MMG Undergraduate Research Showcase

Tuesday, April 10, 2018
12:30pm - 2:30pm
BPS North Atrium
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1. Migratory ability of human trophoblast cells exposed to bisphenol S
   Tori Adomshick, Yong Pu, and Almudena Veiga-Lopez
   Department of Animal Science, Michigan State University, East Lansing, MI

During a healthy pregnancy, first trimester placental (trophoblast) cells implant in the maternal endometrium to enable embryonic development. The migratory ability of these trophoblast cells is of great importance because improper invasion of the endometrium can lead to pregnancy complications such as a miscarriage or preeclampsia. Previous studies have shown endocrine disrupting chemicals (EDCs) can impair trophoblast cell function. Recent studies have demonstrated that gestational exposure to bisphenol S (BPS) can impair endocrine placental function and reduce trophoblast cell number. The goal of this study is to investigate BPS’s effect on the migratory ability of first trimester human trophoblast (HTR8/SV_{neo}) cells. Because BPS has been shown to increase intercellular communication, it is hypothesized that BPS will enhance the migratory ability of HTR8 cells. To test this hypothesis, a scratch assay was performed on the HTR8 cells. The HTR8 cells were exposed to BPS (0, 10, 200, and 1000 ng/mL) for five days before, followed by the scratch migration assay. Images were captured after 24 hours and 48 hours. Preliminary results suggest that BPS increases the migratory ability of HTR8 cells. To further assess the migratory ability of HTR8 cells treated with BPS, additional replicates will be performed, as well as gene expression studies to identify migratory markers such as vimentin, E-cadherin, and N-cadherin. An increase in the expression of these markers would indicate that exposure to BPS during pregnancy could have the ability to negatively affect trophoblast placentation and fetal development.

2. Rare damaging missense mutations in IRF6 found in sequence control populations
   Kyleigh Buckley and Brian C. Schutte
   Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI

One of the most common birth defects is cleft lip and palate, and DNA variants in the IRF6 gene account for 12% of clefts worldwide. However, not all DNA variants in IRF6 lead to cleft. We hypothesize that rare IRF6 DNA variants found in cleft patients are pathogenic for disease and will not be found in sequence control populations. To test this hypothesis, we compiled three lists of DNA variants in IRF6: from cleft, from gnomAD, a sequence database from 122,000 individuals, and from an evolutionary data set. We used predictive algorithms that assessed the pathogenicity of each DNA variant and assigned a score. We found 161, 123, and 535 missense DNA variants in IRF6 from the patient, gnomAD, and evolutionary dataset, respectively. As expected, variants from the cleft list were more likely to be damaging (OR = 29.4; 95% CI = 15 to 56; p < 0.00015). However, contrary to our hypothesis, we found 8 in both the patient and gnomAD lists and 2 in both the patient and evolutionary lists. In gnomAD, 3 were found in 1 individual, and 5 were found in multiple samples. Overall, our data support our hypothesis, but a small but significant subset of variants was found in the sequence control lists. Further studies are needed to determine whether the 8 variants shared in the patient and gnomAD lists are non-pathogenic or low-penetrant variants, and whether the 2 variants shared in the patient and the evolution population lists are functional and may contribute to speciation.
3. Comparative Analysis of Hyperalkaliphiles from Serpentinite-influenced Environments

David Chalmers, Lydia Hayes, Kati Ford, and Matthew Schrenk
Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI

Microbial communities in soils are generally high diversity due to micro-niches that shelter organisms from the environment, facilitate cell-cell interactions, and allow access to surface derived nutrients. Serpentinite soils, however, are known to be harsh with low amounts of calcium and nitrogen, high pH, and high concentrations of magnesium and heavy metals. Serpentinite-influenced environments are also used as analogs for understanding soil fertility and habitability on distant planets, such as Mars, and for understanding microbial homeostasis at alkaline pH. Samples from Tablelands Ophiolite in Gros Morne National Park, Newfoundland, Canada were used to test whether microorganisms attached to solids differed from those in liquid sources in serpentinite-influenced environments. Culture-dependent and culture-independent approaches were taken for observing the microbial diversity. For culture-dependent approaches, rock samples were crushed, suspended, and grown on pH 11 plates. Once microbial isolates were obtained through streaking, pH 11 liquid media was inoculated with individual colonies. Pigmentation, size, shape, and ease of growth on solid and liquid media were observed. For culture-independent approaches, DNA was extracted and used to sequence the SSU rRNA gene to aid in taxonomic identification. Taken together, these results will provide insights into microbial diversity and function across distinct niches in a serpentinite-influenced ecosystem.

4. Identifying the Mechanism of Activation of the Phospholipase CapV by cyclic-GMP AMP in Vibrio Cholerae

Alyssa M. Corpus¹, Geoffrey B. Severin², and Christopher M. Waters¹
Departments of Microbiology & Molecular Genetics¹ and Biochemistry & Molecular Biology², Michigan State University, East Lansing, MI

The bacterial pathogen Vibrio cholerae is the causative agent of the diarrheal disease cholera, for which multiple pandemics in the past 200 years have resulted in millions of deaths. The current pandemic (7th) is perpetuated exclusively by strains of the El Tor V. cholerae biotype. One of the greatest genetic differences between this biotype and those responsible for the previous six pandemics is El Tor’s maintenance of the unique genomic island, VSP-1. A novel second messenger signaling network encoded entirely in VSP-1 has recently been characterized. The enzyme DncV synthesizes production of the cyclic dinucleotide (cdN) cGAMP, which activates the phospholipase activity of the enzyme CapV. Ectopic expression of DncV results in rapid degradation of El Tor’s membrane, leading to Cap-V dependent cell death. However, the molecular mechanism by which DncV-derived cGAMP activates CapV has yet to be elucidated. We propose to identify CapV’s cGAMP binding site by utilizing targeted site-directed mutagenesis to generate CapV variants and assessing their activation following DncV expression. Guided by a computationally-derived model of CapV, we have identified two unique residue loops which lie outside of the enzyme active site and are involved in regulating substrate accessibility. The conspicuous location of these loops and the presence of arginine residues, which are common in other cdN binding sites, gives this region high priority for
mutagenesis. Understanding how CapV is regulated by cGAMP will have implications for identifying other cGAMP-dependent enzymes in other bacterium, as DncV and CapV orthologs are frequently found together in other pathogenic bacterium.

5. Identification and Quantification of Antibiotic Resistance Genes from Canadian Geese Feces

Gabrielle Curtis and Poorna Viswanathan
Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI

The aim of this study was to discern whether bacteria found in fecal samples of Canadian Geese are a threat to human health. This animal inhabits areas in or near wetlands and comes in close contact with people. A strain of Escherichia coli was isolated from the feces sample and subject to various tests to determine if it contained the antibiotic resistant genes bla-TEM or tet(w); in addition to this, the gDNA from the feces was analyzed with qPCR to determine the quantity of bacteria in feces with these antibiotic resistant genes. A significant number of bacteria were found to have at least one of the two tested genes. The presence of these genes validates concerns for public safety; however further analysis is needed to determine the threat level presented by the Canadian goose.

6. Validation of FGF-independent Cell Fate Markers of the Primitive Endoderm Lineage

Eli Falk, Tristan Frum, and Amy Ralston
Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI

An important area of research in developmental biology has been to uncover the mechanisms that determine cell fate during embryological development. One such cell-fate determination occurs during the blastocyst stage of early mammalian development, where the inner cell mass (ICM) differentiates into primitive endoderm (PE) and epiblast (EPI) cell lineages. Previous research into the PE and EPI cell fate decision in mouse embryos has led to the model that the FGF-signaling pathway promotes all PE genes and represses EPI genes, and therefore determines whether a cell differentiates into the PE or EPI lineage. However, researchers in the Ralston lab, using RNA sequencing, have identified a set of transcripts that are enriched in PE cells but not regulated by FGF-signaling, suggesting that there exist FGF-independent cues that drive PE cell fate. In this experiment, our goal was to examine putative FGF-independent PE enriched transcripts to validate which, if any, are truly FGF-independent. Using QPCR, we compared the levels of putative FGF-independent PE enriched transcripts between embryos in which FGF-signaling was either stimulated or inhibited. In this presentation, I will summarize our analysis that confirms some PE enriched transcripts are likely FGF-independent. This analysis provides a roadmap to focus efforts in the Ralston Lab to refine the model of this critical cell-fate determination step, which has significant implications for our understanding of embryonic development and stem cell research.
7. **Identifying the Actinobacillus Succinogenes Glucokinase Gene**
   Meghan Grossmann and Claire Vieille
   Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI

Actinobacillus succinogenes is a fermentative bacterium isolated from the cow rumen, and is the best known natural succinate producer. If produced cost-competitively, succinate could replace oil as the feedstock to produce many industrial products. Today the succinate production is not cost effective but with genetic engineering the consumption of one glucose molecule could yield two moles of succinate.

How A. succinogenes uptakes glucose to produce succinate is unknown, as this organism does not have a glucose phosphotransferase system. Our working hypothesis is that A. succinogenes uses an ABC transporter (ATP-dependent system) followed by glucose phosphorylation by glucokinase to glucose-6-phosphate, which then enters glycolysis. No gene annotated as aglucokinase gene is present in the genome sequence, and no sugar kinase genes are upregulated in the transcriptome of glucose-grown cultures. Thus, the goal of my project was to identify what could be a completely new glucokinase gene in Actinobacillus.

My approach was to build an Actinobacillus genomic plasmid library of partially digested Sau3A fragments, and use it to complement an Escherichia coli strain devoid of glucokinase for growth on minimum medium-glucose plates. The only colonies growing on these plates will have a plasmid carrying an Actinobacillus glucokinase-encoding gene. By sequencing the plasmid inserts of these colonies, we hope to identify the A. succinogenes glucokinase gene. In the future, we could overexpress this gene to increase glucose uptake and succinate production. This would result in a cheaper succinate production, and succinate replacing oil in the production of goods.

8. **In Utero vs. in Vitro: The Differences in Embryonic Gene Transcription**
   Hannah Guider, Amy Ralston, and Tristan Frum
   Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI

The preimplantation period of embryonic development makes in vitro fertilization technologies possible. However, the embryo culture conditions used for in vitro fertilization were developed over thirty years ago, long before it was possible to examine embryonic development at the molecular level. During the preimplantation period, embryonic cells undergo many changes in gene expression and transcription factors that are necessary for the healthy development of the embryo. We previously discovered that cultured embryos display defects in the abundance of proteins essential for embryonic development in comparison with embryos developed in utero. In order to further examine the phenotype of cultured embryos by qRT-PCR, we identified a normalization control suitable for the comparison of gene expression levels across multiple developmental stages. We show that, in comparison to RNA-seq data, this normalization control generates more accurate stage-specific patterns of gene expression than the standard normalization controls used for qRT-PCR in the field. By comparing the abundance of
transcripts in embryos developing in culture and in utero, we identified several transcripts that are sensitive to culture conditions. Our results suggest culture induces defects in gene transcription, providing molecular phenotypes to further optimize embryo culture conditions.

9. **Identification and Characterization of Phage in the Environment**

  Madeline Hilton, Sarah Doore, and Kristin Parent
  Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI

Viruses are widely known to cause disease in eukaryotic organisms. Less known is that a subgroup of viruses called bacteriophage infect only bacteria. These ‘bacteriophage’ are highly specific in their selection of host and are found any and everywhere on the planet. Only a small number of these phages have been identified compared to how many are estimated to exist. Here we show that by sampling from different areas in the environment, new samples of phage can be isolated and characterized to expand our knowledge of phage infection and discover phages previously unknown.

