L-1G

BIOLOGICAL RESEARCH FROM ANTIBIOTICS TO ZEBRAFISH (A-Z)

Session Moderator: Ann Murkowski, Biology
9:30 AM to 11:00 AM

* Note: Titles in order of presentation.

The Effect of Helminth Infection and Notch Signaling Pathway Manipulation on CD4 T Cell Response and Gene Expression

Pavithra Sundaravaradan, Senior, Microbiology
UW Honors Program
Mentor: Elia Tait Wojno, Immunology

Gastrointestinal helminth infections are among the most common infections worldwide affecting about 1.5 billion people. Previous work shows that during infection with *Trichuris muris*, a mouse model of human whipworm in the large intestine, basophils, rare innate immune cells, accumulate at the site of infection and upregulate the expression of Notch receptors, part of a signaling pathway that regulates gene expression. Due to this discovery, we are investigating the importance of Notch receptor expression in basophils for the anti-helminth immune response and parasite clearance during infection. With single-cell RNA sequencing (scRNAseq) we are able to analyze the expression of individual cells to better understand immune cell response to infection. To investigate how Notch signaling in basophils impacts CD4 T cell function during infection, we performed scRNAseq on CD4 T cells from the intestine of naive and T. muris infected wild-type mice and mice without Notch signaling specifically in basophils. Using this approach, for the first time, we have characterized the gene expression profile of CD4 T cells at the site of infection, allowing us to identify different CD4 T cell subpopulations and their differential gene expression. This will provide insight into how T. muris infection impacts CD4 T cell gene expression and function, and the importance of Notch signaling in basophils for promoting T cell function during infection. Ongoing work is addressing how CD4 T cells and basophils interact in the large intestine, how the highly regulated environment impacts the development of the CD4 T cell response and the T cell receptor repertoire in T. muris infection, and the importance of basophil Notch signaling for this process. A better understanding of the response to helminth infection and manipulation of Notch signaling-dependent cellular responses can lead to the discovery of novel treatments for helminth infection and allergic diseases.

Using Literature Review to Develop a CubeSat Research Question to Study the Effects of the Space Environment on *C. elegans*

Julia Walker, Junior, Molecular Biosciences, Bellevue Coll
NASA Space Grant Scholar
Keith Duc Nguyen, Junior,
Ezgi Ayaz, Sophomore, Bioengineering , Sociology , Bellevue Coll
Amanda Swenson, Sophomore, AAS-T Engineering , Bellevue Coll
Mentor: Jacqueline Gapinski, Molecular Biosciences, Bellevue College
Mentor: Michael Reese, RISE Learning Institute, Bellevue College
Mentor: Jennifer Pritchard

With the impending implications of climate change and global warming, space colonization in the near future will be necessary for the long-term survival of humans. We are examining how exposure to space affects biological aspects of humans by studying a model organism using a CubeSat. A CubeSat is a miniaturized satellite used in space research, built to a set of standardized measurements (10 cm per side), allowing small research projects to be launched simultaneously. We asked the questions: what would the experimental design look like investigating microgravity and UV radiation's impact on the model organism *C. elegans* and what is a feasible experiment to design on a CubeSat? In this project we developed this research question to design a molecular biosciences project on a CubeSat. As part of this project, we conducted a literature review to determine 1) how small-scale satellites can be used for this kind of research, 2) what model organism is best suited for our project, and 3) how we can build upon the existing body of knowledge. The literature review, done in consultation with experts in the field, focused on the effects of microgravity and UV radiation on living or-

ganisms in the space environment. We didn't limit our model organism research to *C. elegans* in the literature review. We used the constraints of the CubeSat to determine our data sampling methods and developed a research question. We anticipate research from our literature review will help us determine the next steps to take in designing our project. We hope to continue this research in preparation for implementing our work in collaboration with UW CubeSat. Research projects and experiments done in CubeSats like this one can advance the research and help address challenges our Earth faces with a rapidly expanding human population and ongoing climate change.

Investigating the Role of Tubulin Hyperglutamylation in the Neurodegeneration of *Caenorhabditis elegans*
Rose Fridman, Senior, Molecular Biosciences, Bellevue Coll
Helena Ochoa, Senior, Molecular Biosciences, Bellevue Coll
Mentor: Jacqueline Gapinski, Molecular Biosciences, Bellevue College
Mentor: Stacy Alvares, Science Division-Molecular Biosciences and Life Sciences, Bellevue College

