10:30 AM - 11:00 AM	*Attendees arrive, put up posters, and mingle over coffee and tea*
11:00 AM	Opening remarks
11:10 AM – 11:30 AM	Akshada Shankar Ganesh, Erceg Lab *The functional organization of chromosome territories in single nuclei during zygotic genome activation*
11:30 AM - 11:50 AM	Vedansh Patel, Inaba Lab *Dynamic Histone Turnover Facilitates Cell Fate Transitions during Male Germline Differentiation*
12:00 PM	Lunch (*provided to all registered attendees*)
1:00 PM - 2:00 PM	Poster session
2:00 PM - 2:20 PM	Blanka Rogina, Faculty talk *Caloric Restriction and Aging in Drosophila*
2:20 PM - 2:40 PM	Sydney Ballou, Menuz Lab *Role of adult antennal support cells in Drosophila olfaction*
2:40 PM - 3:00 PM	Andre Jang, Menuz Lab *The Role of Kinase CG7236 in Insect Olfaction*

3:00 PM - 3:20 PM	Natalie Aloisio, Sun Lab *DILP8 functions as a mature follicle sensor to prevent excessive accumulation of mature follicles in Drosophila ovaries and oocyte aging*
3:20 PM - 3:30 PM	Coffee Break
3:30 PM - 4:20 PM	Keynote lecture: **Erica Larschan** Associate Professor of Molecular Biology, Cellular Biology and Biochemistry at Brown University. **"X marks the spot: Targeting of the X chromosome for coordinated gene regulation"**
4:20 PM	Awards and Closing Remarks

Talk Abstracts

11:10 PM – 11:30 PM

Akshada Shankar Ganesh

Erceg Lab, Graduate Student

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

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 with extensive homolog pairing at the whole-chromosome level that decreases locally due to spatial variability in chromosome conformations. In the absence of one homolog copy, 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.

11:30 PM - 11:50 PM

Vedansh Patel

Inaba Lab, Undergraduate Student

Dynamic Histone Turnover Facilitates Cell Fate Transitions during Male Germline Differentiation

Histones possess post-translational modifications that can determine the local transcriptional status and thus an epigenetic memory of a cell. Although recent histone replacement techniques have led to significant advancements in understanding how histone modifications regulate gene activity in vivo, the role they play during the transition of cell fates remains unclear. We have generated two histone transgenes with mutations on their modification sites and simply overexpressed them in male germline stem cells (GSCs) and differentiating descendants.

First, we overexpressed histone H3 with a mutated phosphorylation site (Th3, H3T3A). Previously, H3T3A expression was shown to disrupt the distinction between sister chromosomes, each incorporated new vs. preexisting histones during GSC division. However, the effect on cell fate determination was not apparent. To sensitize this genotype, we induced tissue regeneration after experimentally depleting GSCs. The recovery phase of the GSC through the mechanism of “dedifferentiation” is susceptible to intrinsic fate changes. Therefore, we hypothesized that this method may enhance the phenotype caused by subtle changes in cell fates. As we expected, we found that overexpression of non-H3T3A results in the enhancement of recovery associated with an accumulation of DAPI-high, stem-like cells surrounding the niche, suggesting that dedifferentiating cells obtain enhanced stem-cell-like properties due to a potential regeneration signal, and the histone phosphorylation may influence this process.

Second, we tested overexpression of histone H2A with mutated four potential ubiquitination sites under the nosGal4 driver (nos>H2A K4R). We first noticed early termination of expression of the transgene during the transit amplification stage compared with the wild-type histone H2A transgene (nos>H2A). When H2A K4R is expressed in spermatocytes, we observed swollen chromosome territories with massive accumulation of ubiquitinated H2A (H2Aub) in the spermatocyte nucleoplasm. These results suggest that the overexpression of non-ubiquitatable H2A may cause changes in H2A dynamics and chromosome organization.

We will further dissect these phenotypes to understand how histone modification influences cell differentiation.

