Microbiology, Genetics, & Immunology

Undergraduate Research Showcase 2024

Abstract Booklet

Table of Contents:

1) Analysis of cardiolipin synthases' role in Bacteroides fragilis resistance to increased osmolarity.

Timothy Baldwin, Matthew Schnizlein, Aretha Fiebig, Sean Crosson
Department of Microbiology, Genetics, & Immunology. Michigan State University, East Lansing, USA

Cardiolipins play a major role in the regulation of inner membrane function in the bacteria *Bacteroides fragilis*. *B. fragilis* is a Gram-negative, obligate anaerobe, that colonizes the human large intestine. The synthesis of cardiolipins, catalyzed by cardiolipin synthases, in the inner membrane, contributes to cell membrane fluidity, and is vital in minimizing ion leakage across gut bacterial membranes. I hypothesize that increases in osmolarity will increase the level of cardiolipin synthase activity to resist osmotic stress.

Growth curve analysis was performed using *B.fragilis* wildtype(WT), *ΔclsA, ΔclsB*, and *ΔclsAΔclsB*. Growth of each strain was compared across conditions with four sodium concentrations (i.e.,1.7%,1.3%,0.9%,0.5%) and a growth medium control. Optical density at 600 nm was measured every 10 minutes over a 24-hour period using a microplate reader. *cls* gene expression was measured using RT- qPCR in WT, *ΔclsA*, and *ΔclsB* strains exposed to 1.3% sodium for 20 minutes.

Growth curve analysis showed OD600 values of 0.35A for the *ΔclsB* strain at 10 hours in late log phase, compared to WT measured at 0.48A in 1.3% sodium. *ΔclsA* strain phase measured at 0.25A compared to WT *ΔclsAΔclsB* levels measured at 0.17A and was in an early log phase compared to WT. qPCR analysis showed increased expression of *ΔclsA* by 2-fold in WT sodium treated samples compared to untreated.

Increasing osmolarity negatively affects *B. fragilis* growth. When exposed to high solute concentrations in the environment, internal turgor pressure increases. This puts pressure on the membrane lipids, ands pushes the cell to the point of lysis. Cardiolipin synthase activity increases to produce more cardiolipins to stabilize the membrane and prevent lysis. This increased activity utilizes more ATP for production of cardiolipin synthases and cardiolipins. This increase in activity would slow cell division and lead to longer lag phases and decreased concentrations of growth. Further qPCR analysis may show how important cardiolipin synthase activity is to the survival of *B. fragilis*, and cardiolipin contribution to membrane function.

2)

Achala Bannur, Lee Kroos

Intramembrane proteases (IPs) are proteins that are found within cell membranes and can cut other proteins located in the same membrane or near its surface. IPs play critical roles in various signalling pathways and protein degradation processes in different organisms. Each family has specific functions and targets in the cell. Metallo IPs, like SpoIVFB, activate transcription factors in all three domains of life (bacteria, archaea, and eukaryotes). They are involved in processes such as cholesterol homeostasis, stress responses, viral infection in mammals, chloroplast development in plants, and fungal virulence. For example, SpoIVFB is crucial for the formation of endospores in bacteria like *Bacillus subtilis*.

The inhibition mechanism of SpoIVFB by BofA and SpoIVFA is different from the regulation of other IPs. In this case, the second transmembrane segment of BofA occupies the active site of SpoIVFB, blocking access to its substrate, Pro-σ^K. This unique inhibition mechanism provides

valuable insights for potential strategies to design therapeutic IP inhibitors that could have broad applications in various organisms.

We made changes in SpoIVFA (F132C) and SpoIVFB (F66C) to test if we could see any disulfide cross-linking between them. However, we did not find evidence to support this hypothesis.

Now, we are working toward testing the model-based hypothesis that SpoIVFA P129 and/or L130 are near SpoIVFB L23, T27, and/or H29, using a disulfide cross-linking approach. We also plan to test whether SpoIVFA A131 and/or F132 are near SpoIVFB S183.

