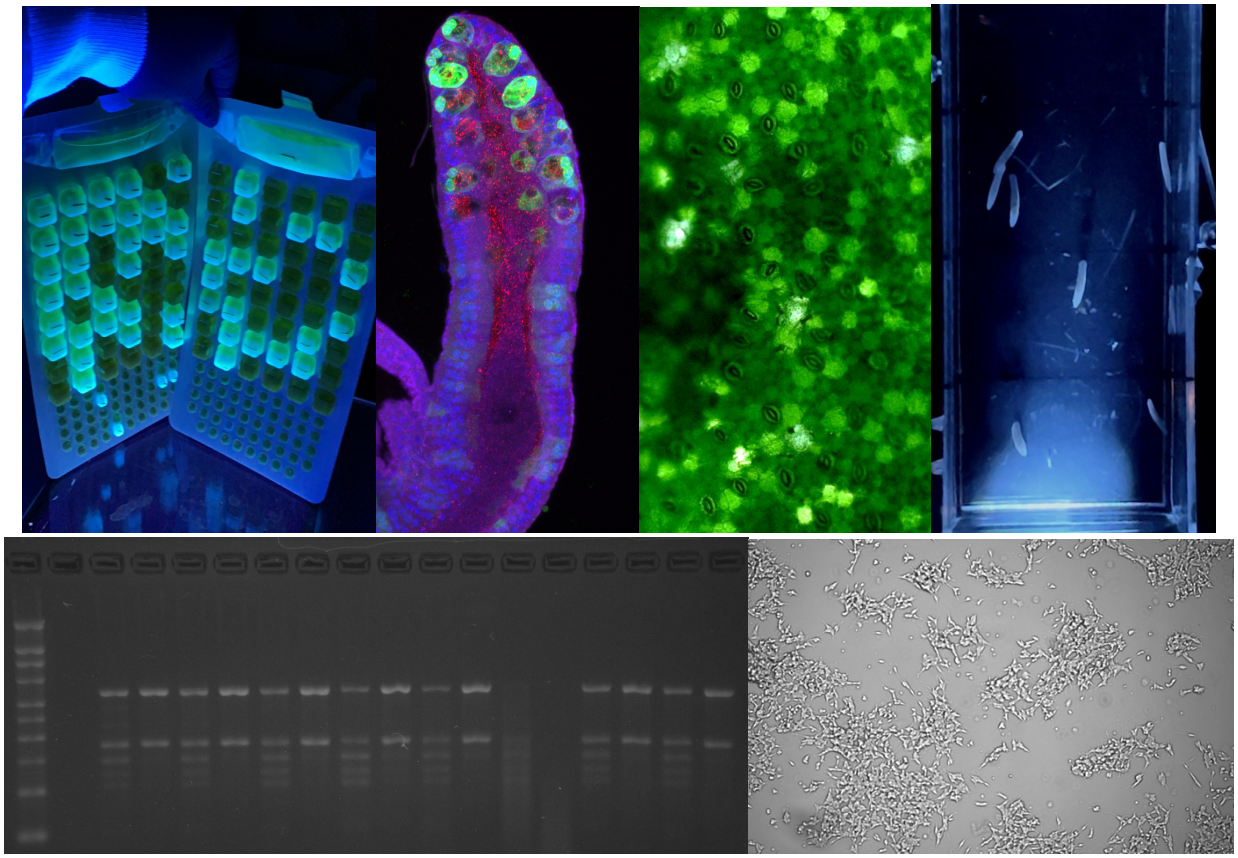
Book of Abstracts

9th Annual CMB Symposium

Winter 2023



Grand Valley State University
Cell and Molecular Biology Department

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Studying GmSLY1a and GmSLY1b Gene Expression in Soybean Nodules

Lyla Dao
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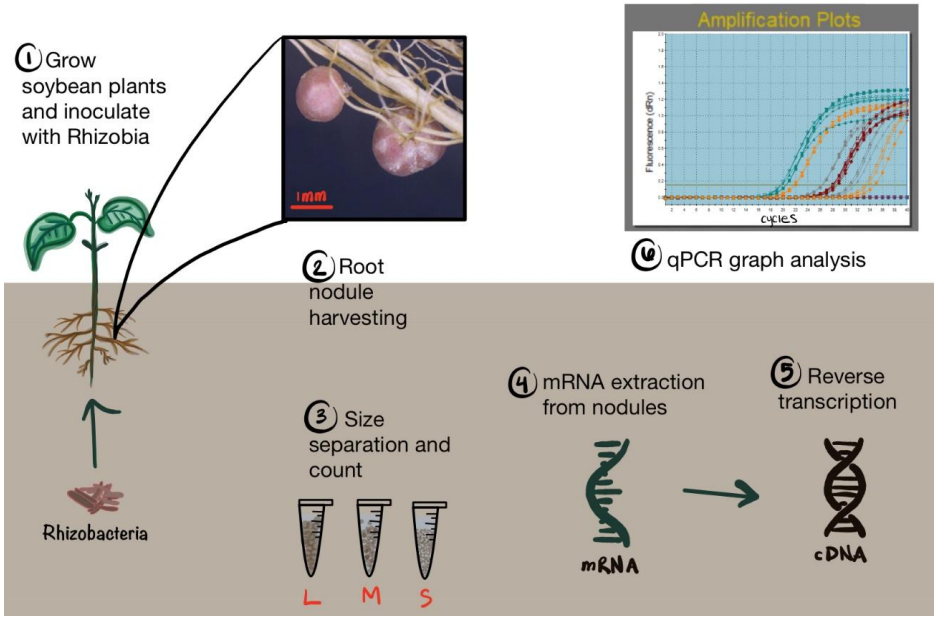

Food security is becoming a crisis due to extreme climate change and less cultivable land worldwide. Learning about hormone signaling pathways involved in plant development and growth can potentially help improve crop yield in the future. Soybeans are one of the most important crops worldwide. Like all legumes, it is a nitrogen fixating plant—meaning it can convert atmospheric nitrogen into ammonia within the nodules of the roots mediated by N-fixing Rhizobia bacteria. Gibberellic acids (GA's) are important plant hormones that regulate growth, cell division, seed germination, and even nodule formation in the root. In *Arabidopsis*, the SLEEPY1 (SLY1) gene functions as a positive regulator of GA signaling. There have been two orthologs identified in soybean: GmSLY1a and GmSLY1b. Here, we investigate the gene expression of the GmSLY1a and GmSLY1b genes in soybean nodules. Soybean plants were grown and inoculated with Rhizobia, the specific nodular root tissue was harvested, mRNA was isolated from the various tissue samples and converted to cDNA, and then gene expression was measured through qPCR. The results showed that GmSLY1a and GmSLY1b were both expressed in nodules. In addition, we found that GmSLY1b expression was higher in the medium nodules than that of GmSLY1a. These results shed light on the gibberellic acid signaling in plant physiology.