2:00 PM - 2:20 PM

Blanka Rogina

Faculty talk

Caloric Restriction and Aging in Drosophila

The Rogina lab is interested in the basic biology of aging, including the molecular genetic determinants of aging and longevity. Over the past few years, her work has concentrated on understanding some of the mechanisms that regulate gene expression during adult life in Drosophila melanogaster. Her lab conducts large-scale genetic screening to obtain genes that are involved in life span determination. Another interest is to investigate the biological determinants of female longevity. Female life spans depend on reproductive status. Her lab's goal is to understand the relationships between reproduction capacity and female longevity.

2:20 PM - 2:40 PM

Sydney Ballou

Menuz Lab, Graduate Student

Role of adult antennal support cells in Drosophila olfaction

Insects utilize their antennal olfactory system to detect numerous environmental odorant stimuli to aid in behaviors such as identifying food sources, host seeking, and mating. While Olfactory Receptor Neurons (ORNs) and the odors which they respond to are extensively studied, there is much less known about another major class of antennal cells, the support cells. The roles of support cells are defined during development, but in the adult antenna their function remains elusive. It's hypothesized that support cells might metabolize odors, since many putative Odorant Degrading Enzymes (ODEs) are found expressed in the antennal transcriptome. One abundant class of these putative ODEs are the Cytochrome P450 (Cyp) gene superfamily. Of the approximately 87 Cyps expressed in the Drosophila melanogaster genome, ~57 are detected in the antennal transcriptome at significant levels. However, the contribution of antennal Cyps to odorant detection and their localization within the antenna is poorly understood. Here, we selected thirteen antennal Cyps to investigate, including those most highly expressed and those with antennal-enriched expression compared to other tissues. Using newly generated transgenic LexA lines, immunohistochemical analysis revealed that most of the Cyps are broadly expressed across the antenna, whereas a few are more narrowly expressed. Comparison with the neuronal marker, elav, revealed that one Cyp is expressed in olfactory neurons, whereas the majority are found partially expressed in one or more support cell types of the antenna. Additionally, very few functional studies have been done to investigate Cyps role as ODEs. Here, we identify one Cyp, Cyp313a4, that is uniquely expressed in the support cells of a specific class of ORNs. Since the antennal ORNs odorant response profiles have been very well-studied, it makes it straightforward to test if Cyp313a4 has an effect on those particular odorant responses. Ongoing work is testing a Cyp313a4 null mutant for any differences in odorant responses and kinetics in comparison to wildtype responses. Together, this work will be the first map of Cyp expression in the Drosophila antenna and will serve as a roadmap for future studies on their functional roles in odorant degradation.

2:40 PM - 3:00 PM

Andre Jang

Menuz Lab, Graduate Student

The Role of Kinase CG7236 in Insect Olfaction

Insects use olfaction as one of their primary senses to find food sources. While insect anatomy is conserved between different insect species, odorant receptors that mediate the odor response profile of olfactory sensory neurons are not well conserved. Our previous work led to the identification of conserved candidate genes that may have critical roles in insect olfactory signaling. Here, we examine one of these conserved candidate genes known as CG7236, a member of the serine/threonine CDKL protein kinase family. CG7236 is found to be expressed in not only all antennal neurons but also broadly expressed in sensory neurons ranging from mechanosensory neurons to olfactory neurons found in sensory organs outside of the antenna. The subcellular localization of CG7236 in antennal neurons has been determined to be localized to the olfactory neuron dendrite and cell body. Further investigation has demonstrated that neuronal spontaneous activity and odorant responses were significantly reduced in CG7236 mutants, particularly in the large and small basiconic sensilla subclass. Lastly, the loss of CG7236 does not result in odorant receptor mislocalization or change in dendritic length, however there is a significant change in odorant receptor fluorescence intensity in the small basiconic subclass. Our results suggest that the loss of CG7236 leads to reduced multibranched dendrites at the end of olfactory sensilla leading to reduced odorant receptor neuron responses expression. This is a novel study into CDKLs in the Drosophila model system.