10. **Aquaporin Expression in Barley Following Partial Root Excision**

    Jillian G. Howland\(^1\), Wieland Fricke\(^2\), and Philip Strong\(^3\)

    \(^1\)Department of Microbiology and Molecular Genetics, Michigan State University
    \(^2\)School of Biology and Environmental Science at University College Dublin
    \(^3\)Lyman Briggs College, Michigan State University, East Lansing, MI

The purpose of this study was to investigate the changes in aquaporin gene expression when the transpiration pathway of barley, *Hordeum vulgare* L., was interrupted through partial root excision. It presents the essential relationship between leaf transpirational water loss and root aquaporin activity, to maintain water balance within the system of the plant. We hypothesized that with the removal of approximately half of the seminal root, the plant would increase the activity of aquaporins in the remaining root tissue to maintain the same volume of water passing through the plant for transpiration. This concept is assumed based on changes in plant transpiration observed from previous studies in literature. We found that when approximately half of the root system was removed, transpiration did not decrease, but rather increased slightly by an average of 17.10%. This shows that the remaining roots must have taken more than twice as much water per unit root-surface-area (RSA) than before partial root removal. This increase in transpiration could have been achieved through up-regulation in AQP gene expression, which could have led to the increase in AQP activity.

11. **Isolating Mycobacterium Smegmatis Mutants Resistant to a Novel Tuberculosis Antimicrobial**

    Emily Juzwiak, Jacob Baker, and Robert Abramovitch

    Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI
Tuberculosis is caused by the bacterium Mycobacterium tuberculosis (Mtb) which slowly colonizes the acidic environments of macrophages or granulomas. This process is vital to Mtb’s establishment as a chronic infection and its development of drug resistance. My project attempts to understand pH-dependent mechanisms that allow for a productive infection using a non-pathogenic strain related to Mtb, Mycobacterium smegmatis. Our lab performed a high throughput screen to identify novel antimicrobial compounds, such as AC2P017, that produce pH-dependent growth arrest. This mechanism was tested using half-maximal effective concentration (EC50) assays. I generated AC2P017 resistant M. smegmatis mutants and picked twenty-one resistant colonies. Then, I verified each mutant was resistant to pH-dependent growth inhibition using the EC50 assay in duplicate. The mutants that demonstrated significant growth arrest resistance were sent for whole genome sequencing. Analysis of the Illumina results indicated that the M. smegmatis gene 5340 (MSMEG5340) was associated with resistance to AC2P017. Therefore, my project will proceed by complementing this target gene to observe if the introduction of the wild type allele into mutant bacteria will produce susceptibility to the compound or if the addition of the mutant allele into wild type bacteria will produce resistance to AC2P017. Furthermore, functional studies of MSMEG5340 will be utilized to determine how the gene mediates resistance. CRISPR methods will produce a knock-down of MSMEG5340 to test if the resistance phenotype is a loss of function. Finally, Mtb mutants that are resistant to AC2P017 will be isolated and characterized to further elucidate the resistance mechanism.

12. Non-invasive imaging to assess new therapies for targeting solid tumors
Nathan Kauffman and Dr. Kurt Zinn
Department of Radiology, Michigan State University, East Lansing, MI

