightless alleles can be used for population suppression applications in the future.

856F A single-cell transcriptomic atlas of Drosophila metamorphosis

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Cell atlases have become an essential tool in the analysis of single-cell and single-nucleus RNA-seq data. The Fly Cell Atlas(FCA), among several others, compiles transcriptomic profiles of millions of nuclei, along with providing ground truth cell type labels and marker genes to which researchers can compare their own snRNA-seq results. These atlases largely focus on adult organisms, leaving biologists interested in earlier stages of development without a reliable reference. Applying snRNA-seq to pupae presents unique challenges, which is perhaps why they are relatively under studied. This is despite their relevance to human research, which stems from parallels in hormonal signaling drawn between insect metamorphosis and human puberty. To begin filling this knowledge gap, we have created an atlas spanning the timeline between the early pupal stage and early adults.

Widespread programmed cell death occurring during metamorphosis results in reduced RNA synthesis and degradation of already synthesized RNA, reducing the total number of molecules available to be quantified. Damaged and dying cells can lead to increased levels of ambient RNA being encapsulated into the GEMs used for snRNA-seq. For these reasons, analysis methods proven to work with adult samples can be suboptimal when applied to pupae, thus we have developed a pipeline optimized for their study. For example, the popular software package CellRanger discards multimapping reads, but we recover these reads via expectation maximization. This leads some genes, for example CG42586, to go from near undetectable to become a significant marker of the larval fat body. Notably, as many as 3 times more ambient counts were removed from some pupa samples vs. adults. In the end, we recovered approx. 100k nuclei from 12 snRNA-seq libraries, with two libraries for each 24-hour increment between the white prepupa stage and newly emerged adults. Machine learning methods were used for dimensionality reduction and to project cell type annotations from the FCA onto our nuclei. Compared to adult cells, many pupa cell types show differing gene expression patterns, so our annotations have been manually verified via comparison with existing literature. This yielded 26 broad cell type clusters, 8 more than are in the FCA. Based on these cell types, we provide an examination of their evolution through developmental time. One interesting finding is that mature oenocytes, as expected, increase in number throughout metamorphosis, but upon reaching adulthood, they split into two distinct subtypes.

857F Characterization of the light-dependent phosphoproteome in *Drosophila melanogaster*

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Drosophila phototransduction is a G-protein-coupled receptor (GPCR) phosphoinositide signaling cascade that allows for rapid response to light, enabling flies to adapt to changing light conditions in milliseconds. Proteins involved in this light response cascade have been reported to be phosphorylated in a light-dependent manner by eye-specific Protein Kinase C (INAC), which is necessary for correct phototransduction termination. These proteins include the TRP calcium channel and the INAD scaffolding protein. Light-dependent and INAC-dependent phosphorylation of these proteins has been reported in previous studies using targeted mass spectrometry (MS) and western blot analysis. Since determining light-dependent and INAC-dependent phosphorylation has only been conducted with targeted approaches on specific proteins, the potential for identifying other light-dependent and INAC-dependent phosphoproteins remains. Due to these missing data, we sought to determine a light-dependent phosphoproteome in *Drosophila* heads along with determining novel INAC-dependent substrates that may contribute to phototransduction termination. We first built a *Drosophila* head phosphopeptide library by utilizing offline peptide fractionation and a data-dependent acquisition (DDA) MS method on flies collected under light and dark conditions. This library contains 63804 unique phosphopeptides and 5349 unique phosphoproteins for reference in future data-independent acquisition (DIA) MS experiments. We then conducted unbiased phosphoproteomic experiments on whole fly heads from WT or INAC-null flies collected under light or dark-adaptation conditions, utilizing a DIA-MS method. We identified 2732 unique phosphosites corresponding to 1096 phosphoproteins that are differentially phosphorylated between experimental conditions in WT flies. Many of the upregulated phosphorylation sites in WT flies are seen under prolonged blue light exposure which hyperactivates *Drosophila* phototransduction. Along with this, we have identified several candidate INAC-dependent phosphosites, and we will be exploring their role in phototransduction termination.