3:00 PM - 3:20 PM

Natalie Aloisio

Sun Lab, Graduate Student

DILP8 functions as a mature follicle sensor to prevent excessive accumulation of mature follicles in Drosophila ovaries and oocyte aging

Excessive mature follicle accumulation in ovaries harms oocyte health and offspring viability while also wasting energy. As such, the number of mature follicles in ovaries is tightly controlled. In Drosophila, each ovary is comprised of ~16 ovarioles, each containing 1-2 mature follicles, regardless of the female's mating status. The mechanism by which the female flies count the number of mature follicles to coordinate egg release and oogenesis remains a mystery. Previous work, along with our RNAseq and antibody analysis, demonstrated that Drosophila insulin-like peptide 8 (DILP8) is expressed in somatic follicle cells of mature follicles but not in younger follicles. Contrary to previous findings, we found that DILP8 is not essential for mating-induced ovulation/egg laying. In contrast, global depletion or follicle-cell-specific knockdown of dilp8 leads to defective ovulation/egg laying and a significant increase of mature follicles in virgin females. In addition, we found that excessive accumulation of mature follicles in dilp8-knockdown females leads to poor oocyte quality. Furthermore, both global and neuronal knockdown of lgr3, encoding a previously identified DILP8 receptor, showed similar ovulation/egg laying defects, accumulation of mature follicles, and poor oocyte quality in virgin females. Therefore, DILP8 functions as a mature follicle sensor to prevent excessive accumulation of mature follicles and maintain oocyte quality through the neuronal Lgr3 receptor in virgin females. Because mating is not always available in the wild and the DIP8/Lgr3 pathway is highly conserved across multiple species, our findings suggest that DILP8/Lgr3 is likely critical for maintaining the optimal reproductive fitness of virgin females and for species survival in the wild.

Poster Abstracts

Poster #1 **Teng Long**

Menuz Lab Graduate Student

Identifying the Molecular Components that Generate the Transepithelial Potential in Insect Sensilla

Insects rely on olfaction to navigate their environment, find food, locate mates, and avoid predators. Odorants are detected by sensory neurons housed in olfactory sensilla, the hair-like protrusions on the antenna. While sensilla serve various sensory functions, including taste, mechanosensation and hygrosensation, olfactory sensilla share a similar structure with other types of sensilla. A key feature across all sensilla is a voltage difference between the sensillar lymph and hemolymph, called the transepithelial potential (TEP), with the lymph being more positive. This potential is thought to drive cations into sensory neurons when receptors are activated, initiating neuronal firing. The TEP may stem from high potassium concentrations in the sensillar lymph relative to the hemolymph, and is likely generated by support cells transporting potassium. However, the molecular basis of TEP generation remains entirely unknown.

Here, we use the Drosophila olfactory system to characterize the TEP and investigate its role in odor detection. Using single sensillum recordings, we measured TEPs in olfactory large basiconic sensilla and found them consistent across sensillum subclasses, sex, and age. An RNAi screen identified candidate genes involved in TEP generation. Finally, we show that loss of TEP reduces odor-evoked responses, suggesting the TEP plays a critical role in insect olfactory signaling.

Poster #2 **Samaneh Poursaeid**

Inaba Lab Post-doc

Heterogeneous Regulation of Brinker mRNA by BMP Signaling in Drosophila Testes

Bone morphogenetic protein (BMP) signaling plays critical roles in various biological processes. In Drosophila testis, the niche-derived BMP ligand is essential for maintaining germline stem cells (GSCs) by repressing a key differentiation factor, bag of marbles (bam). However, in contrast to its role in GSC maintenance, BMP signaling promotes differentiation in gonialblasts (GB) and spermatogonia (SGs). How the same signaling pathway is interpreted differently among germline cells to generate distinct cell fate outcomes remain unclear. Our previous findings suggest that the variability in the levels of downstream effector contribute to regulate cell fate decisions and behavior.

One such effector is Brinker (Brk), a well-characterized transcriptional repressor of BMP signaling in Drosophila embryos and wing imaginal discs. However, its role in testis development remains unknown. Here, we show that, brk is specifically transcribed in clusters of 4–8 interconnected spermatogonia (SG), where it overlaps with bam expression. Using bam transcriptional reporter flies, we found that Brk represses bam expression in SGs and its own expression is negatively regulated by BMP signaling. Interestingly, we found that brk mRNA levels were not uniform among interconnected SGs; some cells displayed high expression, while others exhibited low or undetectable levels This variation in brk mRNA levels corresponded with differences in Brk protein expression, suggesting distinct cellular responses to BMP signaling.

