UNIVERSITY of WASHINGTON

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Online Proceedings

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MEASURING CELL GROWTH AND EVOLUTION

Session Moderator: Kristin Anderson, Immunology

MGH 271

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Persistence of Extrachromosomal Plasmid DNA in *Escherichia coli*, *Klebsiella pneumoniae*, and *Shewanella oneidensis*

Reilly Virginia (Reilly) Falter, Senior, Biology (Molecular, Cellular & Developmental)

Mary Gates Scholar, UW Honors Program

Mentor: Olivia Kosterlitz, Department of Biology

Mentor: Benjamin Kerr, Biology

Extra chromosomal pieces of DNA, called plasmids, exist within bacterial populations. Many scientists believe that the relationship between plasmids and their bacterial hosts is mutually beneficial. Plasmids use replication machinery from their host to ensure their continued persistence. Meanwhile, the plasmid carries genes that can benefit the bacteria, such as genes that can provide resistance to antibiotics. In environments that contain antibiotics, bacteria that contain plasmids with antibiotic resistance genes would have a higher fitness and be able to survive and reproduce at a higher rate compared to bacteria that don't carry plasmids. Contrary to popular belief, research by members in our group showed that different plasmids actually persisted in bacterial populations when they were not under selection. The purpose of this project is to see if the trend of plasmid persistence would continue in a nutrient limited environment. We performed a long term evolution experiment on three types of bacteria containing plasmids in a nutrient limited environment. Next, grew the evolved descendants in an environment containing antibiotic to see what portion of the population is still containing the plasmid. The results of this experiment will help scientists have a more holistic understanding of plasmid biology which could have implications on antibiotic treatments.

Characterization of Tse7 in *Pseudomonas aeruginosa*

Savannah Bertolli, Senior, Biochemistry

Mentor: Kaitlyn LaCourse, Microbiology

Mentor: Joseph Mougous, Microbiology

Bacteria inhabit a world filled with threats, including antagonism from other species throughout different environmental conditions. One mechanism microbes employ as protection from these hazards is the type VI secretion system (T6SS) - a system bacteria utilize to inject toxic proteins into neighboring cells, leading to cell death. The H1-T6SS of *Pseudomonas aeruginosa* comprises 7 pairs of toxins and cognate immunity proteins (which prevent self-intoxication). I hypothesized that each of these toxins could be maximally effective against specific kinds of competing bacteria, however the function of many effector proteins is unknown. My research focuses on characterizing the protein Tse7, encoded by the gene PA0099, and elucidating its potential role in species-specific antagonism. To begin, I identified its key functional amino acids and the gene encoding its immunity protein. I designed and created mutant strains with several genes adjacent to PA0099 deleted and co-cultured these with wild-type *Pseudomonas aeruginosa* to identify any mutants with a loss in competitive fitness. This lead to the discovery that the gene PA0100 encodes the cognate immunity protein. To determine key functional residues, I identified potential candidates using conserved motifs in the toxin's amino acid sequence. By creating mutants of these residues and analyzing their change in competitive fitness compared to wild type, I recognized histidine 230 as the residue vital for Tse7 function. Going forward, I will attempt to determine whether Tse7 improves fitness of *P. aeruginosa* against any specific families of bacteria, indicating the toxin targets that particular bacteria. Almost one-third of Gram-negative bacteria have T6SSs. These systems largely dictate the ability of bacterial species to compete with one another, dramatically affecting bacterial community structure and the landscape of human infections. Therefore, this deepened understanding of its function could further our knowledge of and ability to manipulate bacterial interactions' impact on environmental and human health.