858S High-Resolution Automated *Drosophila* Geotaxis Analysis Elucidates Clinically Relevant Phenotypes of Genetic Neuropathies

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The innate negative geotaxis response of *Drosophila* has long served as a readout for locomotor function, widely used to assess the impact of aging and neurological diseases. Existing assessment methods are predominantly manual and rely on basic metrics, limiting both throughput and analytical insights. Here, we present an automated, high-resolution geotaxis assay combining a custom-built, fully automated apparatus with advanced multi-object tracking software capable of following sub-second movements of individual flies, allowing for reproducible and quantitative analysis of geotactic behavior. Our system reliably initiates and monitors geotaxis in up to 10 cohorts—each comprising up to 7 flies—concurrently, increasing experimental throughput. The tracking algorithm achieves ~97% accuracy in resolving individual fly trajectories at a temporal resolution of 1/30 second, enabling robust, reproducible quantification of complex behaviors within and across groups. From these high-fidelity output datasets, we extract detailed climbing curves, speed, linearity, and movement vectors, while also detecting and quantifying transient movement events, such as pause, drop, jump, and flight behaviors that are overlooked in geotaxis studies due to limited spatio-temporal resolution. Such granular metrics have been shown to model clinically relevant phenotypes in genetic neuropathies such as hereditary spastic paraplegia and sorbitol dehydrogenase deficiency capturing subtle but significant motor and sensory changes. By integrating automation with sophisticated tracking analytics, our platform is therefore poised to advance Drosophila geotaxis analysis, facilitating deeper exploration of the genetic and physiological determinants of locomotor behavior.

859S Developing a Novel Dual-driver Model to Facilitate Mechanistic Studies of Cancer Cachexia in *Drosophila melanogaster*

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Cancer cachexia is a complex, multi-organ syndrome whose early initiating events remain poorly understood, in part due to limitations in existing model systems. Our lab's GAL4-driven *Ras*^{V12}, *scrib*^{RNAi} larval model (hereafter Gal4-model) has yielded extensive data on cachexia and pre-cachexia, but it has significant limitations for mechanistic studies. The Gal4 system is a powerful tool for gene expression, functional, and rescue studies due to the vast number of both target and driver lines available. This makes it desirable to convert the tumor generation to an alternative technology, thus freeing Gal4 for use in the peripheral tissues. To overcome this, dual-driver models (DDM) have been developed, utilizing the alternative "Q-system" binary expression tool to drive tumorigenesis, thereby freeing Gal4 for targeted manipulation of specific muscles (Lodge et al. (2021) and Hodgson et al. (2021). To accomplish our longer term goals, we have decided to build an additional DDM to complement the utility of existing models. We designed our model to limit the number of transgenes needed, and to enable testing of transgenes in the peripheral tissues as an F1 cross. To this end, we constructed a dual promoter vector to express QUAS-RasV12 and QUAS-scribRNAi elements to drive tumor formation and had the construct injected at the su(Hw)attP5 site on the second chromosome. This resulted in weak tumor induction (hereafter QQ-weak), which surprisingly recapitulated early pre-cachectic changes in the muscle. We therefore established this model for pre-cachexic mechanistic studies. To generate a stronger model that recapitulates the full spectrum of GAL4 model phenotypes, including delayed pupation, giant larvae, and invasive tumorigenesis, we used conventional stock building to integrate a previously published QUAS-RasV12 and QUAS-scribRNAi chromosome with our hh-QF chromosome, which we generated with the HACK methodology (Lodge et al., 2021; Potter et al., 2010b). Together, this study generated two dual driver tumor models, QQ-weaker and QQ-strong which will improve the toolbox for mechanistic cachexia studies in *Drosophila melanogaster*.