Microtubules play an essential role in neuronal health as they contribute to the regulation of neuronal morphology, transport and polarity. Our post-translational modification of interest, polyglutamylation, involves the elongation of glutamate chains of microtubules by a set of enzymes called glutamylases. Polyglutamylation is involved in the regulation of microtubule functions, however, the specific role of tubulin glutamylation in its impact on neurodegeneration is not well understood. While there are five glutamylases encoded in the *C. elegans* genome, there are only two carboxypeptidase enzymes, *ccpp-1* and *ccpp-6*, which catalyze the shortening of polyglutamate chains. A deletion of these carboxypeptidases can cause hyperglutamylation since the shortening mechanism is absent. The purpose of this study is to utilize the loss-of-function mutation in carboxypeptidases to investigate the connection between hyperglutamylation and neurodegeneration using *C. elegans* as a model organism. We will utilize an Alzheimer's model strain, *GMC101*, in which an overexpression of amyloid-beta protein in the body-muscle tissue causes a paralysis phenotype.Using GMC101 helps us explore the neurodegenerative effects of hyperglutamylation in the *ccpp-1* and *ccpp-6* mutants. We will establish baseline data of *ccpp-1*, *ccpp-6* and *GMC101* mutant strains using dye filling and paralysis assays to characterize each strain individually. Next, we will develop a double mutant *ccpp-1/GMC101* and *ccpp-6/GMC101*, to investigate whether these mutations improve or worsens the neuronal morphology and the paralysis phenotype. Based on the literature, we expect the double mutant strains to have a worsened paralysis phenotype as well as a worsened neuronal morphology determined through dye filling assays. Investigating the specific contributions of hyperglutamylation to modulating microtubule properties in neurodegeneration is essential since there is a link between an increase in hyperglutamylation enzymatic activity in neurodegenerative regions. Further understanding of this mechanism could contribute toward developing therapeutics involved in polyglutamylation for individuals with neurodegenerative diseases.

What is in Your Soil? Could it be Antibiotic Producers?
Hadiya Amjad, Freshman, Biology, Lake Wash Tech Coll
Lidiia Gagarina, Sophomore, Biology, Lake Wash Tech Coll
Hanah Nguyen, Sophomore,
Mentor: Kimberly McClure, Natural Science, Lake Washington Institute of Technology

Antibiotics are medicines that inhibit the growth of or kill microorganisms. The discovery and production of new antibiotics has greatly decreased in the past 30 years, while the number of antibiotic-resistant microorganisms has increased. Microorganisms growing in soil are a potential source to discover new antibiotics. The goal of our study was to identify and characterize soil microbes that are antibiotic producers. A soil sample was diluted in water and plated onto complex media. Of the 300-400 colonies that grew, 26 soil isolates were screened for the ability to produce antibiotics and inhibit the growth of the five ESKAPE-safe relatives *(Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and several species of *Enterobacter).* Of the 26 soil isolates screen, 7.7% were found to produce antibiotics. The identity of the antibiotic producers was determined by PCR of the 16S rRNA gene and DNA sequence analysis. One of the antibiotic producers was of the genus *Flavobacterium* and the other is *Streptomyces.* Our plan is to perform future biochemical analysis of the antibiotic producers. In conclusion, our study supports the hypotheses that soil is an excellent source for potential antibiotic discovery.

Activity of Broad Spectrum Polyimidazolium Antibiotics in a "Host-Mimic" Medium
Ching Wen Chiu, Senior, Microbiology
Mentor: Amy Schaefer, Microbiology
Mentor: E Peter Greenberg, Microbiology

Antimicrobial peptide (AMPS) hold promise as novel antibiotics for treatment of human infections. However, in general, they fail to reach clinical application because of issues with human cell toxicity or other factors. The AMP colistin (CST), is a rare AMP in clinical use and despite its nephrotoxic effects, has seen increasing use as a last resort to antibiotics. There's now widespread CST resistance among bacterial pathogens. Our chemist colleagues recently synthesized a new class of AMP mimics, main chain cationic polyimidazoliums (PIMs). Their lead PIMs are potent broad spectrum antibiotics with a novel mode of action. We are interested in developing PIMs for inhalation therapy to treat respiratory

infections in people with the genetic disease cystic fibrosis (CF). The bacterium, *Pseudomonas aeruginosa*, is the major contributor of disease in patients with CF lung infection. Unlike CST, PIM-resistant *P. aeruginosa* do not emerge in laboratory evolution experiments. We wanted to assess a particularly potent PIM called PIM1D as a potential CF therapeutic. Many antibiotics are inhibited by the secretions in CF lung and we expected this might be true of PIM1D. We used a special *in vitro* culture medium designed to mimic CF sputum, synthetic CF medium (SCFM). The medium contains the nutrient composition of CF sputum (SCFM-1) along with mucin and DNA (SCFM-2), polymers present at high concentrations in CF lungs. These polymers are known to inhibit many antibiotics' activity. PIM1D retains high activity in SCFM-1 but activity is reduced by 90% in SCFM-2 compared to SCFM-1 and standard media. The inhibition might be due to either mucin, DNA, or both together. We aim to answer which of these molecules is responsible for inhibition of PIM1D activity. In collaboration with chemists who synthesized PIMs, they will provide PIM1D derivatives and PIMs packaged in nano-carriers to test for better activity in SCFM-2.