Funded by: Michigan Invasive Species Grant

Molecular Determination of Branch Angle: Examining the *LAZY1* Gene in Native Tree Species

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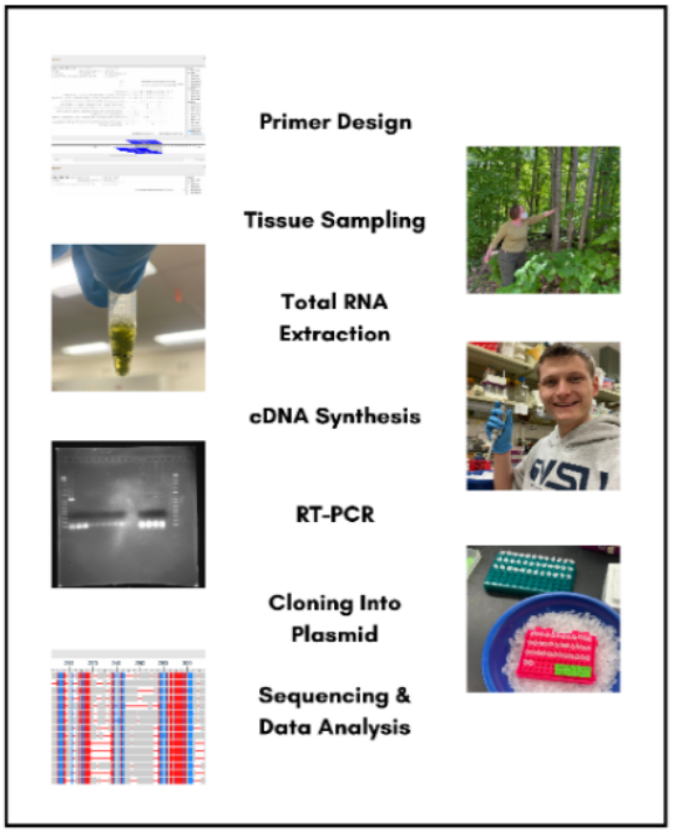

The architecture of tree branches, that is their size and angles, in part determines the potential for a tree to efficiently transport water and support the mass of its branches. Tree architecture is relevant in answering ecological questions about species distribution and phenotypic plasticity. It is also an area of interest for developing more efficient agriculture and forestry operations. The *LAZY1* gene plays a role in determining branch angle, however it has mostly been studied in agricultural or ornamental trees. It is involved in a gravitropic response that promotes narrow branch angles. Here, we obtained *LAZY* transcript sequence data from several native Michigan tree species using RT-PCR and degenerate primers. Sequence data for native species furthers our understanding of the *LAZY* family of genes and branch architecture more broadly. This work builds on previous research I have done to develop an easy method of quantifying branch architecture using photographs. Differences between transcript sequences of *LAZY1* among various tree species will be correlated with the difference in branching architecture determined in our previous study. This provides insight into natural forest systems and could be useful in the development of better orchard and timber tree varieties.

Monitoring of Hemlock Wooly Adelgid (HWA) in High-Risk Forest Areas Using 3D-printed eDNA Traps

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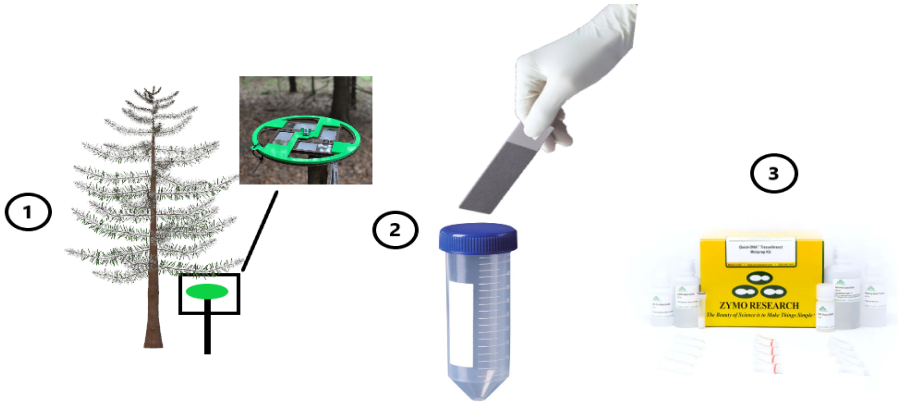

[Biweekly process]
Placement of four petroleum jelly coated slides in eDNA trap [week 1] ***(1)****, sample slide collection/replacement [week 2]* ***(2)****, DNA extraction from collected slides using the Zymo insect kit* ***(3)***

Hemlock wooly adelgids (Adelges tsugae, HWA) were first detected in Michigan in 2016. Less than 1mm in length, nymphs are easily translocated by birds, small mammals, and humans and have spread up the western coast of Michigan. A member of the aphid family, HWA colonize branches of the Eastern hemlock, insert their mouthpiece, a stylet, through the base of the needle and feed directly from the tree's storage cells. Eastern hemlocks affected by HWA will experience needle die-off and the resulting nutrient deficiency makes the tree susceptible to other pests and illnesses. To monitor spread, various types of insect traps were tested in 2021 for efficacy of capture. Building from that research, a novel 3D-printed trap was created in 2022, for environmental DNA (eDNA) collection and deployed at 100 sites in Michigan. These included control areas with known infestations and high-risk areas, where HWA had not yet been detected. DNA was extracted from collected samples and quantitative polymerase chain reaction (qPCR) was used to determine if HWA were present, using HWA-specific primers. A previously undetected zone adjacent to a residential area tested positive for HWA DNA at 2/10 sites. The location of the affected site was noted, and appropriate management agencies were informed. Monitoring creates a partnership with management agencies to help reduce the spread and treat affected hemlocks. Early detection and subsequent insecticide treatment are key to the tree's survival, as an affected tree can die in four to ten years without it.

Funded by: Michigan Invasive Species Grant

Can Increasing the Density of External Media Mimic Microgravity in Plant Roots?

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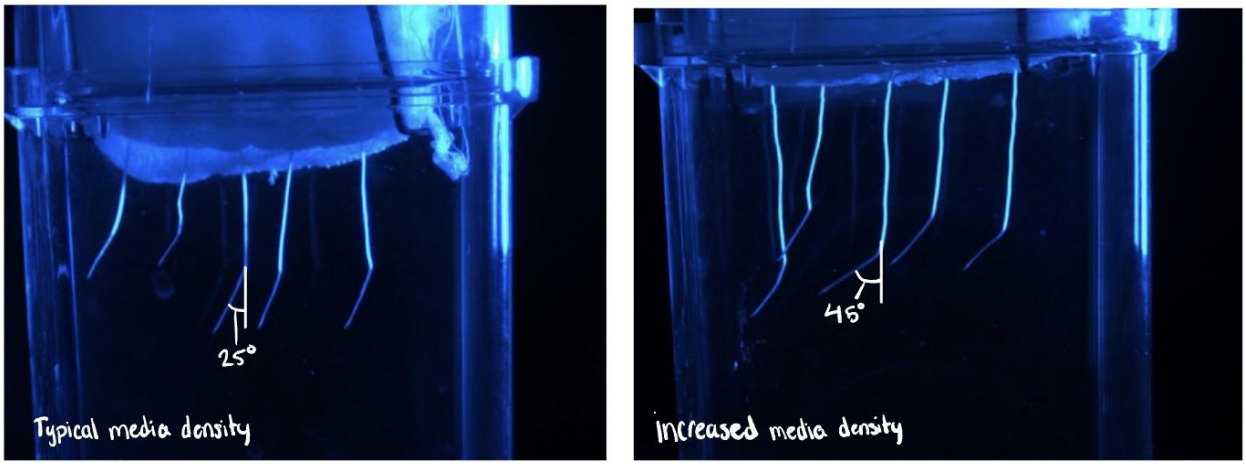