860S A Rapid Whole-Genome Pipeline for Lesion Identification in Chemically Mutagenized Drosophila

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Ethyl methanesulfonate (EMS) mutagenesis has generated extensive collections of *Drosophila melanogaster* mutants with well-defined phenotypes but unknown causal genotypes. One such large-scale EMS screening of over 26,000 chromosomes identified 566 pupal-lethal mutants, among which 26 strains in 19 complementation groups displayed a pronounced small pupa (sP) phenotype. Further characterization of this phenotype provides a chance to identify essential components of growth and developmental regulation. Additionally, this sub group presents an opportunity to develop a method to characterize which proteins have been disturbed and can be expanded out to broader swaths of the screen.

To identify causal variants underlying these mutants, we applied short-read whole-genome sequencing to previously unmapped sP EMS lines. Genomic DNA was isolated from sP mutant lines, the parental mutagenized background, and previously mapped EMS mutants used as controls. Sequencing data were analyzed using a variant-filtering framework designed to retain EMS-induced single-nucleotide mutations while removing background and shared variants. All analyses were restricted to the third chromosome to reflect prior genetic mapping. Functional annotation was used to prioritize variants predicted to have strong effects on protein function.

For a subset of lines, prior genetic characterization, including complementation tests between mutants and defined chromosomal deficiencies, was incorporated to further refine candidate loci. Candidate genes identified through this combined approach were tested by fly crosses to confirm causal mutations that lead to the small-pupa phenotype.

Together, this work establishes a scalable and reproducible strategy for moving EMS mutant lines from phenotype to genotype. Systematic genotyping of small-pupa mutants will enable comparative analysis of genes and pathways required for normal growth and developmental progression, and demonstrates the continued value of EMS mutagenesis as a discovery tool in the genomics era.

861S DrOVi: An Interactive Web Platform for Synteny-Guided Orthology Exploration in *Drosophila*

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Orthologous genes, which diverge as a result of speciation, are fundamental to understanding gene function and evolutionary processes. In Drosophila, where gene turnover and structural variation occur frequently, precise identification of orthologs is challenging. Although sequence- and phylogeny-based methods are commonly applied, integrating conserved local synteny can greatly enhance prediction accuracy, particularly for complex gene families. Existing synteny-based tools, however, often suffer from constraints such as limited taxonomic coverage, inflexible parameters, or the need for advanced computational skills. To overcome these weaknesses, we developed the Drosophila Orthology Viewer (DrOVi)—an interactive platform for investigating orthology relationships across multiple Drosophila species. For identifying orthologous genes, DrOVi provides answers from both public repositories and de novo predictions, combining sequence similarity with synteny-based comparisons. Through an intuitive interface, users can adjust parameters such as similarity cutoffs and syntenic window sizes, enabling customized exploration of the conserved gene order. By integrating flexible orthology inference with local synteny information, DrOVi provides a useful tool for studying gene evolution, functional conservation, and genomic organization in Drosophila. DrOVi can be accessed through https://cosbi9.ee.ncku.edu.tw/drovi/GeneOrder/

862S *Drosophila* Genome Atlas (DGA): An Integrated Platform for Comparative Genomics and Functional Annotation of Non-model *Drosophila* Species

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Genomic databases serve as fundamental infrastructures for modern biological research. In the field of *Drosophila* research, while the model organism *Drosophila melanogaster* is well-supported by comprehensive resources like FlyBase, similar high-quality databases for non-model *Drosophila* species remain limited. To address this gap, we present the ***Drosophila* Genome Atlas (DGA)**, a web-based platform for comparative genomics across *Drosophila* species. The database integrates high-quality assembled and annotated genomes from **20 *Drosophila* species,** along with one outgroup species from *Scaptodrosophila*. Currently, *Drosophila simulans* serves as the fully implemented pilot species. DGA integrates a wide range of functional annotations for non-model *Drosophila* species, including InterPro domains, Gene Ontology (GO) terms, and KEGG pathways inferred through orthology with *D. melanogaster*. These resources allow users to efficiently query gene functions in less-studied species.

In addition to the basic genomic tools, DGA provides an **advanced local synteny visualization tool that leverages orthology information from NCBI and OrthoDB**, allowing users to directly visualize microsynteny relationships between a target species and the reference *D. melanogaster*. This capability is particularly valuable for identifying evolutionary events such as gene duplication and genomic rearrangement.