Impact of Rapamycin on Larval Size

Bill Young, Senior, Psychology, Biology (Molecular, Cellular & Developmental)

Levinson Emerging Scholar, Mary Gates Scholar

Mentor: Daniel Promislow, Department of Lab Medicine & Pathology, University of Washington School of Medicine

The mechanistic target of rapamycin (mTOR) pathway is a central nutrient signaling pathway involved in regulating cell proliferation and metabolism. Targeting this pathway has promising implications for treating a variety of diseases, especially cancer and age-related diseases. Rapamycin is an allosteric inhibitor of mTOR that has been shown to extend longevity in the fruit fly *Drosophila melanogaster*. Despite this, rapamycin can interfere with healthy mTOR pathways necessary for survival, which is why further research on rapamycin's mechanism is necessary to improve clinical usage. Previous research has shown that larval size is significantly decreased in fly larvae following inhibition of the mTOR pathway. However, this effect has not been studied across fly strains with differing levels of rapamycin sensitivity. In the Promislow Lab, we have found that fly strains vary in sensitivity to the effect of rapamycin on developmental timing. We are now comparing variation in sensitivity of development time with variation in rapamycin's effect on larval size. Using *D. melanogaster*, we are able to selectively expose larvae to rapamycin from embryogenesis to model the effects of rapamycin. We are collecting larvae at various time points throughout their development, comparing individuals exposed to rapamycin or control conditions. We use ImageJ software to quantify larval size. Because of mTOR's involvement in growth pathways, we hypothesized that larvae with greater rapamycin sensitivity would have significantly smaller sizes at the same time points compared with larvae with lesser rapamycin sensitivity following rapamycin exposure. These experimental results will provide insight into how rapamycin sensitivity is linked to the phenotypic effects of rapamycin on larval size. They will also help us investigate what contributes to the sensitivity differences seen between genotypes. The spectrum of rapamycin resistance in humans is unknown and so our work could help us identify targets for treatments and therapies of age-related diseases.

Using Proximity Labeling to Investigate Mitochondria Associated Signaling

Hayden Gizinski, Senior, Biology (Molecular, Cellular & Developmental)

Mary Gates Scholar

Mentor: Yasemin Sancak, Pharmacology

Organelles - membrane bound structures within eukaryotic cells - allow compartmentalization and simultaneous execution of thousands of biochemical reactions within one cell. Rather than serving just one purpose, they are constantly interacting with outside molecules and each other to complete processes. This function is described as being signaling platforms, surfaces that can facilitate interactions between proteins. Mitochondria house signaling proteins that are important for innate immunity and mitochondrial quality control on their surfaces and has been shown to have signaling complexes that associate with the outer mitochondrial membrane to coordinate local protein synthesis. Based on these findings, we hypothesized that mitochondria function as signaling platforms for protein interactions. We chose to focus on two signaling cascades, insulin and calcium, due to their significance in metabolic processes and clinical relevance. To investigate, we used proximity labeling technology, in which a molecular tag is attached to proteins within a certain cellular location. We labeled proteins that alter their mitochondrial association in response to these two stimuli. After cells were treated with stimuli, the lysed and tagged proteins were visualized through Western blots. This process also enables their identification through mass spectrometry. Our goal is to have a detailed characterization of mitochondria associated proteins after insulin and calcium stimulation. Such data will create openings for further research into the mitochondria as a signaling platform and potential for implications in the medical world.

Nicotinamide Mononucleotide Triggers Inflammation in Aged Kidneys Barring Protective Rescue by Mitochondrial-Targeted Tetrapeptide, Elamipretide

