Undergraduate Research Symposium May 19, 2017 Mary Gates Hall

Online Proceedings

POSTER SESSION 1

MGH 206, Easel 177

11:00 AM to 1:00 PM

Identifying Active Gene Regulatory Networks Important for Adaptation to Fluctuating Resource Environments

Ayodale Bukari (Ayodale) Braimah, Senior, Microbiology
Mentor: Nitin Baliga, Microbiology, Institute for Systems Biology
Mentor: Serdar Turkarslan, Baliga Lab, Institute for Systems Biology

Gene regulation lessens the cost of cellular operations by altering physiological processes in an as-needed manner depending on resource availability. Previously, it was shown that repeated ecologically-relevant shifts in combination with conditional gene regulation can lead to a population collapse phenomenon. In this prior work, wild-type and regulatory mutant strains of *Desulfovibrio vulgaris* Hildenborough (DvH) were grown in co-cultures together with wild-type *Methanococcus maripaludis* in an environment where they were continuously transitioned between sulfate respiration and syntrophy conditions. While the wild-type DvH co-cultures collapsed after few transitions in this environment, the regulatory mutant persisted and never collapsed. This work showed that conditional gene regulation can drive a microbial community to collapse when the environment fluctuates too rapidly. In addition, it provided us with an experimental framework for probing the impact of gene regulation in adaptation of microbial communities to rapidly fluctuating environments. In this work, we further combined the experimental framework of alternating transitions together with transposon mutant libraries to identify active gene regulatory networks. The random barcode transposon-site sequencing (Rb-TnSeq) DvH library was rapidly transitioned between high and low sulfate concentrations and samples were collected for barcode sequencing to determine transcription factors that were enriched over the course of transitions. We will present fitness data and a list of enriched regulatory genes to shed light on how the regulatory mutations impact the organism's ability for adaptation to fluctuating environments.

POSTER SESSION 1

MGH 241, Easel 144

11:00 AM to 1:00 PM

The Antiviral Activity of Chimeric APOBEC3 Proteins Against HIV

Elisa Aiko Cano, Senior, Biology (General)
Mentor: Michael Emerman, Microbiology, Fred Hutchinson Cancer Center

The APOBEC3 proteins are a family of antiviral proteins encoded by 7 genes in humans. They act by hypermutating the viral genome due to their cytidine deaminse activity (mutation of cytidines to uracils). However, they are inactivated by the HIV Vif proteins. The APOBEC proteins have been the subject of much research over the years due to their evolutionary arms race with the HIV protein Vif and their restrictive properties the against the Vif-deficient virus. Moreover, some of the APOBEC3 proteins encode two deaminase domains, while others encode only one. It is known that the most restrictive APOBEC3s tend to localize mostly in the cytoplasm and have the propensity to dimerize. It is unknown, however, whether either of these factors cause the proteins to be packaged into the virions more readily, consequently causing them to be more restrictive, or if these factors by themselves are the cause of higher restriction of the virus. In this experiment, I am looking to see if the artificial linking of two weakly antiviral APOBEC3s that encode single deaminase domains and do not naturally link in vivo will increase their restriction factor against HIV. First, the chimera will be made by PCR, then ligated into a mammalian expression vector, pcDNA 3.1, and cloned into bacteria. Next, the plasmid will be prepped and used to do a viral infectivity assay with Vif-deficient HIV by the transfection of HEK293T cells and analyzing the data. Based on the findings in other research, I expect to see an increase in restriction of Vif-deficient HIV, and possibly also Vif-proficient HIV. If this is found to be true, then further research could be done to find the localization of the chimera within the cell. Furthermore, research on the viral packaging of these proteins could reveal more concerning their antiviral activity.