DGA provides a centralized resource to support the *Drosophila* research community in exploring the genomic diversity and evolutionary history of the genus. The platform is freely accessible at cosbi10.ee.ncku.edu.tw/Drosophila/SelectSpecies/

863S PolyORF: A plasmid cloning strategy for polycistronic expression of ORFs in Drosophila and beyond

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Exogenous expression of multiple genes is a highly valuable technology in biology, medicine, and industrial biotechnology. In particular, expressing multiple genes from one transgene is ideal to facilitate straightforward genetics in model organisms such as *Drosophila*. While modular cloning strategies such as Golden Gate Assembly allow multi-fragment DNA construction, they have not yet been adapted to generate single-gene expression transcripts encoding >3 open reading frames (ORFs). To address these limitations, we developed a Golden Gate Assembly-compatible platform that supports the modular assembly of up to eight different open reading frames (ORFs) under the control of a single promoter. Our system leverages “self-cleaving” 2A peptides to link ORFs to ensure coordinated separation and expression of multiple proteins from a single transcript. We show that eight ORF module plasmids can be efficiently assembled into one destination plasmid using a set of nine compatible Type IIS restriction enzyme sites. We are currently testing 2A peptide cleavage efficiency using a panel of eight ORFs of varying size. We plan to apply this technology in two ways. First, we aim to express six proteins of the bacterial “iLux” pathway to generate an autobioluminescence reporter in *Drosophila*. Second, we aim to express six proteins of the evoCAST CRISPR-associated transposase system to enable programmable gene insertion in *Drosophila*. This highly customizable system expands the toolkit for modular cloning in Drosophila by allowing for the creation of large complex polycistronic constructs, and as a result of using the viral 2A peptide sequences for cleavage, can be delivered into any eukaryotic destination vector.

864S Resources for *Drosophila* embryo cryopreservation at lab and stock center scale.

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Cryopreservation methods to vitrify *Drosophila melanogaster* embryos were originally developed and described in the early 1990s (Steponkus et al., 1990; Mazur et al., 1992). However, the adoption of cryopreservation of *Drosophila* has lagged other model systems, likely due to differences in permeability and recovery rates across strains. We revisited these methods and developed a more streamlined approach in Zhan et al. (2021). Here we report on updated methods to permeabilize and cryopreserve *Drosophila* embryos with improved survival. We have combined butanol and heptane to achieve better permeabilization and improved cryoprotective agent (CPA) loading across *Drosophila* strains with increased survival rates using lower concentration CPA and an improved CryoMesh warming technique. We have designed liquid nitrogen storage boxes to easily store vitrified samples on CryoMesh for later recovery of strains. We are collaborating with the Bloomington Drosophila Stock Center to investigate how cryopreservation would help eliminate genetic drift and reduce costs to maintain *Drosophila* stocks. We have designed a Canvas website which guides users through the cryopreservation process and disseminates the protocol to the community. Beginning in 2026, we will host week-long in person workshops at the University of Minnesota to train interested individuals or *Drosophila* labs to cryopreserve and store their own strains. Current efforts are underway to evaluate the mutagenic effects of cryopreservation using next generation sequencing.

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Drosophila Embryo Injection Services

- ***User friendly system!*** *Real time tracking of service progress online.*
- ***Inject 200+ embryos per service.***
- ***Deliver transformed and/or balanced flies.*** *We do the crosses!*
- ***One shipping & handling charge per order!*** *No matter how many services purchased.*
- ***Selection of w^{1118}, yw or your own strain for transposable-element injection.***
- ***PhiC31 integrase-mediated site-specific transgenesis - the broadest selection of attP sites.***
- ***Screen for white, yellow, vermillion, and/or GFP/RFP/DsRed/YFP/CFP.***
- ***MiMIC injection service.***
- ***CRISPR injection service.***
- ***Over 110,000 individual constructs were successfully injected and over 580,000 transformants delivered!***