Tara Asal Saleh, Senior, Global and Regional Studies

UW Honors Program

Mentor: Mariya Sweetwyne, Laboratory Medicine and Pathology

Mitochondrial dysfunction is characterized by loss of structural integrity and decreased efficiency of the Electron Transport Chain. These changes are linked to cellular senescence, an irreversible end to the cell-cycle that contributes to aging phenotypes and is marked by a cellular senescence-associated secretory phenotype. Understanding this impact of late age mitochondrial dysfunction interventions will contribute to the field of, 'senolytics', anti-senescent drugs dedicated to improving health span. Previously, systematic treatment of old mice with tetrapeptide, Elamipretide (ELAM), which interacts with the inner mitochondrial membrane to improve cristae structure and function, reduced mitochondrial dysfunction and senescence in kidney and heart. We hypothesized that additional intervention with Nicotinamide Mononucleotide (NMN), a NAD+ precursor, which contributes to adenosine triphosphate generation, would supplement this finding by enhancing mitochondrial energetic capability and decreasing mitochondrial dysfunction and senescence in aged kidneys. Liver was selected to clarify intervention effects as global vs. kidney-specific mechanisms. Old mice were treated at 24 months-old (mo) for 8 weeks with: ELAM through osmotic pump (3mg/kg), NMN via drinking water (300mg/kg), or both simultaneously. Untreated control mice were 4 mo (young) and 26 mo (old). Contrary to our hypothesis, NMN treatment proved detrimental in kidney by increasing mRNA expression of IL-1b, an inflammatory cytokine, relative to untreated aged mice. Further exploration of IL-1b downstream targets showed consistent upregulation of CCL2, an inflammatory chemokine and KIM-1, a marker of proximal tubule injury in NMN treated mice. ELAM, however, provided rescue in the coupled treatment group, by significantly reducing mRNA expression of IL-1b and CCL2 relative to NMN alone. These data suggest that ELAM lessens senescent burden by reducing renal inflammation; conversely NMN exacerbates existing inflammation pathways in aged kidneys. This result was not observed in liver, demonstrating tissue specificity. Further work will investigate the cellular source of inflammation through RNA in situ hybridization.

Skin Appendage-Dependent Development of Touch-Sensing Cells in Zebrafish

Everett Fan, Senior, Biology (General)
Mentor: Jeff Rasmussen, Biology

Skin is a very important organ that facilitates our sense of touch. The touch system is complex and versatile. For example, specialized cells detect a variety of tactile stimuli, including temperature, pain, and textures. Merkel cells are specialized skin cells responsible for detecting light touch and textures in most vertebrates. Despite being discovered over a hundred years ago, their development remains poorly understood. Zebrafish are a good model organism for studying Merkel cells because the fish skin is transparent and easy to image. In wildtype zebrafish, Merkel cells are distributed in clusters, corresponding to the location of scales. The ectodysplasin (Eda) signaling pathway regulates the formation of many types of skin appendages, including mammalian hair follicles and zebrafish scales. In eda mutants lacking scales, Merkel cells appear at a lower density and are uniformly distributed across the trunk. This suggests that blocking Eda-dependent scale formation inhibited Merkel cell development, but what would be the effect of altering scale shape and size? To address this question, we examined the skin of hagoromo mutant fish. The hagoromo mutation is a viral insertion that causes an overexpression of fgf8a, another important signal pathway for scale formation. By imaging transgenic zebrafish with marked Merkel cells and osteoblasts, we used ImageJ to calculate Merkel cell density, scale area, scale Feret's diameter, and scale aspect ratio. We found that juvenile zebrafish with the hagoromo mutation had highly variable scale shapes and sizes. Interestingly, the clusters of Merkel cells expanded or shrank to match the new scale shapes in the mutants. However, there seemed to be no significant difference in Merkel cell density. We expect to see similar results in adult zebrafish. Our research will help us understand the development of Merkel cells, therefore helping us understand skin development in vertebrates better.

Reestablishing Touch: Examining the Regeneration of Merkel Cells in Zebrafish

Nathaniel Yee, Senior, Biology (Physiology)
Mary Gates Scholar
Mentor: Jeff Rasmussen, Biology

Touch is an extremely important sense to any organism's understanding of their environment. The anatomy of the touch system is well-characterized: sensory neurons project axons to the skin where they form complexes with many specialized cells. One such specialized skin cell is the Merkel cell, known for its ability to sense gentle touch and texture. Although Merkel cells have been well studied in rodents during adult stages, little is known about Merkel cells during development. The Rasmussen lab recently identified a novel population of zebrafish skin cells sharing many characteristics of mammalian Merkel cells. In both zebrafish and mammals, Merkel cells arise from basal keratinocytes, a stem cell population in the epidermis, during normal skin development. However, the precise mechanisms involved in this process remain unclear. Stem cells can undergo two types of cell division: asymmetric or symmetric division. I hypothesize that Merkel cells develop from asymmetric division of basal keratinocytes, which would allow the production of new Merkel cells while also maintaining a certain level of stem cells. To test my hypothesis, I examined the Merkel cell lineage during zebrafish scale regeneration (to simulate recovery after injury) which we have established as an experimentally tractable system to study Merkel cell differentiation. Testing of my hypothesis has been conducted through two methods:

EdU labeling/antibody staining of Merkel cells and photoconversion of Merkel cells. Preliminary results suggest asymmetric division to be occurring, with no "doublets" of recently divided Merkel cells shown in either method to support the occurrence of symmetric division. My results provide novel insights into the lineage connecting basal keratinocytes to differentiation of specialized sensory cells in the skin. I hope these findings can be used to better our understanding of the restoration of touch systems after injury.