SESSION 1K

MOLECULAR BASIS FOR HUMAN DISEASE

Session Moderator: Caroline Harwood, Microbiology

MGH 271

12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

KSHV Modulates the Expression of Genes Involved in Peroxisome Biogenesis

Yashmira (Mira) Naidoo, Senior, Microbiology
Levinson Emerging Scholar, Mary Gates Scholar, UW Honors Program
Mentor: Michael Lagunoff, Microbiology

Kaposi's sarcoma-associated herpesvirus (KSHV) is the causative agent of Kaposi's Sarcoma (KS), a cancer of endothelial cell origin that is the most common malignancy among AIDS patients worldwide. Previous research in our lab has established that the number of peroxisomes is increased during latent KSHV infection. Peroxisomes are multifunctional cellular organelles involved in a variety of metabolic pathways important to KSHV pathogenesis. My project is to evaluate the cellular mechanism by which KSHV induces peroxisome biogenesis, thereby elucidating one of the key pathways involved in KSHV latency. I hypothesize that KSHV increases the transcription of specific regulatory genes responsible for peroxisome biogenesis. I evaluated gene expression of a known transcription factor, peroxisome proliferator-activated receptor alpha (PPARA), that has been implicated in peroxisome biogenesis during mock and KSHV latent infection of endothelial cells. I used real-time PCR to quantify gene expression of PPARA, in addition to other genes involved in peroxisome formation and function. My data demonstrates that KSHV infection upregulates PPARA and peroxisome-associated genes, suggesting that PPARA may be a key regulator of the expression of peroxisome biogenesis. To further establish the role of PPARA in peroxisome biogenesis, I am currently working on silencing PPARA expression using small interfering RNA (siRNA). I will determine if the knockdown of PPARA prevents KSHV upregulation of peroxisome-associated genes. In the absence of PPARA, I expect that expression of peroxisome-associated genes will be downregulated, suggesting that PPARA regulates them at the transcriptional level. These results will establish a key mechanism in KSHV pathogenesis, and potentially contribute to the development of novel therapeutic avenues for KS treatment.

POSTER SESSION 2

MGH 241, Easel 135

1:00 PM to 2:30 PM

Internal Deletion Induced Interferon Response to Influenza A

Jacob Richard Kowalsky, Senior, Microbiology
Mary Gates Scholar
Mentor: Jesse Bloom, Division of Basic Sciences
Mentor: Alistair Russell, Basic Sciences

Influenza is a prolific and hazardous virus, affecting even those in the population who have been previously infected or vaccinated. The innate immune system serves as a key first line of defense against this pathogen, with the signaling components, called interferons, driving the production of a potent cellular antiviral response. Studies have indicated that viral populations replete in defective virus particles, virions with a deletion in a portion of their genome, are less efficient at blocking the antiviral response, as shown by increased interferon in the host. Our project seeks to explore this phenomenon of RNA deletions leading to increased interferon expression in host cells by testing the hypothesis that deletions in the three polymerase genes of influenza alone are sufficient to cause an increase in the interferon response. To begin answering this question, an interferon reporter system was used to analyze the viral genome of interferon positive cells, and deletions within various lengths of the PB1, PB2, and PA polymerase genes were found to be enriched. I then created pure populations of influenza with these empirically derived gene deletions in PB1 while my mentor, Dr. Alistair Russell, created pure PB2 deletion populations, which were grown on cell lines expressing functional PB1 and PB2 proteins respectively. When used to infect unmodified cell lines, it was found that pure populations of both PB1 and PB2 defective influenza were sufficient to induce the host interferon response. In the future, I will create PA expressing cell lines and influenza with deletions in PA to analyze the effect of PA deletions within the influenza genome on the host interferon response, and it is hoped that these results can be used to explore a mechanism for how influenza occasionally fails to escape the innate immune response.

POSTER SESSION 2

MGH 206, Easel 178

1:00 PM to 2:30 PM

Hypoxia Induced Factors in Latent Kaposi's Sarcoma Herpesvirus Infected Endothelial Cells

Jie Yin, Senior, Biochemistry, Microbiology
Mary Gates Scholar, UW Honors Program
Mentor: Michael Lagunoff, Microbiology
Mentor: Daniel Holmes, Microbiology