Radiation therapy is widely used as a both a primary and adjuvant treatment to kill malignant cells in cancer patients. Pretarget radioimmunotherapy (PRT) is new radiation therapy delivery system that involves high affinity peptides and antibodies as well as in vivo conjugation to deliver radiation. The success of this approach has many key components including specific cancer targeting accuracy, therapeutic radionuclide choice, kinetics of uptake, complex affinity, and dose amount. Proteins with different targets and affinities can be chosen for the radionuclide delivery. My project involves studying the movement and uptake of these PRT targeting agents in rodent tumor models. With the use of modern imaging technologies, it is possible to track and quantify molecules of PRT on their journey from the site of entry to point of tumor binding, allowing for determination of proper dose, understanding of specific drug kinetics, and efficiency of retention in tumor and other tissues. Once these are known, different therapeutic radionuclides can be attached to these agents and tested for tumor reduction and side effects on other tissues. This research is important because cancer is unique depending on its location, breadth of dissemination, and the individual it is attacking, so treatments must be just as unique in their therapeutic approach and targeting to properly treat all patients. Therefore, perfecting the key components of a radiotherapy can prove to be significantly beneficial to cancer patients worldwide.
13. Methylotrophs, an Efficient Platform for Critical Metal Recovery
Adam Kibiloski, Zach Jansen, and Cecilia Martinez-Gomez
Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI

The Martinez-Gomez lab studies single carbon metabolism in the α-proteobacterium Methylobacterium extorquens AM1. Lanthanides are considered ‘rare earth elements’ not because they are difficult to find in nature, but because they are biologically unavailable. This is due to the fact that they are commonly found ionically bound to carbonates or phosphates. However, lanthanides are as abundant as copper and zinc. Current lanthanide mining techniques are hazardous both to the workers and the ecosystem. The extraction process requires the use of concentrated acid paired with high temperatures and pressure. Lanthanides typically coexist with radioactive elements such as uranium and thorium. Therefore, waste produced by these mines are both highly radioactive and acidic. These waste products can cause irreversible damage to both the environment and the workers. There are only two mines left open in the world, one in Belgium and one in China. It has been recently shown that M. extorquens AM1 can efficiently sense, solubilize, and store lanthanides. We are engineering M. extorquens AM1 to effectively recover lanthanides from used batteries and speakers to generate a safer and more economically viable technology for lanthanide supply.

14. Effects of combination endocrine treatments on breast cancer cells
Richard Kim, Ramya Erasala, and Susan Conrad
Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI

Many different proteins regulate the balance between cell proliferation, cell metastasis, and cell death. One example is a family of proteins known as Mixed Lineage Kinases (MLKs), which function in intracellular signaling pathways. Previous studies demonstrated that inhibition of MLK activity using the MLK-inhibitor CEP-1347 led to a cell cycle arrest in early mitosis and reduced cell viability in estrogen receptor (ER)-positive breast cancer cells, while not effecting non-tumorigenic cells. This suggested that CEP-1347 has potential as a therapeutic drug to prevent tumor growth. ER-positive breast cancer is currently treated with drugs that target ER, including antiestrogens and aromatase inhibitors, and these drugs block the cell cycle in G1. In the current research, we used the FDA approved antiestrogen ICI 182,780 (clinically known as fulvestrant) and CEP-1347 to test the hypothesis that combination treatments would be more effective than either agent alone to decrease cell proliferation and/or viability. A live/dead experiment was conducted to show the effects of single and combination treatments on cell viability using trypan blue exclusion and colony formation assays. The result of this experiment will provide evidence for or against our hypothesis that a combination of fulvestrant plus CEP-1347 could reveal another method of combating (ER)-positive breast cancer.
15. A new DNA fingerprinting tool for honey bees: 12 multiplexed tetraSTR markers
Brenna Kizer and Patrick Venta
Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI

Conservation efforts for the European honey bee, Apis mellifera, have become increasingly important with the rise of colony collapse disorder. Genomics studies have the potential to elucidate the role of genetics in honey bee behavior and disease resistance, among other traits. Previous studies have documented the use of dinucleotide simple tandem repeat markers (STRs) for DNA fingerprinting of honey bees, but tetranucleotide STRs (tetraSTRs), which have greater potential for studies of mixed DNA samples, have not yet been explored. Our objective was to design a set of 12 independently assorting genetic markers based on tetraSTRs which could be combined in a PCR multiplex for identification of individual honey bees. 12 tetraSTR markers were designed and amplified in genomic DNA extracted from honey bee workers. Products were analyzed for variability using a combination of gel electrophoresis and high-resolution genotyping. Currently, three multiplexes of four markers each have been developed with a mean allelic richness of 4 (range 2-5), a mean observed heterozygosity of 0.50 (range 0.08-0.88), and a mean stutter ratio of 0.065 (range 0.06-0.10). A full 12-marker multiplex will be assembled from these smaller sets to maximize fingerprinting efficiency. These results have implications for simple, cost-effective fingerprinting of honey bee individuals, which will be useful in understanding the role of genetics in the well-being of bee populations.