Reversing Rate-Adaptation with Water-In-Oil Emulsions

Jordana K. Sevigny, Sophomore, Pre-Health Sciences
Mary Gates Scholar
Mentor: Benjamin Kerr, Biology
Mentor: Katrina van Raay, Biology

Twelve replicate populations of the bacterium *Escherichia coli* have been evolving in Lenski's Long-Term Evolution Experiment (LTEE) for over 67,000 generations in a shared nutrient limited environment. The evolved bacteria grow 70% faster than their ancestor but experience a decrease in number produced during a growth cycle. This is consistent with a trade-off between growth rate and yield (here defined numerically). We explore if populations are constrained by their previous ecolution, and if populations with high growth rate can evolve to have a higher yield (and if so, does this happen at a cost to growth rate?). We do this by adding population structure to growing populations, where selection is relaxed on growth rate and strengthened on yield. Water-in-oil emulsions provide a structured environment where millions of nutrient-filled droplets are isolated by an oil phase. We manipulate population structure by inoculating droplets with either one bacterial cell (low starting density) or more than two bacterial cells (high starting density). We observe that selection acts on faster growing cells in our high density emulsion treatment and higher yield cells in our low density emulsion treatment. We also observe a change in cell size: cells in the high density emulsion treatment get bigger over time, and cells in the low density emulsion treatment get smaller. We explore if there is a relationship between cell size and growth rate/yield trade-off.

***Staphylococcus Aureus* Strain Switching during Chronic Lung Infections among Cystic Fibrosis Patients Treated with Ivacaftor and Antibiotics**

Madeline Grace Fisher, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Samantha Durfey, Microbiology
Mentor: Pradeep Singh, Microbiology

In cystic fibrosis (CF), a genetic defect in the CFTR anion channel compromises host defenses and causes chronic lung infections with organisms like *Staphylococcus aureus.* Our lab has been studying the effects of combining ivacaftor, a CFTR modulator which increases CFTR channel activity, with a period of intensive antibiotic treatment. We found that *S. aureus* lung infections generally persisted despite this aggressive treatment. However, most subjects undergoing treatment were found to be infected by different *S. aureus* strains one year after treatment than were present before treatment was initiated. Understanding the dynamics of strain switching provide new knowledge about the natural history of chronic CF infections, help define the effects of CFTR modulators and antibiotics, and inform new approaches that might produce infection eradication. We hypothesize that (1) strain switching is most likely to occur during the period of combined ivacaftor and antibiotic treatment, as sputum bacterial burdens were lowest during combined treatment; and that (2) strain switching is rare in the absence of combined treatment. To test this, we used a new population-based multilocus sequence typing (PopMLST) method we developed to perform strain-level genotyping on *S. aureus*. PopMLST uses PCR amplification and next generation sequencing of housekeeping genes from bacterial isolate pools cultured from sputum. Sequencing determines the number and relative abundance of unique sequence types present, and the data can be used to infer the number of strains present. This analysis was performed on samples obtained before treatment, during treatment with ivacaftor alone, and during combined treatment. We also examined a cohort of subjects receiving usual care. These data improve understanding of strain dynamics during CF infections and suggest new strategies to eliminate infection.

Stage-Specific Molecular Markers in *Giardia lamblia* Membrane Trafficking

Renaldo Sutanto, Senior, Biology (Molecular, Cellular & Developmental), Biochemistry
Mentor: Alexander Paredez, Biology
Mentor: Elizabeth Thomas

Giardia lamblia, a microscopic flagellated parasite that causes giardiasis, is a highly divergent eukaryote in which conventional Golgi, endosomes, lysosomes, and mitochondria are absent. Similar to other parasites of medical importance, Giardia lamblia has two life cycle stages - proliferative trophozoite form and water-resistant, nonmotile, infectious cyst form. During encystation when Giardia trophozoites transform into infectious cysts, they secrete cyst wall proteins (CWP1-3) that are trafficked and processed in Encystation Specific Vesicles (ESVs). These vesicles are thought to be stage-induced Golgi in Giardia. Previous work in the lab has shown that the signaling activities of G. lamblia's single Rho family GTPase, GIRac play an important role in regulating this encystation process. The aim is to characterize proteins in Giardia lamblia that potentially interact with GIRac, currently focusing on homologs of known players in membrane trafficking by examining their order of arrival using morphology of the ESVs based on CWP1 staining. Since this is subjective, there is a need for stage-specific molecular markers. In other eukaryotes, Rab GTPases have been established as markers of membrane identity and directionality of trafficking. Only two out of nine Giardia's Rab GTPases have been localized and reportedly found at ESVs and based on published images, they appear to be recruited at different stages of ESV maturation. By tagging the N-terminus of all 9 Giardia Rab GTPases with fluorescent tags, we can screen them for their localization to ESVs and perform multi-color imaging to determine the order of arrival of these markers. Ultimately, this finding of stage-specific molecular markers could