Kaposi's Sarcoma-Associated Herpesvirus (KSHV) is the etiological agent of Kaposi's Sarcoma (KS), a highly vascularized tumor predominantly made up of cells of endothelial origin. KSHV establishes a predominantly latent infection in endothelial cells in culture and in the KS tumor. KSHV latent infection alters cellular metabolism to improve the survival of the infected cells. These metabolic changes resemble a common alteration in cancer cells, termed the Warburg effect. This refers to an increase in glycolysis in the presence of oxygen and a decrease in oxidative phosphorylation. The mechanism of KSHV induction of the Warburg effect is currently unknown. Hypoxia-induced factors (HIFs) are

likely candidates for KSHV induction of the Warburg effect as both HIF1α and 2α are induced by KSHV latent infection. To determine if HIF1α or 2α is required for the survival of KSHV infected endothelial cells, I constructed HIF1α or 2α knockouts using the CRISPR/Cas9 gene editing technique in a lentivirus vector. I expressed the endonuclease Cas9 and guide RNAs that lead Cas9 to cut at the HIF1α or 2α genomic sequences leading to genomic mutations in HIF1α and 2α respectively. To determine if HIF1α or 2α is required for the survival of latently infected endothelial cells, I infected the HIF1α and 2α knockout cells with KSHV and looked for cell death at 48 hours after infection. To evaluate if HIFs are required for KSHV induction of the Warburg effect, I will determine if one or both of the knockout cells produce less lactic acid and increase oxygen consumption at 48 hours post KSHV infection as compared to infection of wild type cells indicating the Warburg effect is not being induced by KSHV in the knockout cells. These results will aid in the future efforts to develop antiviral drugs by inhibiting viral latency.

POSTER SESSION 2

MGH 241, Easel 152

1:00 PM to 2:30 PM

Characterizing the Immune Response of Gastric Epithelial Cells to *H. pylori* Infection

Rohan Hassan, Senior, Biology (Molecular, Cellular & Developmental)

Mary Gates Scholar

Mentor: Nina Salama, Human Biology, Fred Hutchinson Cancer Research Center

Mentor: Tina Gall, Molecular and Cellular Biology, Fred Hutchinson Cancer Research Center

Helicobacter pylori is a gastric bacterial pathogen that infects 50% of the world's population. *H. pylori*'s helical shape is presumed to help it drill through the mucus lining of the stomach and arrive at the surface layer of gastric epithelial cells, its preferred niche. The bacteria then use a type IV secretion system to deliver toxins to the gastric epithelial cells. Once infected, the gastric epithelial cells initiate an immune response via numerous receptors resulting in a signaling cascade that leads to activation of pro-inflammatory genes including a cytokine called interleukin 8 (IL-8). Gastric epithelial cells secrete IL-8 in an attempt to recruit immune cells to fight the bacterial infection. However, this immune response seldom results in clearance of the infection. This is because *H. pylori* controls the host's immune response through a set of virulence factors that are responsible for invoking and maintaining a pro-inflammatory response. Inducing inflammation allows *H. pylori* to thrive, however, too much inflammation will result in a clearance of the infection. My goal is to understand which *H. pylori* genes are responsible for modulating the immune response in gastric epithelial cells. To find these genes, I am screening a mutant library that targets virtually all non-essential genes (approximately 1000 out of 1500) in the *H. pylori* genome. I co-culture individual *H. pylori* mutants with gastric epithelial cells and assay their response to the bacterial infection. Using enzyme-linked immunosorbent assay (ELISA), I can quantify the amount of IL-8 gastric epithelial cells secreted into the supernatant. By determining which mutant bacteria in our library induce more or less IL-8 secretion in gastric epithelial cells, I can identify bacterial pathways that are important for modulating the host immune response. This approach may potentially uncover bacterial factors that can be targeted therapeutically.

POSTER SESSION 2

Commons East, Easel 46

1:00 PM to 2:30 PM

The Presence of Rare Earth Elements (REEs) Determine the Competitiveness of Methane-oxidizing Bacteria in Synthetic Microbial Communities from Lake Washington