16. Student conceptions of structure-function relationships in cell membranes
John Knapp, Kamali Sripathi, Kevin Haudek, John Merrill, and Mark Urban-Lurain
Center for Engineering Education Research, Michigan State University, East Lansing, MI

Student understanding of the structure-function relationship is an important core concept for undergraduate biology education as seen in “Vision and Change In Undergraduate Biology Education: A Call to Action” (AAAS, 2011). Structure-function specifically regarding cellular membranes is an important underlying concept for understanding most cellular processes. The goal of this research is to identify the most common student ideas about cellular membranes. Multiple Choice (MC) questions may not reveal the complete thought processes of students. Constructed response (CR) questions offer a more complex view of student thinking than MC questions. The Automated Analysis of Constructed Response (AACR) research group uses machine learning to provide lexical and statistical analysis of student constructed responses that predict expert human scoring. To understand student thinking about structure-function, we are developing a question that asks students to explain why individual phospholipid molecules in a membrane do not flip sides. We collected responses from 777 students from two universities. Consensus scoring between three scorers was done to develop consistent scoring and to uncover other emergent student conceptions that may be added to the scoring rubric. The most prevalent concepts about why membrane phospholipids will not flip include: unfavorable interactions between hydrophobic/hydrophilic parts of the lipids, unfavorable energy requirements, and integral proteins preventing movement. To further understand student thinking, eleven student
interviews were conducted. Next steps include analyzing the student interviews, and obtain inter-rater reliability among human scorers. Our goal is to provide reports to instructors that represent the complexity of student thinking about this question.

17. Weight Gain and Insulin Resistance in Mice Lack Cannabinoid-2 Receptor
Emily Kurjan, Omayma Alshaarawy, and L. Karl Olson
Department of Physiology, Michigan State University, East Lansing, MI

The discovery of cannabinoid receptors (CB1R and CB2R) has provided a platform for investigating the health effects of marijuana. The CB1R is predominantly expressed in the central nervous system, whereas the CB2R is primarily expressed in the immune system. Activation of CB1R is associated with increased food intake and obesity. Epidemiological studies, however, have shown a decreased prevalence of obesity and type 2 diabetes among cannabis users. Here we use high-fat feeding (HFD, 45% of calories from fat vs. control LFD with 10% of calories from fat) to study the associated metabolic changes in male mice lacking CB2R (CB2−/−) when compared to wild-type (WT) C57BL/6 mice. After 12 weeks, WT mice gained an average of 20 grams on HFD compared to 9 grams on LFD (183% vs. 138% increase of basal weight). CB2−/− mice gained an average of 19 grams on HFD (173% increase of basal weight), and an average of 10 grams on LFD (134% increase of basal weight). Importantly, CB2−/− mice fed a HFD were glucose intolerant relative to WT-mice on HFD. No significant differences in the calorimetry parameters measured were detected. In summary, CB2R deficient mice when fed a HFD displayed impaired glucose metabolism despite the absence of significant weight differences, highlighting the potential role of CB2R in insulin resistance.