3) Visualization of MHC-I degradation via the Autophagy Pathway in HPV+ Head and Neck Cancer

Prerna Chahal, Lexi Vu, Mohamed Khalil, Dohun Pyeon
Department of Microbiology, Genetics, & Immunology

Human papillomavirus (HPV) is the causative agent of approximately 25% of head and neck cancers (HNCs). Immune-recognition of HPV-positive (HPV+) tumor cells is critical for preventing progression of HPV+ HNC. An important component of the immune system that is specifically involved in immune-recognition is major histocompatibility complex class I (MHC-I), found on all nucleated cells in the human body. MHC-I is downregulated in HPV+ HNCs, but the mechanism has yet to be elucidated. Our lab has shown that the HPV oncoproteins, E6 and E7, upregulate MARCHF8, an E3 ubiquitin ligase that ubiquitinates surface immune receptors including MHC-I. Following ubiquitination, most proteins are degraded via the proteasome. Interestingly, treatment of HPV+ HNC cells with inhibitors against the major protein degradation pathways in the cell identified not the proteasome, but autophagy as the major degradation pathway of MHC-I. Therefore, we hypothesize that the upregulation of MARCHF8 by HPV directs MHC-I for degradation via autophagy. To test this hypothesis, we evaluated the co-localization of MHC-I with subcellular organelles involved in the autophagy pathway in HPV+ HNC cells, with and without MARCHF8, using confocal microscopy. Following MARCHF8 knockdown, co-localization of MHC-I with the endoplasmic reticulum increased, while co-localization with the autophagosomes decreased. Our results suggest that MARCHF8 ubiquitinates MHC-I to be localized to the autophagosome, leading to decreased cell-surface expression of MHC-I in HPV+ HNCs.

4) Investigating the Role of Histone Tri-methylation Markers in the Efficiency of Cloning through H3K9me3 Characterization in Bovine and Zebrafish Somatic Fibroblasts

Veona Cutinho, Fermin Jiminez, Jose Cibelli
Department of Animal Science and Department of Large Animal Clinical Sciences, College of Natural Science

Somatic Cell Nuclear Transfer (SCNT), also commonly known as cloning, is a technique that allows the transformation of a differentiated somatic cell into an undifferentiated zygote. Theoretically, the manipulated zygote has the potential to develop into a fully functioning, normal organism (clone) within the conducive environment of an enucleated oocyte. However, a somatic cell acquires epigenetic modifications as it progresses in differentiation which results in variation in its gene expression compared to a naturally fertilized zygote. These differences can impediment the development of a clone, decreasing the efficiency of successful nuclear

transfer. Previous studies have shown that persistent H3K9me3 in the nucleus of the somatic cells prevents zygotic genome activation during SCNT, thus being an epigenetic barrier to cloning. We hypothesise that the transfer of genetic material from somatic cells with lower H3K9me3 expression will yield better embryonic progression of the reprogrammed clone. In this study, H3K9me3 expression will be characterised in bovine somatic cells and 24 hours post fertilization zebrafish embryos. Cells with low expression of H3K9me3 will be further phenotyped using whole embryo immunocytochemistry. Understanding the abundance and localisation of cells bearing the lowest levels of H3K9me3 will facilitate their live isolation using flow cytometry. Finally, these isolated cells will be tested for their efficiency as donors for SCNT.

5) Generating an Induced Pluripotent Stem Cell Line to Follow Heart Chamber-specific Cardiomyocyte Differentiation in Human Heart Organoid Model.

Veona Cutinho, Aleksandra Kostina, Aitor Aguirre
Department of Biomedical Engineering, College of Engineering

Congenital heart disease (CHD) is a leading cause of morbidity and mortality in developed countries. Hypoplastic Left Heart Syndrome (HLHS) is a life-threatening condition, and yet its etiology and possible risk factors remain elusive. Ondansetron is an FDA-approved drug commonly prescribed to pregnant women to treat symptoms of vomiting and nausea during the first trimester of gestation. Epidemiological studies suggest a possible risk of CHD in embryos from mothers who have been prescribed ondansetron. This study aims to create a system for studying the effect of ondansetron on early cardiac development, particularly on the fate of First Heart Field (FHF)-derivatives mainly contributing to left ventricle structures. A pluripotent stem cell line containing the sequence encoding the reporter fluorescence protein surrounded by loxP sites and expressing Cre recombinase under the promotor of the *HAND1* gene – the primary marker of FHF – will be created. This genetically engineered cell line will be used to differentiate heart organoids that recapitulate the main aspects of early cardiogenesis. Life imaging techniques will be used to track FHF-derived cardiomyocytes under exposure of ondansetron. In addition, a stem cell line with the expression of a reporter gene under the control of a proliferative marker will be developed to examine the expansion of embryonic cardiac ventricles in the presence of ondansetron. We will perform lentiviral transduction, clonal selection, and expansion of genetically modified pluripotent stem cell lines.