Hannah Lee (Hannah Erdy) Erdy, Junior, Pre-Sciences

Mentor: Sascha Krause, Chemical Engineering

Lake Washington is a freshwater ecosystem, which is characterized by a dynamic turnover of methane, an important greenhouse gas, serving as both a major source and a major sink. In this environment, methanotrophs are a key aerobic microbial group that oxidizes methane preventing its release into the atmosphere, thereby acting as a natural filter. Consequently, methanotrophs have been studied intensively in this environment. However, the data from different approaches have not always agreed with each other. In particular, DNA stable isotope probing (DNA-SIP) with 13-C-methane suggested that the genus *Methylobacter* species might be the dominant active type in laboratory setups approximating natural conditions. In contrast, pure culture experiments with other methanotrophs, including *Methylobacter* demonstrated the genus *Methylomonas* to display robust growth and the highest competitiveness. This suggested that there must be biotic and/or abiotic factors that act differently in pure culture and setups approximating natural conditions. In this study, we established synthetic communities to test the effect of Rare Earth Elements (REEs) and microbial diversity as explaining variables on the competitiveness of the dominant methanotrophs. We focused on Lanthanum as a representative of REEs because of its increasing importance in methanotrophy. Quantitative PCR and spectrophotometry were used to determine growth of cultures and individual abundances of methanotrophs. Over a time span of 4 weeks synthetic communities resulted in variable dynamics whereas two species synthetic communities clearly showed *Methylomonas* to be the stronger competitor compared to *Methylobacter* in the presence of Lanthanum. The distinct effects of Lanthanum

are still being investigated, yet these results suggest that both biotic and abiotic factors determine the species interactions of this environmentally important group of bacteria. Future studies will aim to further disentangle the contribution of these factors to better understand the functioning of an important microbially driven biogeochemical process.

SESSION 2B

CHEMISTRY, BIOCHEMISTRY, AND MATERIALS SCIENCE

Session Moderator: Sharona Gordon, Physiology and Biophysics

MGH 228

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Heterogeneous Production of C-Di-GMP during Early Stages of *P. aeruginosa* Biofilm Formation

Jessica Lynn Parker, Senior, Microbiology
Mentor: Matthew Parsek, Microbiology
Mentor: Catherine Armbruster, Microbiology

Biofilms are surface-associated aggregates of bacteria encased in a protective, extracellular matrix. Biofilm bacteria resist antibiotic treatment and killing by the host immune response, leading to persistence in a variety of chronic infections. *Pseudomonas aeruginosa* is an opportunistically pathogenic bacterium and a model organism for studying biofilm formation. One major factor that drives biofilm formation in *P. aeruginosa* and other bacteria is the intracellular second messenger signaling molecule cyclic diguanylate monophosphate (c-di-GMP). Elevated c-di-GMP levels promote cell surface adhesiveness by up-regulating production of biofilm extracellular matrix components and down-regulating motility genes. In contrast, planktonic *P. aeruginosa* cells are known to have comparatively lower c-di-GMP levels than their biofilm counterparts. While factors contributing to the formation of mature biofilms have been well-characterized, early biofilm formation, when a bacterium first senses a surface and transitions from a planktonic state to a surface-attached state, remains largely understudied. Using a fluorescent, transcriptional reporter of intracellular c-di-GMP and confocal microscopy, we have monitored the dynamics of c-di-GMP production at the single cell level. Our major finding is that c-di-GMP levels are elevated in only a subpopulation of *P. aeruginosa* cells (30-69%) during this time. We have used flow-assisted cell sorting (FACS) to separate surface-attached cells with high and low c-di-GMP, to examine ways in which these populations are physiologically distinct. We have found by lectin staining and by qRT-PCR that surface-associated cells with high intracellular c-di-GMP produce more polysaccharide than their surface-associated, but low c-di-GMP counterparts. We hypothesize that the heterogeneity in c-di-GMP observed during *P. aeruginosa* surface sensing represents a specialization among the genetically homogenous population into subpopulations of non-motile, early polysaccharide producers and motile, surface-exploratory cells, both of which contribute to downstream biofilm maturation.