Given Brk's role as a bam repressor, this heterogeneity may influence cell fate decisions, potentially giving SGs with high brk expression a competitive advantage over their neighbors during de-differentiation. Understanding the mechanisms underlying this heterogeneity will provide deeper insight into how BMP signaling is differently interpreted among germline cells.

Poster #3 **Kaylah Samuelson**

Hanlon Lab Graduate Student

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

The supernumerary B chromosomes recently found in D. melanogaster exhibit biased transmission, or drive, during female meiosis, which is has enabled them to be maintained over many generations despite being nonessential. This drive is dependent on a genetic reduction of matrimony (mtrm), which normally regulates Polo kinase (Polo) in a 1:1 ratio. We found that B chromosome transmission decreases as the genetic levels of Mtrm increase relative to Polo, suggesting that an excess of unregulated Polo promotes B chromosome drive. We then tested if the direct interaction of Polo and Mtrm is necessary to suppress the drive of the B chromosomes and found that expressing an allele of mtrm that cannot bind Polo is unable to reduce B chromosome transmission, indicating that the direct interaction of Mtrm and Polo is necessary to suppress drive. 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 preliminary data show a downward trend in B chromosome transmission in Gwl[Scant] females, leading us to conclude that unchecked Polo activity promotes drive of the B chromosomes. To link our findings with the mechanism of biased chromosome segregation during the female meiotic divisions, we are refining an assay that will overcome the biological barriers that limit the direct observation of D. melanogaster female meiotic chromosome segregation. Our initial results reveal a high degree of irregular chromosome positioning and segregation in meiosis I and II when Polo kinase activity is elevated. Overall, our work demonstrates that governing Polo kinase activity is necessary to suppress meiotic drive of the B chromosomes, revealing a novel mechanism the genome employs to protect itself from accumulating supernumerary genetic elements.

Poster #4 **Suparna Dutta**

Hanlon Lab Graduate Student

B chromosomes disrupt proper chromosome segregation during female meiosis

The recently discovered B chromosomes in Drosophila melanogaster are nonessential, do not carry any known genic regions, and appear to be composed entirely of heterochromatin. Based on shared sequence elements, these B chromosomes are likely derived from chromosome 4. Interestingly, the B chromosomes can induce high levels of chromosome 4 missegregation during female meiosis, prompting us to investigate the interaction between chromosome 4 and the B chromosomes. We first examined B chromosome transmission during female meiosis when zero, one, or two copies of chromosome 4 are passed on to progeny. We observed an inverse relationship between B chromosome and chromosome 4 segregation: when the female passes on two copies of chromosome 4, fewer B chromosomes are transmitted, and when zero copies of chromosome 4 are passed on, more B chromosomes are transmitted. To further investigate the effect of B chromosomes on chromosome 4 segregation during female meiosis, we measured chromosome 4 nondisjunction as a function of B chromosome copy number. Our preliminary data with a small B chromosome copy number range indicate a positive correlation between B chromosome copy number and chromosome 4 nondisjunction. We are currently expanding this B chromosome copy number range to determine if chromosome 4 nondisjunction continues to rise or if it plateaus at high B chromosome copy number. Additionally, we plan to explore the interaction between B chromosomes and chromosome 4 cytologically by examining heterochromatic threads. These threads form between homologous chromosomes during meiosis, and if they are improperly forming between the B chromosomes and chromosome 4, it may be affecting the proper distribution of chromosome 4 on the metaphase I spindle. Overall, our research aims to enhance our understanding of how supernumerary, nonessential chromosomes influence the segregation of essential chromosomes during female meiosis.