There are two leading models of plant gravity sensing: the statolith model, where statoliths sense gravity through sedimentation, and the gravitational pressure model, where the protoplast acts as the gravisensor. The statolith model is widely accepted, but plants without statoliths and plants with statoliths removed retain their ability to sense gravity. In this project, we tested these models using *Oryza sativa* (rice) as it contains statoliths and grows well in liquid media. We grew the plants with the roots exposed to unilateral light in artificial pond water (APW) and OptiPrep solutions. The APW experiments gave a baseline for how roots respond to the light conditions. OptiPrep increased the density of the media and the root curvature increased, suggesting the gravity response was reduced. We compared sections of rice roots to check statolith distribution, which was not affected by our experiments. These results lead us to conclude that increasing the density of external media can mimic microgravity in plants. Taken together, our data are consistent with the gravitational pressure model and inconsistent with the statolith model.

This research was supported by the Modified Student Summer Scholars program, the RISE program, ACF, the McNair Scholars program, and the Michigan Space Grant Consortium.

The Impact of Abscisic Acid on Ubiquitin Mediated Protein Degradation in *A. thaliana*

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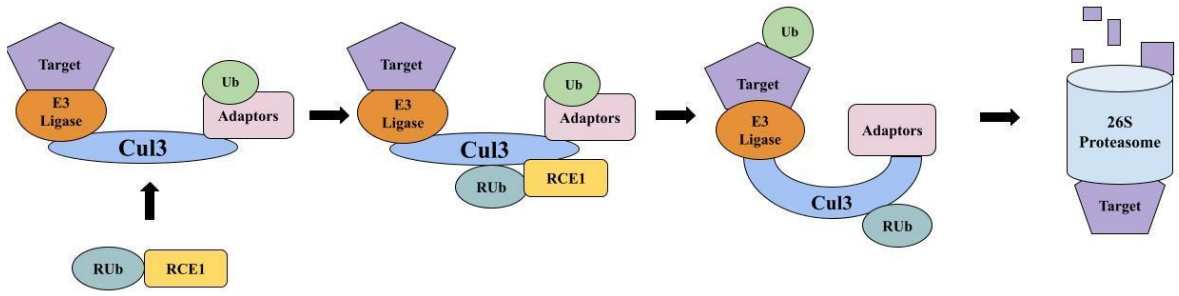

Protein degradation is a highly regulated process in eukaryotic organisms and plays a major role in general cell function. One of the key events in selective protein degradation is the addition of ubiquitin (Ub) to the target protein which signals the cell to degrade that protein using the proteasome. This allows the cell to recycle components of proteins that are no longer functioning or necessary for cellular function. E3 ligase protein complexes select specific target proteins for degradation and add Ub to them, but for the E3 ligase complex to become active, Rub (related to ubiquitin) must be covalently ligated to the Cullin protein of the E3 complex. This project investigates the impact that exogenous Abscisic Acid (ABA) has on the ratio of Rubbylated (active) to unRubbylated (inactive) Cullin-3 proteins present in *Arabidopsis thaliana*. Using immunoblotting, this project quantifies the amount of selective protein degradation through Cullin3 ligases, specifically investigating the ratio of Rubbylated to unRubbylated Cullin3 proteins. This investigation revealed a statistically significant increase in the ratio of Rubbylation upon increasing concentrations of ABA in the plant growth media. Phenotypic changes in plant growth consistent with ABA exposure were observed and documented through imaging. To further elucidate the molecular mechanisms involved in the ratio of Rubbylation trend, RT-qPCR will be performed to investigate the expression of proteins associated with the Rubbylation cascade. Investigating selective protein degradation pathways allows for further understanding of how a plant's developmental cues can impact its rates of protein degradation.

Funding provided by the Department of Cell and Molecular Biology and the Modified Student Summer Scholars Grant.

Comparative Assessment of Low Input ChIP-seq Library Kits

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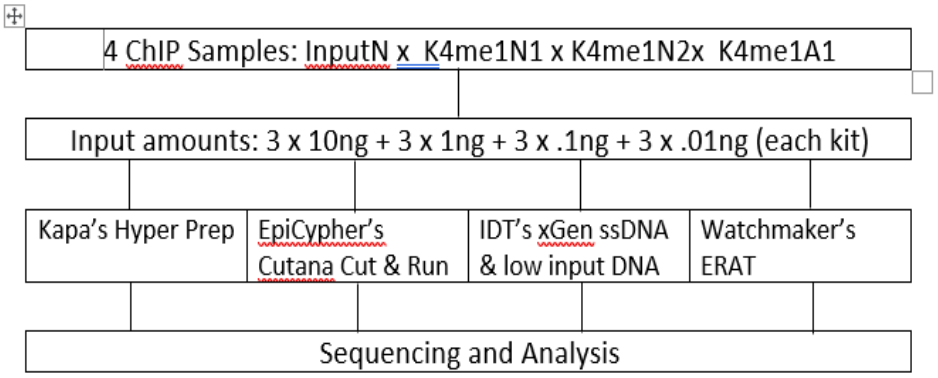

Chromatin Immunoprecipitation (ChIP) is a powerful technique used to investigate genome wide protein-DNA interactions. It is often used to identify the DNA sequences targeted by transcription factors (TF) or associated with various histone modifications. Due to the limited regions of DNA that many TFs interact with, it is not uncommon for a miniscule amount of material to be collected. Sequencing library preps starting with limited material can yield low quality sequencing results. Low input ChIP-seq libraries are often beset by low complexity, a high degree of PCR duplicates and unmappable reads. When preparing quality low input ChIP libraires, optimization of adapter ligation and avoidance of PCR amplification bias must be accounted for. This makes the choice of library preparation kit an important factor to consider. The main question is: given the varying underlying methodologies of different library prep kits, which kits will provide the highest quality sequencing data from low input ChIP samples in terms of complexity, duplication rate and mappable reads? In this project we assessed the results returned from low input ChIP libraries by surveying four different library preparation kits including KAPA's Hyper Prep, Epicypher's CUTANA, IDT's ssDNA and Low Input DNA, and Watchmaker's ER/AT, and comparing the quality of the sequencing data from each. Varying amounts of input DNA ranging from 10ng down to .01ng were utilized and three technical replicants were implemented for each sample of varying input to make statistical analysis possible. The criteria used to assess and compare these kits included duplication rate, alignment rate, map to read percentage, uniquely mapped read percentage, peak comparison and changes in library complexity given varying input amounts.