SESSION 2M

FUNDAMENTAL IMMUNE MECHANISMS

Session Moderator: Alanna Ruddell, Comparative Medicine

MGH 287

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Inhibition of *Staphylococcus aureus* Protein A by Nitric Oxide

Helen Ivory (Helen) Warheit Niemi, Senior, Microbiology
UW Honors Program
Mentor: Ferric Fang, Laboratory Medicine & Microbiology
Mentor: Rodolfo Urbano, Microbiology

Nitric oxide (NO) is a reactive radical produced by cells of the innate immune system. NO reversibly binds to protein cysteine residues through a process called S-nitrosylation, often resulting in reduced protein function. This antimicrobial molecule serves as an important defense mechanism used by the immune system to fight off infections by many different pathogens, including the Gram positive bacterium *Staphylococcus aureus*. *S. aureus* is a common opportunistic pathogen and a major cause of wound infections, food poisoning, pneumonia, and invasive disease. Its ability to cause acute and chronic infections throughout the body is the result of numerous virulence factors. While many of these virulence factors have been extensively studied, little is known about how *S. aureus* pathogenesis is affected by its interaction with NO. We show that NO inhibits the expression of protein A, an extracellular IgG binding protein that is a key immune evasion factor in Staphylococcal disease. Reverse transcriptase quantitative PCR (RT-qPCR) shows that *S. aureus* cultures treated with NO exhibit dose-dependent inhibition of protein A transcription with increasing concentrations of NO. Western blots further confirm that transcription inhibition results in reduced protein A levels. Using allelic exchange mutagenesis, we determined that inhibition is likely due to NO targeting XdrA, a transcription activator of the *spa* gene encoding protein A. Using the Biotin-switch method, we have shown that XdrA is directly modified by NO and structural analysis of the protein suggests that NO modifications could detrimentally affect its ability to bind the *spa* promoter as it does when exogenous NO is not present. These findings introduce a novel mecha-

nism by which the innate immune system could compromise the ability of *S. aureus* to evade immune detection during infection through NO inhibition of protein A.

SESSION 2M

FUNDAMENTAL IMMUNE MECHANISMS

Session Moderator: Alanna Ruddell, Comparative Medicine
MGH 287
3:30 PM to 5:15 PM
* Note: Titles in order of presentation.

The New Pattern Recognition Receptor RECON Acts as Part of the Intestinal Immune System
Alexie Anne Carletti, Senior, Biology (Molecular, Cellular & Developmental)
Mary Gates Scholar, Innovations in Pain Research Scholar
Mentor: Joshua Woodward, Microbiology
Mentor: Adelle McFarland, Molecular and Cellular Biology/Microbiology

We have recently identified a new cytosolic sensor for bacterial cyclic dinucleotides, the aldo-keto reductase RECON. RECON plays a critical role in surveying the host cytosol for intracellular bacterial pathogens, orchestrating the innate immune response to those pathogens and aiding in bacterial clearance. In the host, RECON is most highly expressed in the small intestinal epithelium, enterocytes and M cells. Its high expression at these important mucosal immune sites raises the question as to whether RECON is part of the intestinal immune system. We have recently made a RECON knockout (KO) mouse and found significant alterations in intestinal inflammation and commensal abundance, particularly an increase in segmented filamentous bacteria (SFB). Using quantitative real-time PCR copy number analyses of SFB 16S gRNA, we detected high SFB burden in the RECON KO mice while SFB was undetectable in wild-type mice. SFB have been shown to impact the development of the immune system, particularly T cell populations, in rodents by colonizing and attaching to the follicle-associated epithelium of the small intestines. We predict that the high abundance of SFB in RECON KO mice may either be the result of immunosuppression due to the presence of too many T regulatory cells or will associate with the increased presence of proinflammatory T helper type 17 cells. Current work is aimed at establishing whether there are changes in these T helper cell types in the intestine, as well as looking at the broader dysbiosis by 16S deep sequencing. This project will establish which immune axes are dysregulated in the absence of RECON and will direct future mechanistic investigations into how loss of RECON enzymatic activity is driving immune dysregulation.