Poster #5 **Edward Russell**

Hanlon Lab Graduate Student

Accelerating chromosome evolution using the Drosophila melanogaster B chromosome

The genome is in constant flux as its composition and chromosome complement undergo frequent changes. An example of this are B chromosomes, which are nonessential accessory chromosomes that are often maintained in a population through mechanisms that "drive" their biased inheritance. The current model for B chromosome evolution begins with an essential chromosome-derived fragment that cannot bias its inheritance. As this "proto-B" matures, it's thought to accumulate unique sequences and develop a successful drive mechanism. Though several established B chromosome systems support this model, the details of sequence translocation remain unclear. To investigate the evolution of B chromosomes, we are using the B chromosomes recently discovered in a laboratory stock of Drosophila melanogaster. Unlike traditional rye and maize B chromosome models, which are millions of years old, the D. melanogaster B chromosome is a proto-B that is a mere 20 years old. It was derived from an essential chromosome, carries no known genes, lacks unique sequences, and is maintained through a host-derived drive mechanism. To accelerate the maturation of this B chromosome, we are inducing the mobilization of a modified P-element to determine if transposition and expression on the B chromosome is possible. Our goal is to show that sequences from essential chromosomes can transpose into and be expressed by a nonessential B chromosome. The presence of the P-element would provide a landing site for other sequence elements. By placing transgenic constructs onto the B chromosome that carry unique sequence elements or promote B chromosome drive, we aim to further test the model of B chromosome evolution. This research will begin to bridge the gap in our understanding of how newly formed B chromosomes can develop their own drive systems and acquire unique sequences as they mature, as well as interrogate the mechanisms that govern novel chromosome genesis and evolution.

Poster #6 **Stella DiPippo**

Inaba Lab Other

The role of Me31b in germ cell maintenance and dedifferentiation in the Drosophila testis stem cell system

The *Drosophila* testis stem cell system consists of 8-10 germline stem cells (GSCs) attached to a somatic cell hub. Asymmetric division of GSCs results in one differentiating daughter cell and a self-renewing stem cell. Approximately 1 GSC is lost from the niche per day, so the asymmetric division alone cannot maintain stable GSC number. Dedifferentiation, a differentiating daughter cell reverts to a stem cell state to replenish the lost stem cells, has been demonstrated to occur in Drosophila testicular niche, and suggested to be essential mechanism for stem cell maintenance. However, there is a challenge in studying dedifferentiation, as accurately identifying dedifferentiated cells is difficult. Consequently, the mechanism of dedifferentiation is poorly understood.

Previous studies have used abnormal localization of spermatogonia (or SGs) at the hub with fragmented fusomes as the marker of dedifferentiated stem cells. Fusome is the germline-specific organelle that connects spermatogonia (or SGs) in a syncytium through the 4- to 16-cell stages. A previous study observed increased SG cysts at the hub with fragmented fusomes in Maternal expression at 31b (Me31b) knockdown testes, suggesting increased dedifferentiation. Using Me31b as a model gene, we tested several other methodologies to estimate dedifferentiation frequency. Our results indicate that fusome fragmentation is not a reliable indicator of dedifferentiated cells.

Poster #7 **Stella Cho**

Sun Lab Graduate Student

Gγ1, RhoGEF2 and Cysts in the follicular epithelium regulates Rho1-mediated contraction for follicle rupture during Drosophila ovulation

Drosophila ovulation releases a mature oocyte housed within a sac of epithelial follicle cells (known as a mature follicle) through a process called follicle rupture as in mammals. Our previous work demonstrated that octopamine (OA) and octopamine receptor in the mushroom body (Oamb) activate Rho1 GTPase-mediated actomyosin contraction in the follicle cell cortex to generate the mechanical force to facilitate the follicle rupture. However, the detailed signaling pathway downstream of Oamb, a G-protein-coupled receptor (GPCR), has not been explored. Here, we discovered that Gγ1, one component of the heterotrimeric G protein, is essential for the cortical enrichment of both Rho1 and phosphorylated non-muscle myosin II (NMM II; active NMMII) in response to OA signaling. In addition, Gγ1 knockdown caused defects in OA-induced follicle rupture *ex vivo* and ovulation *in vivo,* suggesting that non canonical Gbg may play a critical role downstream of Oamb to induce Rho1 activation and follicle rupture. Furthermore, we found that two guanine nucleotide exchange factors (GEFs), cyst and RhoGEF2, may play redundant roles in activating Rho1 in follicle cells after OA stimulation. Simultaneous depletion of both cyst and RhoGEF2 was necessary to disrupt the cortex enrichment of Rho1 and NMM II, while knockdown of either one alone showed minimal defect. Interestingly, double knockdown of both cyst and RhoGEF2 resulted defects only in OA-induced follicle rupture *ex vivo* but did not significantly impact egg-laying behavior *in vivo*. This implies that other compensatory mechanism may exist *in vivo* to ensure the robustness of egg laying. All these results led us to propose that OA/Oamb signaling activates Gbg, which may further activate cyst and RhoGEF2 to induce Rho1 activation and actomyosin contraction. More work in on the way to thoroughly characterize this pathway in mature follicle cells. Given the conserved nature of GPCRs and Rho1, this work could illuminate the G protein signaling cascade involved in mechanical force generation for follicle rupture in other species or for other cellular processes.