Investigating The Role of Septin Proteins as GTPases and Their Locomotive and Neurological Influence in *Schmidtea Mediterranea*

Dylan Parker
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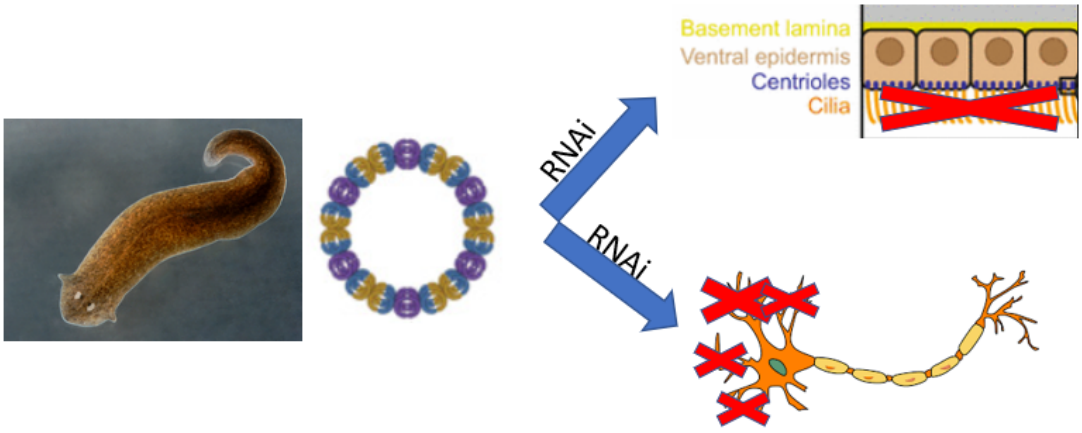

The underlying mechanisms behind neurodegenerative disorders such as Parkinson's Disease (PD) and Alzheimer's Disease (AD) are still being discovered and investigated. Uncovering key players in these pathways can allow for more specific therapies to treat this class of disorders. One postulated key part of these mechanisms is septin proteins, a highly conserved class of GTP-binding proteins. These proteins are highly expressed in the brain and involved in many regulatory neurological functions such as dendrite formation and branching. In this study, the effect of the absence of septin-1198, septin-1509, and septin-1717 are observed. The respective genes were knocked down in planaria, a type of flatworm that contain these septin genes and utilize them for cilia formation, a key component for their locomotion, as well as dendrite formation and branching within their central nervous system (CNS). The genes were knocked down in the worm using RNAi and their locomotive and neurological behavior were measured through maze and locomotive assays.

Results showed that knockdown in these genes resulted in a significant inability to complete the prescribed maze. Experimental groups ranged between 0-50% completion rate compared to a negative control of 70%. Additionally, knockdown of genes resulted in a significant decrease in velocity, ranging from 4.80 lines/min to 6.41 lines/min in the experimental genes and 8.27 lines/min in the control. The absence of these septin genes also resulted in a significant increase in abnormal neurological behaviors such as sudden sharp turns compared to the control.

Overall, these results help define the role of septins regarding motile and neurological behaviors in planaria. These results provide evidence of septin's involvement in mechanisms within the central nervous system and its contribution to neurodegenerative disorders, showing potential as a therapeutic target.

Investigating the Role of PP2A-b55a, PP2twin, Cullin3, Spartan-like and NIMA-kinase on *Schmidtea mediterranea* Behavior

Izzat Teklu
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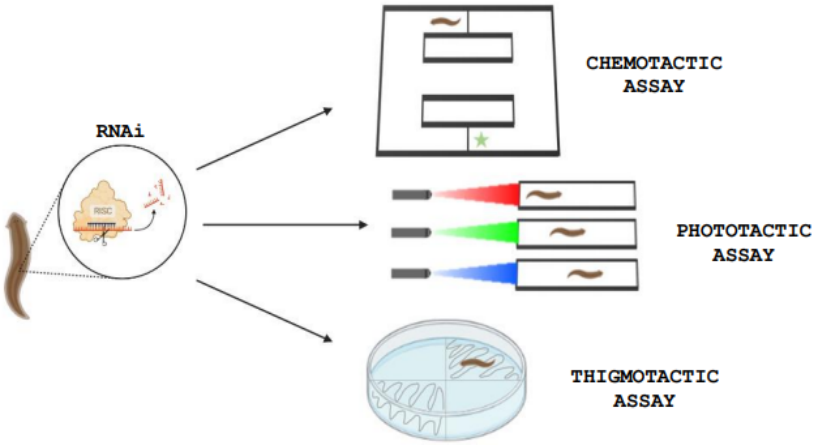

Schmidtea mediterranea, a species of planarian freshwater flatworm, has been a well-established model organism for studying regeneration. They can regenerate all body parts, and most notably the central nervous system (CNS). Their genetic tractability and simple behavior make them an attractive system to investigate the role of specific genes on the CNS. Currently, there is no comprehensive understanding of the several genes involved in the planarian CNS and their role in behavioral responses. Preliminary data and background research have shown five genes of interest, some which have never been studied in planaria, that may have a significant impact on planarian behavior. Here we investigate the role of PP2A-b55a, PP2twin, cullin3, spartan-like and NIMA-kinase on planaria behavior post knockdown. In order to study the impact these genes have on planaria behavior, we performed RNA interference (RNAi) to knockdown the five genes and performed three behavioral assays; chemotactic, phototactic and thigmotactic. We found that knockdown of cullin3, spartan-like and NIMA-kinase negatively impacted the chemosensory behavior in planaria. The thigmotactic assay showed spartan-like knockdown planaria are more attentive of a change in surface area and prefer smoother textures, however, further studies must be done in order to determine whether this change is due to a sensory loss or general abnormal movement. Phototactic assay results did not show any significant behavioral changes in response to blue light across the genes tested. In response to red light, knockdown of PP2twin, spartan-like and NIMA-kinase showed a significant increase in time taken to avoid red light. In response to green light, knockdown of NIMA-kinase showed an increase in time taken to avoid green light. We also observed a trend where the average median time taken to avoid light increased as wavelength of the light decreased. Collectively, these results help us further understand the function in which these genes are involved in planaria. Behavioral responses are essential aspects to consider in order to understand the mechanisms involved in the planarian central nervous system.