POSTER SESSION 3

Balcony, Easel 107
2:30 PM to 4:00 PM

Loss of the Type VI Secretion System from a Human Gut Bacterium via Experimental Evolution
Georgie Lynn (Georgie) Mullen, Sophomore, Pre-Health Sciences
Mentor: Joseph Mougous, Microbiology
Mentor: Benjamin Ross, Microbiology

Human-associated bacteria are important for human health, but little is known about how these bacteria interact with each other. *Bacteroides fragilis (B. fragilis)* is a Gram-negative bacteria that is found in the human gut and assists in nutrition. *B. fragilis* genomes encode the type VI secretion system (T6SS), a needle-like structure that transports toxins into nearby competitor cells. T6SS is used to gain advantage over competitors, yet our lab has observed that *B. fragilis* strains in adults are four times more likely to lack T6SS than strains in infants, suggesting *B. fragilis* may lose this mechanism over time. Why would T6SS be lost if it is used to gain an advantage over competitors? I hypothesize that *B. fragilis* inactivates T6SS because it is no longer required. To test this, I passaged *B. fragilis* strains with T6SS under competition conditions in which the recipient is either susceptible or immune to the effects of T6SS-delivered toxins. I predicted that recipients with immunity will cause *B. fragilis* to inactivate its T6SS mechanism, while predicting recipients that are susceptible to be killed off by the toxins delivered via T6SS in *B. fragilis*. Competitions proceeded for 24 hours, followed with the selection of the donor strain by addition of antibiotics. After selection of donor strains, each were archived in glycerol for evolutionary documentation. Subsequent passages were commenced with newly selected donors. Every 5 generations, competitions proceeded to evaluate competitive fitness of T6SS; whether or not *B. fragilis* has inactivated its T6SS. Whole genome sequencing revealed the mechanism by which T6SS was inactivated at the level of DNA mutations. By using experimental evolution to study interbacterial interactions within the human gut, we will have a better understanding of the long-term dynamics of the human microbiome and its role in influencing human health.

POSTER SESSION 4

MGH 206, Easel 168
4:00 PM to 6:00 PM

Microbiome Analysis of Alder Creek and Fry Creek
Samantha Richardson, Sophomore, Biology, Grays Harbor Coll
Mentor: Amanda Lyn Gunn, Science and Math Division, Grays Harbor College

Identifying the bacteria that inhabit a stream is important to determine the overall health of the stream and its inhabitants. Previous studies have stated *Proteobacteria, Bacteriodetes* and *Firmicutes* to be the dominant phyla found in water microbiomes, while *Flavobacterium*, a microbe responsible for many salmon diseases, is the dominant genus. For this project, we performed a microbial analysis of Alder and Fry Creek through biochemical assays. 8 water samples, each from different sites, were plated onto Mannitol and MacConkey plates to determine the relative proportions of fecal coliforms within the aerobic bacterial population. Additionally, the Alder Creek samples were characterized through 16S sequencing. Our findings are in agreement with previous studies, with the exception of two sites, which were located at the intersection of residential areas and natural habitat. These sites were consistent with the major phyla expectation, except their major genus were *Arcobacter* and *Crenothrix*. *Arcobacter* species are typically found in sewage and some are human pathogens. Infections from some *Arcobacter* species are similar to a *Campylobacter jejuni* infection. The *Crenothrix* genus contains iron precipitating species that have been known to form deposits that clog or stain pipes and make water look unappealing. These findings contribute to our understanding of the health of the water, specifically related to fish bearing streams running through residential and industrial regions. This project will continue as a three-year study to monitor changes in the microbiome during the course of stream restoration.

POSTER SESSION 4

Balcony, Easel 110

4:00 PM to 6:00 PM

Purification of the YjbH Protein in *Listeria monocytogenes*

Emma Bueren, Senior, English, Microbiology
Mentor: Michelle Reniere, Microbiology, UW Medicine at SLU
Mentor: Brittany Ruhland, Microbiology