6) Characterizing Ancestral Ethylene Forming Enzyme

Bryce Delaney, Dr. Shramana Chatterjee, Dr. Robert Hausinger
Microbiology, Genetics, & Immunology

The ethylene-forming enzyme (EFE) is a member of the mononuclear non-heme Fe(II)- and 2-oxoglutarate (2OG)-dependent oxygenase superfamily. EFE can convert 2OG into ethylene and three CO_2. EFE can also hydroxylate L-arginine. Ethylene is one of the most used hydrocarbons commercially. It is used as the feedstock for many polymers and chemicals such as plastic packaging, anti-freeze, and synthetic rubber. Ethylene is commonly derived from a process known as thermal cracking which uses natural gases and leads to a high output of CO_2 into the atmosphere. The use of EFE to produce ethylene through biological synthesis provides a more environmentally friendly alternative for production. The scope of this project encompasses the characterization of the gene product from the *in vitro* recombination synthesis of a computationally generated gene encoding an ancestor of EFE. The primary objective is to map the ancestry of EFE to understand the evolutionary dynamics of ethylene production and L arginine hydroxylation. Implementation involves inserting a pre-synthesized target gene into the

pET28a vector. Subsequently, the gene of interest will undergo overexpression within *Escherichia coli.* The ancestral EFE will be purified by using sonication to disrupt the cells, centrifugation to remove membranes and cell debris, and column chromatography. The enzyme will be characterized for its ability to generate ethylene by using a gas chromatography assay and for its L-Arg hydroxylation activity by a colorimetric assay for the product. The relative activities will provide a comprehensive understanding of the functional properties associated with the ancestral EFE.

7) Impacts of Cell Culturing Methods on Intracellular Metal Ion Fluctuations

Samantha Durrant, Bong Jin Hong, Thomas V. O'Halloran
Department of Chemistry and Department of Microbiology, Genetics, & Immunology at Michigan State University

Maintaining intracellular metal ion homeostasis plays a pivotal role in function and signal transduction, regulating the stability of a cell. In cell culturing conditions, there are numerous factors that play a role in maintaining stability, overall contributing to the metal ion homeostasis within the cell. Fluctuations in such conditions can be highlighted through a number of factors directly related to the technique of cell culturing, stemming from media composition, varying cell densities, chemical makeup of such washing stages, and perturbations in the cellular membrane via cell scraping can all be quantified using ICP-QQQ-MS, a type of mass spectrometry used to measure and quantify intracellular elemental composition. Elucidating the roles played between intracellular metal ion homeostasis and cell culturing methods is an essential quality for performing further cell-based research, paired with an understanding of the roles such metal ions play within the cell. Emphasizing the cell culturing conditions to replicate intracellular conditions can facilitate learning of the true homeostatic mechanisms and give rise to the elements present within such processes. This study outlines the impact of cell culturing conditions and how they translate to intracellular metal ion homeostasis, leading to an understanding of how perturbations to the cell through culturing conditions can be applied to future experimental projects, investigating intracellular metal ion fluctuations and their impact on the behavior of the cell.

8) Autoimmune Regulator (Aire) Deficiency in Mice Causes Immune Cell Infiltration into the Nasal Cavity

Joey Esparza[1], Soo Hyun Ahn[2], Jack Harkema [2], Margaret G. Petroff [1 2 3]
Microbiology, Genetics, & Immunology, College of Natural Science[1]; Pathobiology & Diagnostic Investigation, College of Veterinary Medicine[2]; Cell and Molecular Biology Program, College of Natural Science[3]; Michigan State University, East Lansing, Michigan

Immune tolerance to self is critical for eliminating self-reactive T cells that could cause autoimmune disease. AIRE (autoimmune regulator) is a transcription factor that induces expression of tissue-restricted antigens in the thymus, which in turn causes deletion of self-antigen specific T cells. In humans, mutations of *AIRE* cause autoimmune polyglandular syndrome type 1(APS-1), a multi-organ disease characterized, in part, by infertility. Our mouse model of *Aire* deficiency (Aire$^{-/-}$ mice) recapitulates infertility, and others have shown that *Aire*$^{-/-}$ mice target vomeronasal organ (VNO)-specific proteins. Since the VNO and its associated glands play key roles in pheromone detection and mating, we hypothesized that Aire$^{-/-}$ mice possess immune-associated VNO abnormalities. To test this, we examined the nasal passage histology of 20-week-old wild-type and Aire$^{-/-}$ mice (n=3 each). All three Aire$^{-/-}$ mice exhibited dramatic lesions of the nasal passages, including lymphocyte infiltration and thickening of the respiratory epithelium. The nasal septa contained prominent lymphoid aggregates and

extensive loss of glandular tissue. Using immunohistochemistry, we found that control mice showed scattered CD4+ and CD8+ T cells within the mucosa, and numerous T cells in the nasal-associated lymphoid tissue (NALT). In Aire$^{-/-}$ mice, numerous CD4+ and CD8+ T cells populated the airway mucosa, with concentrated aggregates in the septum in place of glandular tissue. CD4+ and CD8+ T cells were also found in tissue surrounding the VNO. Finally, the NALT appeared organized into discreet CD4+-rich mantle zones. These results suggest immune intolerance to the VNO, which may modify mating behavior and contribute to infertility.