Computational Exploration of Aziridination of Styrene by a Manganese Nitrene Featuring a Tethered Bis-Alkoxide Ligand

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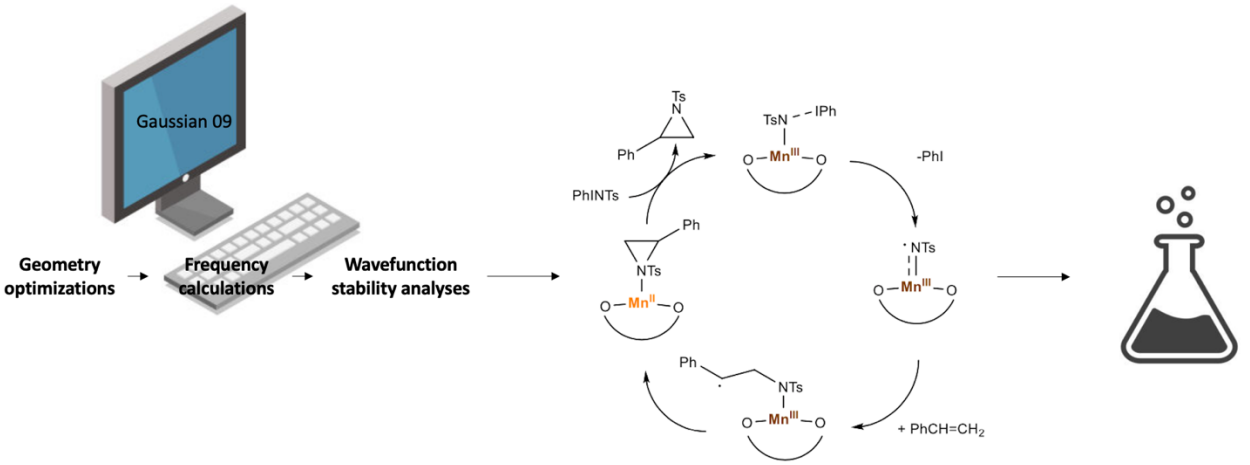

Computational exploration using DFT

Proposed aziridination mechanism
(Kurup et al., 2022)

Test reactivity

Metal-nitrene species have rich and variable stereoelectronic attributes, allowing for unique reactivity, and are a matter of intense investigation. Aziridines serve as valuable building blocks for many chemicals and play a central role in fine chemicals and pharmaceutical agents. The field of Mn-based aziridination catalysis has not yet been highly explored, but the mechanism of aziridination of styrene by a manganese nitrene has been investigated previously. However, metal nitrene formation within that mechanism has not been thoroughly explained. Here, we explore Mn-based aziridination and metal nitrene formation within that reaction when the metal is in a bulky bis-alkoxide ligand environment. To probe the nature of the reaction mechanism and its intermediates, density functional theory (DFT) calculations were used to optimize molecular geometries, calculate vibrational frequencies, and analyze wavefunction stability. DFT calculations suggest efficient styrene aziridination through a nitrene radical reaction intermediate. This advances the field of Mn-based aziridination catalysis by demonstrating the full reaction mechanism of styrene aziridination in a bulky ligand environment.

Research supported by

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Monitoring Millennium Park Beach for *E. coli* and Coliform Using Colilert-2000

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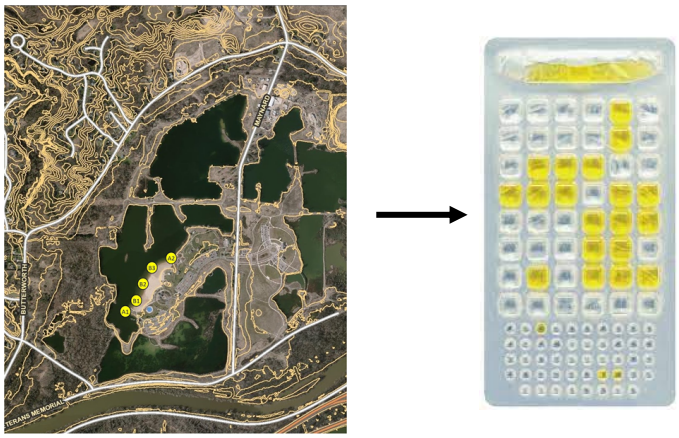

Sample collection **Colilert-2000 analysis**

Millennium Park's public beach is visited by over 90,000 people every summer. Due to warm water temperatures, a low flow rate, and high use of the beach area by children, the lake is at risk for outbreaks of recreational water illnesses of enteric origin. To monitor the cleanliness of the beach water, we worked with the Kent County Health Department and collected samples from three different beach sites and two other sites immediately adjacent, from the end of May to the end of August in the summers of 2021 and 2022. The same methods of collection and analysis were conducted for both years. The samples were analyzed by Colilert-2000, which tests for *E. coli* and coliforms through fluorescent indicators that accumulate over 18 hours. The resulting data from 2021 indicated that the mean of the *E. coli* levels per 100 mL of beach water measured by Colilert was 13.02 (range = 1 to 30.9) and never exceeded Michigan's threshold level (< 130 *E. coli* / 100mL) for restricted body contact. Rainfall data collected from 2021 showed that rainfall in preceding days significantly ($P<0.001$) increased *E. coli* counts in the non-beach areas from a mean of 6.7 (n=14) to 24 (n=4). However, in the beach areas, the effect of rainfall (MPN = 13.3 and 20.0) was not significant ($P=0.07$), suggesting that there is more run-off in the non-beach areas. Data analysis for 2022 samples is under way. Our data showed that Millennium Park Lake, despite its high visitorship and low water circulation, has low levels of *E. coli* in its beach water.

This research was supported by the Michigan department of Environment, Great Lakes, and Energy (EGLE).

Wastewater-Based Epidemiology of Human Pathogenic Viruses

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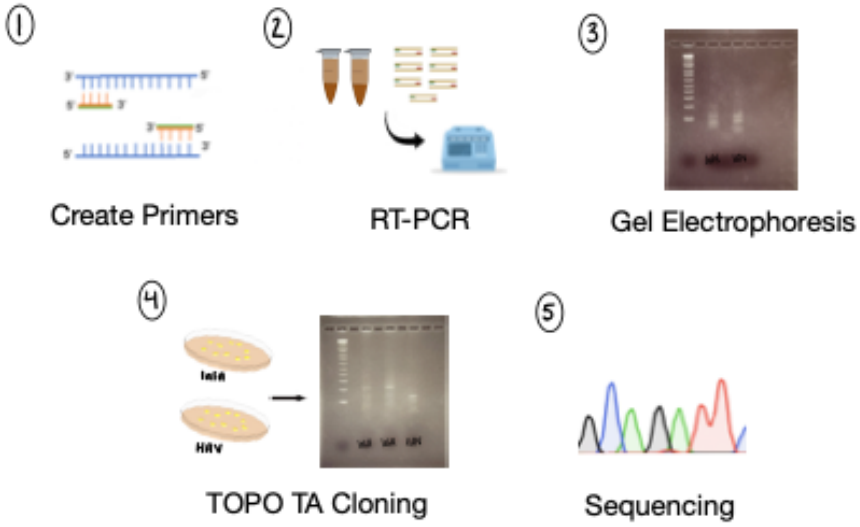

Millions of viral particles are shed from individuals with infectious diseases and introduced into our wastewater. Present in wastewater is a mix of physical, chemical, and biological substances that are discharged to a sewer system, which provides us with a pooled sample from a specific geographical area. Wastewater-Based Epidemiology (WBE) can therefore provide information about a particular population and their exposure to potentially harmful chemical or biological agents, especially viral pathogens. With the use of WBE, the number of harmful properties can be identified both qualitatively and quantitatively from a given population and assessed further. This can be an invaluable tool for virus surveillance when population-wide testing for a virus is not feasible. In this study, wastewater samples obtained from the Kent County health department and primers specific to 7 different human pathogenic RNA viruses were tested through RT-PCR and agarose gel electrophoresis and sequenced using TOPO® TA Cloning. Our preliminary data show that 2 of the 7 viruses being tested, Influenza A (InfA) and Hepatitis A (HAV), are possible to detect. These results revealed the presence of highly contagious viruses in Kent County wastewaters and can allow for public health authorities to alert the public and take precautionary measures.