Listeria monocytogenes is the causative agent of listeriosis, an infection which causes flu-like symptoms in most individuals after ingesting contaminated food, but can severely harm immunocompromised individuals, newborn children, and the fetus of an infected pregnant woman. *L. monocytogenes* is a facultative intracellular pathogen which escapes a phagocytic vacuole early in its life cycle. Once in the cytosol of the enterocyte, the master virulence regulator PrfA activates virulence factor production. However, what initiates PrfA and allows *L. monocytogenes* to sense that it is in the cytosolic environment is still not understood. Recent discoveries have indicated that the translation of at least one of the nine virulence genes, *actA*, requires several genes involved in bacterial redox regulation, including the protein YjbH. In the *Bacillus subtilis* model, a homologous YjbH interacts with the protein Spx, an important regulator of thiol-specific oxidative stress. Under normal conditions, YjbH binds to Spx and facilitates its degradation via the ClpXP protease. During disulfide stress, YjbH aggregates, freeing Spx to regulate oxidative stress response genes. Although the *B. subtilis* and *L. monocytogenes* YjbH are homologs, the function of the *L. monocytogenes* YjbH is uncharacterized. My research project seeks to extract and purify the protein. I first generated two strains of *E. coli* that express a tagged version of either the wild type YjbH or a mutant YjbH which lacks two cysteines that may be involved in its interaction with Spx. The second half of my project will focus on limiting the aggregation of recombinant YjbH. After successful purification of the proteins, these two strains will be a useful tool in characterizing the function of *L. monocytogenes* YjbH, and its role in the virulence pathway. Understanding the role of YjbH in the virulence pathway could ultimately lead to novel ways to inhibit the pathogenicity of *L. monocytogenes.*

POSTER SESSION 4

MGH 206, Easel 176

4:00 PM to 6:00 PM

The Role of Translesion Synthesis Polymerases at Replication-Transcription Conflicts

Chris Hsu, Junior, Biochemistry
Mentor: Houra Merrikh, Microbiology

Since DNA replication and transcription are concurrent in most bacteria, collisions (or conflicts) occur between these two processes. Conflicts have deleterious effects on cells, including double strand breaks and mutagenesis, especially when a gene is transcribed head-on to replication. Because conflicts increase mutagenesis in specifically genes that are oriented head-on to replication, this process may be a conserved strategy for targeted evolution. Consistent with this, a bioinformatics analysis determined that many genes in the head-on orientation are involved in stress response. We previously showed in B. subtilis that the error prone translesion synthesis (TLS) polymerase YqjH is required for the asymmetric mutagenesis of genes in the two orientations and that transcription-coupled nucleotide excision repair (TC-NER) is also involved in this repair pathway. Mutation rate (or reversion assays) and survival assay experiments performed under DNA damaging conditions, however, indicate that a different TLS polymerase, YqjW may be responsible for mutational asymmetry, and that YqjW functions in the same pathway as TC-NER. Thus, YqjW may facilitate targeted evolution under DNA damage conditions.

POSTER SESSION 4

MGH 241, Easel 131

4:00 PM to 6:00 PM

Interaction of Quorum Sensing Systems in Environmental Strains of *P. aeruginosa*

Jose Enrique (Jose) Verdezoto Mosquera, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Ajai Dandekar, Medicine

Quorum Sensing (QS) is a chemical signal based communication system by which bacteria regulate gene expression according to changes in population density. This communication mechanism is used by the environmental bacterium and opportunistic human pathogen *P. aeruginosa*, which is a major cause of Cystic Fibrosis (CF) lung infections. *P. aeruginosa* employs two interconnected QS systems: *las* and *rhl*. These systems are regulated by the transcription factors LasR and RhlR, each of which binds a unique chemical signal that further activates transcriptional regulation. LasR and RhlR together control the expression of virulence factors critical for many infection processes. In laboratory strains, the LasR and RhlR QS systems are arranged hierarchically, such that LasR transcriptionally activates RhlR. Although this QS hierarchy is common to laboratory strains of *P. aeruginosa*, some CF clinical isolates are capable of RhlR activation in the absence of functional LasR. However, the origin of this LasR-independent regulation remains unclear. In this study, we investigate whether the LasR–RhlR hierarchy is conserved in environmental *P. aeruginosa*. We collected several environmental isolates and genetically engineered them through allelic exchange mutagenesis to disrupt LasR function. We then transformed wild type and LasR-null isolates with reporter constructs that produce GFP (Green Fluorescent Protein) in response to either LasR or RhlR. In these two conditions, we measured protein levels of LasR/RhlR, signal concentrations and the production of several QS-regulated products. In most cases, we observed that RhlR activity was substantially reduced in the absence of functional LasR. The observed decrease in RhlR function is so far consistent with the idea that the QS hierarchy is a conserved feature of *P. aeruginosa*. However, mechanisms of quorum regulation in environmental strains may differ from laboratory strains and a proper understanding of them can potentially contribute to the development of non-antibiotic therapies.