Poster #8 **McKenna Rook**

Menuz Lab Undergraduate Student

The Drosophila Odorant Binding Protein 47a (Obp47a) is Enriched in Male Forelegs and Contributes to Male Mating Behavior

Sexual dimorphism is a common occurrence in many organisms, and refers to a difference in behavior, appearance, or gene expression between males and females of the same species. In Drosophila Melanogaster, mating behaviors follow stereotyped, sex-specific patterns which rely on many sensory cues. Forelegs in particular play an important part in the male mating process. Forelegs are used for the courtship behavior known as “tapping” in order to determine if a female is a viable mate. Menuz Lab’s previous RNA sequencing on the legs of both males and females revealed that the gene Odorant Binding Protein 47a (Obp47a) is highly expressed in male forelegs as compared to female legs or other male leg types. Odorant binding proteins are a class of secreted globular proteins found within insect sensilla, whose functions have been mostly undetermined. I tested Obp47a mutant males mating with wild-type females to determine if it had an effect on male mating, and found that Obp47a had a strong effect on male copulation latency. I further tested Obp47a mutant females mating with wild-type males and determined that Obp47a had a mild effect on female copulation latency. Finally, I imaged a reporter line driving membrane GFP in cells expressing Obp47a, and found that Obp47a is expressed in cells at the base of the sex comb in male forelegs. My data suggests that Obp47a may affect the function of the sex comb during fly mating.

Poster #9 **Alexandria Pacrin**

Menuz Lab Graduate Student

Role of jhamt in chemosensory regulation of mating in Drosophila

Juvenile hormone (JH) is a sesquiterpenoid hormone known for its roles in controlling the development of insect larvae and in regulating reproduction in adult insects. More recently, global JH levels have been found to modulate olfactory receptor neuron (ORN) responses to pheromones in adult male Drosophila, resulting in higher success rates of older male flies when compared with younger flies in a mating assay (Lin et al., 2016). JH is known to be synthesized and secreted by the corpora allata (CA), an endocrine gland, but scRNASeq data from the FlyCellAtlas suggest that JH may be synthesized in the antenna due to its expression of jhamt, a key enzyme in JH synthesis. Here we demonstrate that JH is expressed in non-neuronal cells of the antenna, including those that enwrap pheromone-sensing ORNs to regulate mating behavior. Surprisingly, knockdown of jhamt expression specifically in these antennal cells leads to a decreased mating latency of male flies. We present a model that may explain this unexpected phenotype.

Poster #10 **Robin Smith**

Hanlon Lab Other

Mapping matrimony Deficiency Breakpoints in Drosophila melanogaster via Illumina Sequencing