Polymerase Chain Reaction Species Identification of *Aedes Vexans* and Primer Design using COX1

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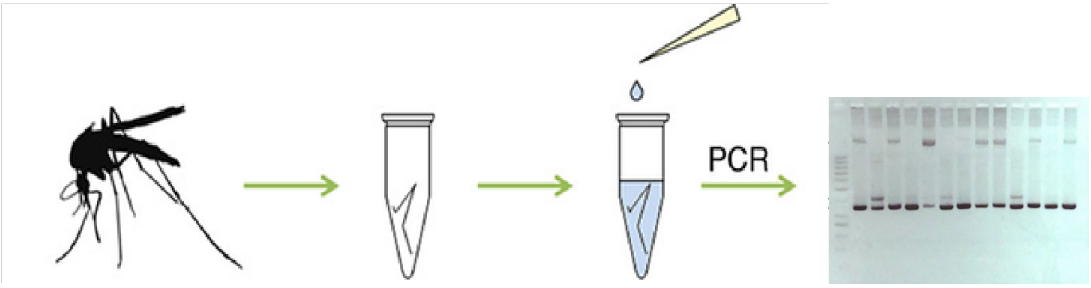

Aedes vexans **purify DNA** **amplify DNA** **gel electrophoresis**

Invasive mosquito species pose a threat to public health, as only some species of mosquitoes carry certain diseases. This makes it critical that the state of Michigan be able to keep track of disease-spreading mosquito populations. The current protocol for mosquito identification from the Kent County Health Department (KCHD) does not include sequencing of any kind. Morphological identification is the method that is used exclusively and has a high frequency of error. The aim of this project is to design species specific primers that allow for a PCR reaction that amplifies the COX1 gene template, to identify *Aedes vexans. Aedes vexans* are responsible for the spread of West Nile Virus as well as canine heartworm, and are currently identified exclusively on a morphological basis. Pre-identified mosquitoes were donated by KCHD and the morphological identifications verified. DNA was extracted and the COX1 gene was cloned and sequenced to verify species by comparison with sequences available in NCBI. A species specific primer was designed and showed specificity when tested against other mosquitoes endemic to West Michigan. This development in molecular identification of *Aedes vexans*, will allow government agencies including local health departments to identify the invasive species and mitigate the spread of the west nile virus and canine heartworm. Additionally, the success of this primer design provides a basis for the development of other assays to identify other *Aedes* mosquitos through PCR and provides insight on sequence variation between species under the genus *Aedes*.

Functional Consequences of Variations in CHEK2

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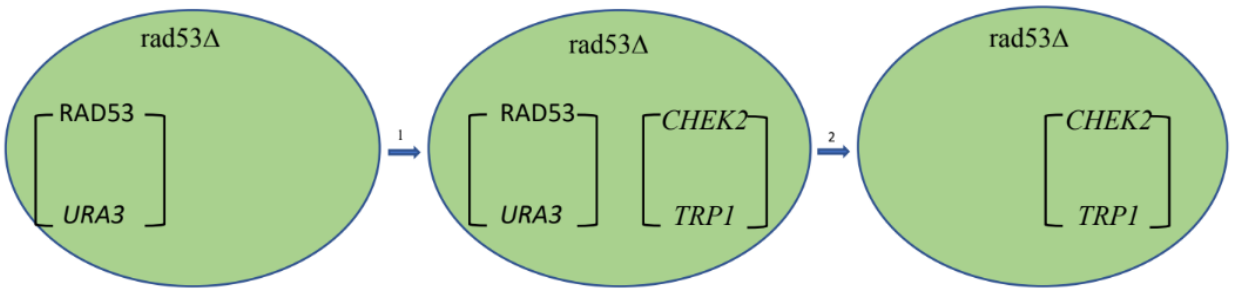

According to the World Health Organization, breast cancer has become the most common cancer worldwide. During the past 50 years, death rates have been dropping steadily in women over the age of 50, but not those under 50. There are multiple breast cancer susceptibility genes, in particular, BRCA1 and BRCA2, but other genes also play a role. In this research we focus on CHEK2. CHEK2 (a protein kinase) plays important roles in the cell cycle, apoptosis, DNA repair, and as a tumor suppressor gene. Most importantly, mutations within this gene compromise multiple functions leading to susceptibility to breast cancer, thus all deleterious SNVs (single nucleotide variations) of CHEK2 must be identified. The overall goal is to define the functional effects of every possible nucleotide change within CHEK2. In this project, we began by analyzing four randomly generated variants in CHEK2 which were tested for complementation in galactose and glucose. We used the yeast-based complementation assay for transformation, followed by a massive parallel DNA sequencing to evaluate a comprehensive library of known CHEK2 SNVs. Our results show that one variant complemented while the other three did not. These results indicate that three of the variants tested may be harmful. Understanding the functional effects of CHEK2 SNVs can help predict the potential consequences of those SNVs in the context of breast cancer.

Investigation of Collagen Fiber Arrangement within the Lobes of Primate Dura Mater on the Closure of Sutures