9) Does Genetic Deletion of *Autoimmune Regulator* Influence Mouse Mating Behavior?

Katrina T. Halgren[1,4], Jack R. Harkema[2], Alfred J. Robison[3], Margaret G. Petroff[2,4], Soo Hyun Ahn[2]
[1]Lyman Briggs College; [2]Pathobiology and Diagnostic Investigation, College of Veterinary Medicine; [3]Department of Physiology, Neuroscience Program, College of Natural Science; [4]Microbiology, Genetics and Immunology, College of Natural Science

Autoimmune regulator (Aire) is a transcription factor expressed in the thymus responsible for generating immune self-tolerance. Deletion of *Aire* causes autoimmune disease and can result in infertility. The vomeronasal organ (VNO) is necessary for pheromone sensing in rodents, which drives mouse mating behavior. We showed that VNO and its surrounding glands are targeted by autoreactive immune cells in *Aire*-deficient (Aire$^{-/-}$) mice. Further, Aire$^{-/-}$ males are severely sub-fertile compared to their wild-type counter parts. Based on these results, we hypothesized that Aire$^{-/-}$ males may be infertile due to their inability to sense female pheromones, resulting in lack of copulatory behavior. To determine if the mating behavior of male Aire$^{-/-}$ mice was significantly different to *Aire*-wildtype (Aire$^{+/+}$) mice, we used the Behavioral Observation Research Interactive Software (BORIS) program to code the behavior of Aire$^{-/-}$ and Aire$^{+/+}$ mice when introduced to female mice in estrus. The male and female mice were recorded for one hour, and then BORIS was used to observe and code sexual behaviors such as anogenital investigation, mounting, intromission, and ejaculation, and non-sexual behaviors such as contact, non-contact, and aggression. The preliminary results of this experiment are not significant due to a small sample size (n=3), but there do appear to be qualitative differences between the behavior of Aire$^{-/-}$ and Aire$^{+/+}$ male mice. In future experiments, we will use a greater number of mice to determine if there are significant differences between the mating behavior of Aire$^{-/-}$ and Aire$^{+/+}$ mice, which may reveal mechanisms of infertility in Aire deficient mice.

10) Investigating Biologically Relevant Metals in *Caenorhabditis elegans*

Kyleen Hall[1,2], Dr. Aaron Sue[1,2], Dr. Thomas V. O'Halloran[1-3]
Michigan State University[1], Department of Microbiology, Genetics, and Immunology[2], Department of Chemistry[3]

The metallome, or the concentrations and distributions of metal ions, plays important roles in key cellular decisions. Understanding how the metallome of an organism changes during various biological processes, and in response to certain environmental cues, contributes to our understanding of biological processes such as reproduction, disease, toxicity, aging, and more. To study the metallome, we employ the use of two instruments capable of sensitive elemental analysis: an inductively coupled plasma triple quadrupole mass spectrometer (ICP-QQQ-MS) and a laser ablation inductively coupled plasma time of flight mass spectrometer (LA-ICP-TOF-MS). The nematode *Caenorhabditis elegans* presents as an ideal model system to study the metallome using these instruments due to its ease of maintenance, small size, genetic

tractability, and many human orthologs. Here, we used *C. elegans* to investigate how the metallome changes in response to overexposure to manganese and iron, two necessary elements that can be toxic at high concentrations. Two C. elegans strains were examined: the N2 (wild-type) and IG6 (*smf-1* metal transporter deletion) strains. Using the ICP-QQQ-MS and LA-ICP-TOF-MS, we observed how the abundance and distribution of biologically relevant metals changed within the worms to gain a better understanding of the mechanism of toxicity of iron and manganese. By more clearly elucidating the mechanism of toxicity of these elements, we hope to shed light on the health consequences of elemental overexposure.