be a powerful tool to further suggests its potential as a novel target for drug development to treat giardiasis.

Creating a Continuous Culture Monitoring Device to Determine Relative Strain Frequency in Solution

Nick Righi, Junior, Bioengineering
Mentor: Maitreya Dunham, Genome Sciences
Mentor: Anja Ollodart, Molecular Cellular Biology

One method of comparing strain fitness is to compete strains head to head; over time, the competitor with a fitness advantage will increase in frequency in the population. To track the frequency of each strain, they must be differentially marked such that their frequencies can be measured by plating the culture and counting colonies of each type - a time and labor-intensive process. One common marker used by our lab and others is using pigment production to produce colonies of different colors. My project is to create a continuous culture monitoring device named a chromostat that uses a color sensor to measure the relative abundance of different colored strains in solution, removing the need for plating and increasing the automation of competition experiments. By comparing the color of the individual yeast strains to the color of them mixed together, the chromostat can calculate, in real time, the relative abundance of each strain in solution and determine which strain is more fit and by how much. I built the chromostat on a raspberry pi minicomputer using an open source Java library, pi4j, to control the attached color sensor. This sensor converts light waves to red, green, and blue color values, which are then converted to frequency values for each strain and displayed to the user. The chromostat is controlled through a text-based interface that operates on the command line and has a variety of functions to modify data acquisition to increase overall accuracy and allow for data analysis. It will be used as part of a high school teaching laboratory in which students conduct evolution experiments and later compete different yeast strains against each other to generate fitness data.

The Epigenetic Computational Remodeler, EED binder, modulates PRC2 Requirements in ZF Embryogenesis and Tissue Regeneration

Alexis Massey, Senior, Biology (General)
Ginger Hojung Kwak, Senior, Neurobiology, Gender, Women, and Sexuality Studies
Mentor: Shiri Levy, biochemistry

The Polycomb Repressive Complex 2 (PRC2) complex is known to be important in the development of zebrafish and Human Embryonic Stem Cells (hESC's) by aiding in the transition from naïve to primed stem cells. H3K27me3 is a PCR2 dependent methylation of histone 3. The catalytic subunit responsible for trimethylation is the methyltransferase EZH2 and is required for correct zebrafish embryogenesis. The EZH2 interacts with the PRC2 complex's EED (Embryonic Ectoderm Development) which is critical for EZH2 activity presumably because EED binds EZH2 to its specific substrate. A computational protein design was utilized to engineer a synthetic, novel protein that is a competitive EZH2 inhibitor with 300 times more affinity to the EED binding site than endogenous EZH2. This synthetic protein is referred to as EED binder (EB). Our previous results show that EB allow hESC exit pluripotency. EB has now been injected into zebrafish embryos in combination with a heat shock protein that will activate the EB, and a GFP indicator. A founder animal has been found that allows us to create an F1 generation of fish that are all positive carriers of the EB protein. These F1 fish can be used to create an F2 generation that receive a heat shock treatment at different times of development in order to observe the affects of inhibiting PRC2. It is assumed that if PRC2 is inhibited in a developing fish it will not survive as this is what was found in hESC's. This is novel research in that we are now able to control temporal aspects of development of PRC2. This research lead to testing tissue regeneration and embryogenesis in zebrafish which will impact new research of regenerative medicine.