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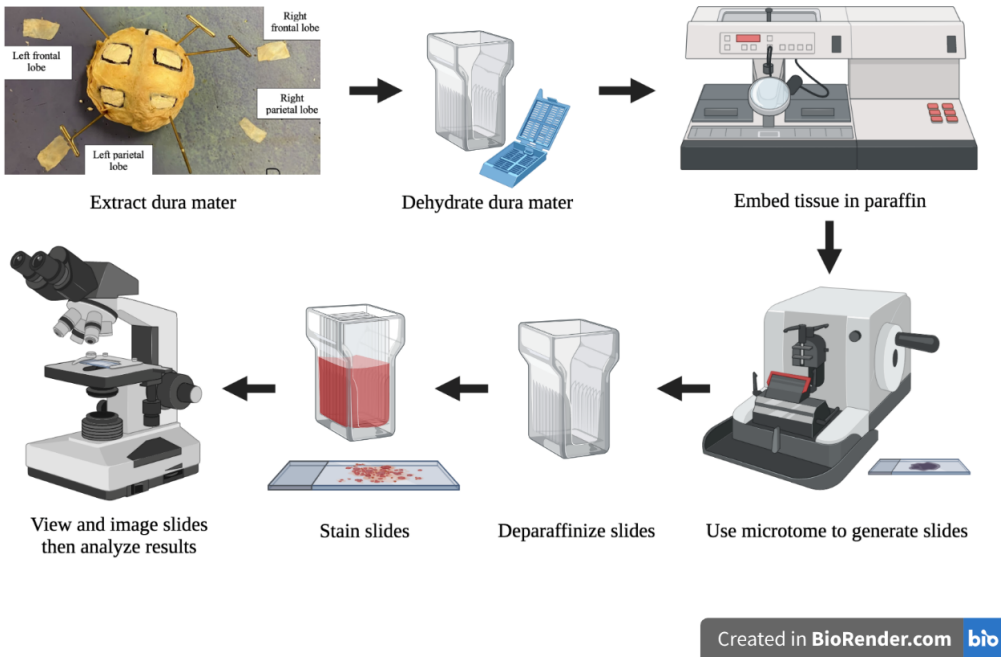

There are many congenital deformities that impact the closure of cranial sutures including craniosynostosis which is characterized by the premature closure of sutures. The presence of disorders such as craniosynostosis calls for a deeper understanding for the underlying mechanism of suture closure. The dura mater is known to influence the development of the human cranium through mechanics and genetics. Based on research done of rabbits, the physical organization of the tissue may give clues about the influence of the dura mater. Primates were used in this study as a model organism to investigate the collagen fiber arrangement and density of cerebral dura mater. The goal was to observe any patterns of collagen fiber arrangement around the lobes of the sagittal suture. A high density of collagen near the sagittal suture could prove that the dura mater can cause the closure of sutures. Using photomicroscopy and computerized image analysis, the trichrome-stained dehydrated-paraffin tissue samples were examined for patterns of density within the arrangement of collagen fibers. Initial data did not display statistically significant results. However, a more efficient methodology is under investigation to verify results with a larger sample size. Future directions include investigating the collagen fiber relationship in human dura if positive results are seen in primates.

Effect of microRNA miR-7 and miR-153 Mimics on Alpha-Synuclein Gene Expression in Parkinson-like Neuronal Cells

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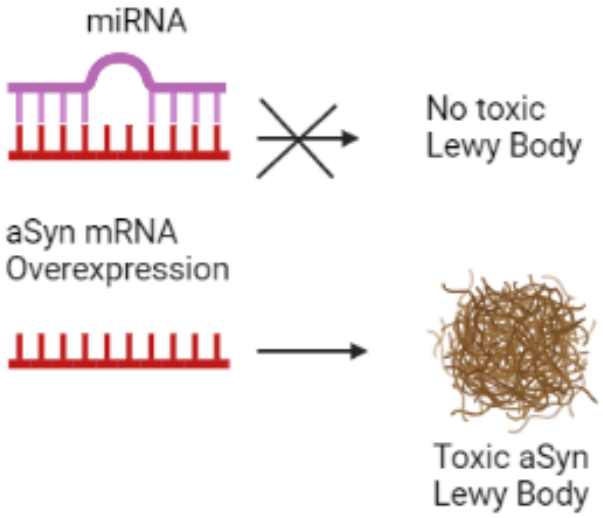

Parkinson's disease (PD) is the second most common neurodegenerative disease, affecting roughly 1% of people over 60 years old worldwide. PD is characterized by dopaminergic neuron deterioration and overexpression/accumulation of alpha-synuclein (aSyn) protein, especially in the midbrain. aSyn expression is naturally regulated by the microRNAs miR-7 and miR-153 in the human body. Our objective is to measure the effects of these microRNAs in SH-SY5Y cell culture, a human neuroblastoma cell line well established as a PD-like cell model. These cells can be differentiated into a PD-like cell model via treatment with retinoic acid, brain-derived neurotropic factor (BDNF), and rotenone. To optimize miRNA transfection, we cultured SH-SY5Y cells in 6-well plates and transfected them with varying concentrations of microRNAs. The microRNA expression of these cells was then measured via quantitative real-time PCR (qRT-PCR) to determine the optimal concentration at which to transfect the cells. Finally, we transfected cells at 50pmol of microRNA and measured aSyn expression via qRT-PCR. These qRT-PCR results showed that miR-7 and miR-153 increased aSyn expression in SH-SY5Y cell culture. This increase may be due to a previously unknown feedback mechanism, which upregulates *SNCA* in response to the downregulation of *SNCA* caused by these miRs. This study elucidates an inconsistency with the preestablished function of miR-7 and miR-153. This discrepancy will require further study before the viability of miR-7 and miR-153 as potential PD treatments can be determined.

Low-Dose Anti-Telomerase Targeted Therapy for Triple Negative Breast Cancer

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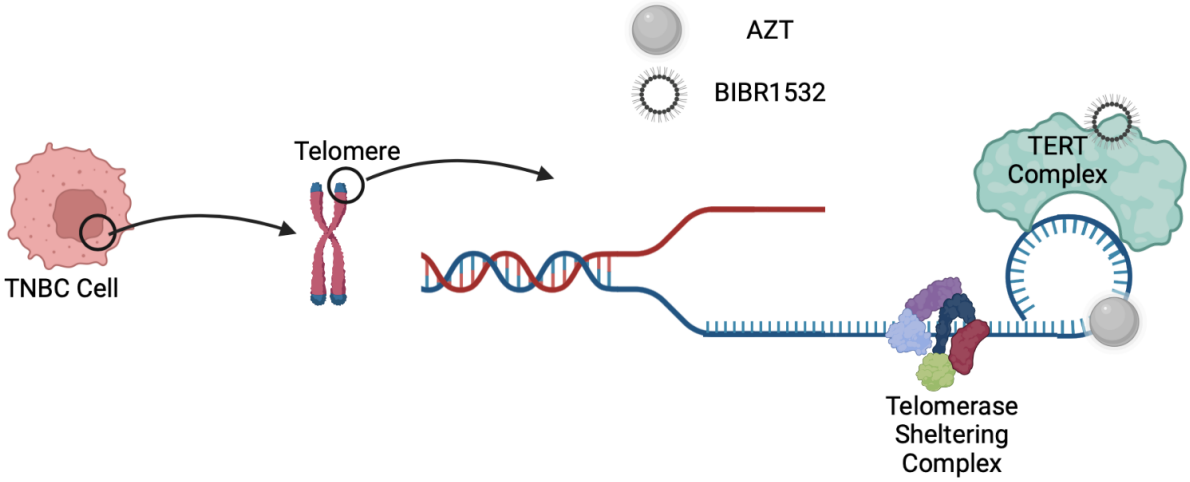