11) Somatic Hypermutation in CH12-F3 Cells and the Role of Bcl6

Kaitlyn Hutchins, Dr. Li Han, Dr. Kefei Yu
Microbiology, Genetics, & Immunology

Although widely used as a model for class switch recombination (CSR), CH12-F3 cells (mouse B cells) are unable to undergo somatic hypermutation (SHM), which is an essential process for optimizing antibody affinity by mutating the sequences encoding the antigen-binding pocket. CH12 cells can be induced to express AID (activation-induced cytidine deaminase), which is the master gene that initiates CSR and SHM. The reason for the inability of CH12 to undergo SHM is unclear. Our previous mRNAseq data revealed that CH12 cells do not express Bcl6 gene, which is an essential regulator of the germinal center where SHM takes place. I will test the hypothesis that restored expression of the Bcl6 gene in CH12 will restore SHM. If so, it will open up additional research opportunities to figuring out what Bcl6 downstream factors contribute to the SHM process. This will lead to novel insight into the differential regulation of SHM and CSR, which are both relying on AID-initiated DNA lesions. We have established a reporter cell line where SHM can be monitored by loss of GFP expression. My work thus far has included cloning and transfection of Bcl6 expression vectors into CH12 cells and confirming Bcl6 transgene expression. High throughput next-generation sequencing will be performed to study the detailed analysis of V region SHM in CH12 cells.

12) Investigating Regulatory Mechanisms of AvcID Phage Defense Systems

Muethel, Aubree C. (*) [1]; Ferrell, Micah J. [1]; Waters, Christopher M. [1]
[1] Department of Microbiology, Genetics, & Immunology, Michigan State University, East Lansing, MI, 48824

AvcID is a novel type III toxin-antitoxin system encoded on the VSP-1 island of *Vibrio cholerae* that functions as an anti-phage defense system with homologous systems found in many medically important bacteria. AvcD (Antiviral Cytidine Deaminase) is an enzyme that deaminates deoxycytidine, effectively starving phages of nucleotides necessary for replication. There are two domains present in AvcD, a C-terminal deoxycytidylate deaminase (DCD) domain which deaminates dCTP and dCMP and an N-terminal P-loop NTPase (PLN) domain of unknown function. Purified AvcD was inhibited by high concentrations of ATP and AvcD overexpressed in bacterial cells was not active in growth inhibition until the stationary phase, when ATP concentrations decline. We hypothesize that AvcD deamination by the DCD domain is regulated by the cell's energy state via ATP binding to the PLN domain. To test this, we have evaluated mutations in a putative nucleotide binding pocket of the PLN domain for enzymatic activity, deoxycytidine starvation, and phage defense. Evaluating PLN domain mutants will elucidate how AvcID toxin-antitoxin systems function in a cellular context to confer anti-phage defense. Understanding regulation of phage defense systems like AvcID is key to future deployment of effective phage therapy as an alternative to antibiotics.

13) Using spotted gar as the outgroup to understand nervous system evolution after whole genome duplication in teleost fishes

Grace Urban (Microbiology, Genetics, and Immunology Department), Jamily Lorena (Department of Integrative Biology), Ingo Braasch (Department of Integrative Biology)

A whole-genome duplication (WGD) event leads to a full duplication or polyploidization of an organism's genome. Throughout evolution, the bony vertebrate lineage has undergone two WGDs. Additionally, lineage-specific WGDs are also observed such as in the ancestor of the teleost fishes (Teleost Genome Duplication, TGD), the most species-rich group of vertebrates. WGD-derived extra copies of genetic elements have been proposed to provide the raw material that can seed evolutionary and developmental innovations, adaptation, and biodiversification, for example in the vertebrate nervous and sensory systems. Genome-wide, around 80% of the extra gene copies from the TGD have been lost secondarily (non-functionalization) in teleosts during their rediploidization process. However, for those genes that have been retained as duplicates, regulatory changes or coding mutations may generate complementary expression patterns and/or protein functions among duplicates (sub-functionalization); and/or gene duplicates may acquire new expression patterns and/or protein functions (neo-functionalization). Yet, the extend of sub- and neofunctionalization following the TGD for genes involved in the nervous systems remains poorly understood. Therefore, my research training will aim to use the closest living outgroup of the teleosts, the non-teleost fish spotted gar (Lepisosteus oculatus) as an outgroup for comparative analysis of gene expression to the teleost model organism zebrafish (Danio rerio). By classifying gar embryos and larvae into developmental stages and preparing brain samples for gene expression assays through sectioning and RNA in situ hybridization approaches, I aim to identify potential cases of sub functionalization and neofunctionalization of TGD-duplicated genes in the zebrafish brain using expression patterns of the single gene in spotted gar as a proxy for the ancestor of teleosts.

Thank you to all the presenters and mentors that participated in this showcase, and to Dr. Vic DiRita and the MGI department for their continuing support!

Best wishes to the graduating seniors for a bright future!