Matrimony (mtrm) plays a pivotal role in female meiosis by ensuring the proper segregation of chromosomes that do not form crossovers. To further study its function, five mutant stocks were created over 20 years ago that each have a large deletion, known as a deficiency, that was believed to encompass mtrm. The approximate breakpoints of each deficiency were assigned to a specific chromosomal location based on the altered cytological banding pattern found in polytene chromosomes, but the precise genomic location of these breakpoints is unknown. Here we used Illumina paired-end sequencing to define the breakpoints of each deficiency with base-pair resolution. Using a suite of bioinformatic tools, we processed and aligned reads to the Drosophila melanogaster reference genome (dm6). Once aligned, we manually identified a deficiency's genomic breakpoints by looking for a sudden ~50% drop in sequencing depth. At the sites of the suspected breakpoint, we also observed a high frequency of split and discordant reads. These reads partially map to two locations on a chromosome, indicating that the split/discordant read spans the deficiency junction. To our surprise, all five stocks had genomically defined deficiency breakpoints that did not align to the previously reported cytological banding location. Despite this, we found that the deficiencies in three of the five stocks included part or all of mtrm and are likely null alleles. In the other two stocks, the deficiencies do not encompass mtrm, which is unexpected because these two stocks have been shown to disrupt noncrossover chromosome segregation during female meiosis. Altogether, our analysis confirms the disruption of mtrm in three separate deficiency stocks and defines the genomic location of their breakpoints, which expands our toolkit of mtrm null alleles to use in future experiments.

Poster #11 **Maddy O'Connor**

Mellone Lab Graduate Student

Determining the localization of centromere-derived transcripts in Drosophila

The accurate segregation of chromosomes relies on the proper functioning of the centromere, the region of the chromosome where the kinetochore and microtubules attach during cell division. The centromere consists of a specialized chromatin domain containing H3 variant CENP-A, which recruits other essential centromeric proteins. If the centromere complex is not assembled properly, it can cause detrimental chromosome segregation errors, leading to cell death or aneuploidy. The mechanism behind how the essential centromeric proteins (CENP-A, CENP-C, and CAL1) are recruited to the centromere has yet to be understood. Studies in human cells suggest that centromere-derived transcripts play a role in the recruitment to the CENP-A domain, but this has yet to be observed in Drosophila melanogaster. Using Drosophila to study transcription at the centromere will help to shed light on the importance of RNA in the recruitment of centromere proteins using systems such as CRISPR-Csm to deplete transcripts in vivo. Work in the Mellone lab revealed the composition of the centromeres of D. melanogaster and discovered that a retroelement, Jockey-3, is transcribed, and the nascent RNAs remain associated with the centromeres they originate from. However, the significance of this localization and its role remains unknown. To determine if other centromere-derived transcripts localize to the centromeres they originate from, I performed RNA FISH (Fluorescent In-Situ Hybridization) using probes designed for simple satellite repeats flanking the centromere. This experiment will show if more than one element is transcribed and retained at the centromere, which could imply a functional relationship. Once the localization of these unique transcripts are identified, I can then determine if they are retained at the centromere due to interactions with different centromeric proteins using CLIP (Cross-linking Immunoprecipitation). This assay uses UV irradiation to irreversibly crosslink RNA to protein, followed by immunoprecipitation and RNA-seq to determine whether these transcripts interact with the centromeric proteins. Following this experiment, using CRISPR-Csm will allow me to investigate the functional role of the transcripts by degrading them and looking at the effects on the centromere complex. The findings from this work will enhance our understanding of the role transcription plays in centromere function and its impact on chromosome segregation across various species.

Poster #12 **Annette R St. Jacques**

Hanlon Lab Undergraduate Student

Investigating How Oocyte Age Impacts B Chromosome Transmission in Drosophila Melanogaster

B chromosomes are nonessential chromosomes that are carried in addition to the essential chromosome set. Recently, B chromosomes arose in a single laboratory stock of the fruit fly, Drosophila melanogaster. These B chromosomes are inherited by the next generation at a frequency that is higher than what is expected, indicating that the B chromosomes are capable of selfish behavior during chromosome segregation in the egg. This biased inheritance of B chromosomes led us to wonder if their selfish behavior may be more severe in other contexts that are known to disrupt chromosome segregation. In D. melanogaster females, older eggs are prone to chromosome segregation defects, therefore we hypothesize that older eggs will promote a more severe bias in B chromosome transmission. To test this, we will compare the B chromosome transmission frequency in females that have aged (old) eggs to females that have fresh (young) eggs. If the age of the egg affects B chromosome transmission bias, then we expect to see an increase in their transmission frequency that correlates with the egg's age. As we begin to understand how age can affect the transmission of B chromosomes, we anticipate using the B chromosomes as a powerful system for evaluating other environmental factors that promote aberrant chromosome segregation.