Breast cancer is the most common cancer found in women globally, accounting for about 25% of all new cancer cases. Triple-negative breast cancer (TNBC) subtype is the most aggressive carcinoma with the highest relapse rate and the worst prognosis. Currently there is no known targeted therapy for TNBC. However, immortalization and malignancy of TNBC is strongly correlated to the telomerase-driven telomere length. Therefore, an anti-telomerase approach provides an attractive strategy to limit progression of TNBC. Our research objective was to evaluate the efficacy of low-dose anti-telomerase agents (Azidothymidine (AZT) and BIBR1532) on TNBC cells. In addition, we also assessed the potency of low-dose anti-telomerase therapy in combination with a low dose of Paclitaxel (PAC), a first-line chemotherapy agent used for metastatic breast cancer. Cell viability, apoptosis rate and cellular energetics were measured post treatment. Monotherapy had showed no significant reduction, but trends did appear that will be examined in future studies. The combination therapy showed a significant ($p<0.001$) decrease in cell counts compared to the control. The AZT+BIBR+PAC reduced the TNBC cell counts by > 60% compared AZT+BIBR alone. The AZT+BIBR+PAC combination therapy decreased ($p<0.001$) the pyrophosphate levels by 30% compared to the control. Our results suggest that low-dose anti-telomerases therapy sensitizes TNBC to the effects of PAC. Further studies are needed to confirm these preliminary results.

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Histamine Immunoreactivity in Secondary Cells of the Male Accessory Gland in *Drosophila melanogaster* Appears during Late Pupal Development

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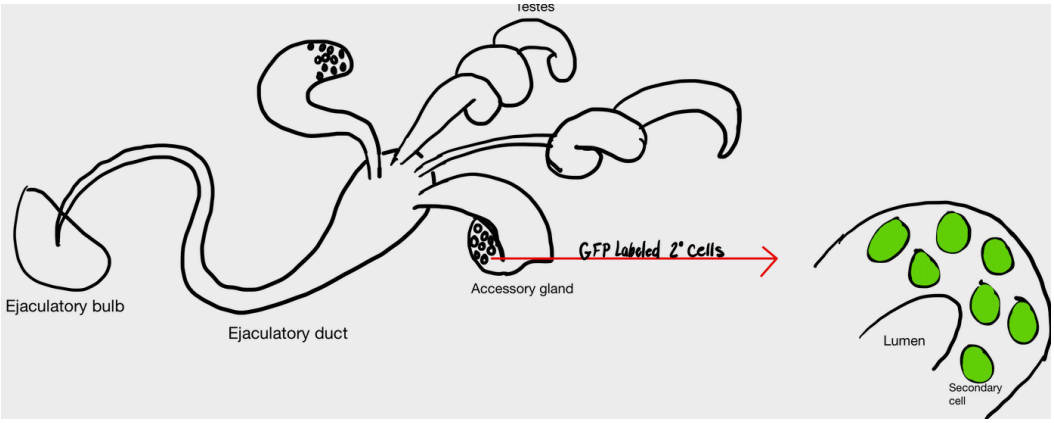

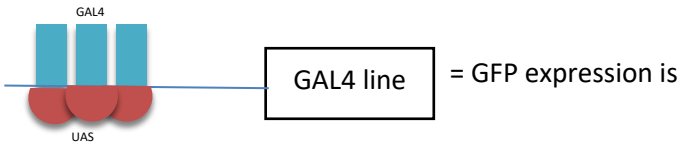

The accessory gland in *Drosophila melanogaster* is composed of primary cells and secondary cells (SCs). Secondary cells are specialized cells in which histamine (or histamine metabolite) immunoreactivity has been detected. Flies with a mutation in the *Hdc* gene, which disrupt histamine synthesis, do not have detectable levels of histamine immunoreactivity, establishing that histamine (or metabolites) are present in SCs. Mutations in the *Hdc* gene alter the male-induced post-mating response (PMR) of female receptivity as well as the number of accessory cells in males. Interestingly, expression of the *Hdc* gene has not been detected in SCs, so it is not known how or when histamine appears in SCs during development. In this project we seek to determine when histamine-like immunoreactivity (HLI) first becomes present in SCs. While the pupal developmental period lasts about 104 hrs, our results indicate that HLI is first detected in the lumen of the AG late, at about 97 hours of pupal development. From 102 hrs to hatching, intensity of the HLI continues to increase in the SCs. These results suggest that either histamine (or metabolites) is synthesized by SCs over a short time-period or that SCs take up histamine from the lumen of the AG.

Does Copulation Result in Movement of Histamine-like Immunoreactivity from the Male to the Female Reproductive System in *Drosophila melanogaster*?

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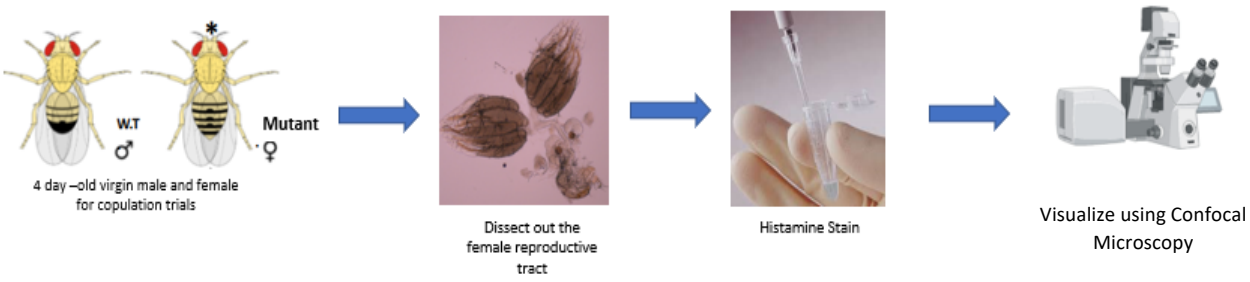

Histamine-like immunoreactivity (HLI) has been identified in the vacuole-like compartments (VLCs) of secondary cells in the male accessory gland of *Drosophila melanogaster*. This histamine-like immunoreactivity is dependent on the *Hdc* gene, as no histamine is detected in secondary cells of *Hdc* mutant accessory glands. To further examine the role that histamine plays in secondary cell function, we have investigated whether copulation can induce the transfer of histamine from secondary cells of males to the female reproductive system via seminal fluid. A four-day old virgin wild-type male and Hdc^{JK910} mutant female (unable to synthesize histamine) were placed in a mating chamber until copulation occurred. Histamine immunostaining was performed on the reproductive system of the Hdc^{JK910} mutant females (and males) that did copulate, ranging from 10 minutes after copulation initiation (during copulation), immediately after copulation had finished, and 30 minutes after copulation had finished. Histamine immunostaining indicated that there was no significant transfer of HLI into the female from the male, unlike other components of the male ejaculate which clearly move into the female and can be detected at the times examined. Histamine-like immunoreactivity did translocate within the male after copulation, moving from the accessory gland to the ejaculatory duct, which suggests that the potential movement of Histamine-like immunoreactivity in the male reproductive system is a result of copulation.

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