18. Identifying Fecal Contaminants using Microbial Source Tracking at Luna Pier Beach
Paige Larner, Jean Pierre Nshimyimana, and Joan B. Rose
Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI

Fecal excretion and associated bacteria due to various animals contaminate public beaches and influence public health. Luna Pier Beach (Monroe County, MI) experienced 72 days of closure during summer months due to an increase in microbial pollution. This study was conducted to determine the source of the high Escherichia coli (E. coli) concentrations. This study was conducted to determine the sources using Bacteroidales-associated microbial source tracking markers. Water samples ranging from 65 to 250 milliliters were collected from five different locations along the beach and filtered on site. DNA was then extracted and processed using droplet digital polymerase chain reaction (ddPCR). Gull (qGull), dog (BacCan) and human-specific (B. theta) Bacteroidales-associated markers based on published primers and probes were used. Thirty water samples were collected between July and August 2017. E. coli counts ranged from 7 colony forming units per 100 milliliters (CFU/100mL) to 517 CFU/100mL. The B. theta human marker accounted for the highest count of positive samples, with 27 of the 30 total samples. Fifteen and zero of the samples tested positive for qGull and BacCan, respectively. The
North and Central locations of the beach resulted in the greatest incidences of fecal contamination for both gull and human markers. B. theta ranged from 97 to 1100 gene copies (GC)/100mL and qGull resulted in 97 to 743 GC/100mL. The results are being used to remediate septic tanks and sewage spills in the area. Storage of environmental samples is essential in the process of analysis. Environmental samples of manure and raw wastewater of Lansing, MI were collected, combined and diluted, then filtered as 10mL volumes and extracted for DNA. Quantifiable differences of DNA stability were measured over a 60-day period between samples-half of which are stored as filters and half stored as DNA extracts. Samples were assayed with B. theta and CowM2 markers using ddPCR. Our current results do not indicate a significant change in DNA concentration over this time period. Results from this study can be used to advance suggested methods of storage for environmental samples.

19. Post-translational modifications regulating Cyclophilin A protein, altering the cell cycle and cytokinesis
Hannah Lufkin and Margaret McGee

Cyclophilin A (CypA) is a peptidyl prolyl isomerase protein which has the ability to change the confirmation of other proteins and thereby their function or structure. CypA is found at the centrosome during interphase in a range of human tumor cells and undergoes a cell cycle dependent relocation from the centrosome to the midbody during mitosis. Regulation of CypA localization to and from these distinct cellular locations in tumor cells remains unknown. It was previously shown that CypA can undergo post-translational modification including phosphorylation and acetylation in vascular endothelial cells and human tumor cells. Thus, it was hypothesized that phosphorylation and/or acetylation may control the subcellular localization of CypA during the cell cycle. A range of human chronic myeloid leukemia cells, K562 and KYO-1, and Jurkat acute lymphoma cells were cultured in vitro and whole cell protein extracts were isolated. The expression and size of CypA was determined in the tumor cells by SDS-PAGE and Western blotting. Cells were synchronized in mitosis by treatment with a low dose of the microtubule disrupting agent, Nocodazole, for 16 hours and confirmed by flow cytometry. Post-translational modification of CypA was confirmed in K562 and KYO-1 by the detection of a protein with altered mobility by SDS-PAGE. Treatment of cell extracts with calf intestinal phosphatase did not alter the protein mobility pattern suggesting that CypA did not undergo phosphorylation. However, a band at approximately 18kDa was detected when probed with an antibody to detect acetylated lysine suggesting that the mitotic cells may undergo acetylation.

20. Interaction of proteins involved in the sporulation of Bacillus subtilis: SpoIVFA and BofA
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**Bacillus subtillis** forms an endospore when under starvation conditions. Transcription factor, $\sigma^K$, is responsible for transcribing the genes that are involved coat formation around the forespore and the release of the mature spore. The precursor, Pro-$\sigma^K$, is cleaved by SpoIVFB which is an intramembrane metalloprotease (IMMP). Late-stage sporulation proteins, SpoIVFA and BofA help regulate the timing of Pro-$\sigma^K$ cleavage by inhibiting SpoIVFB. Previous studies show that SpoIVFA, SpoIVFB, and BofA are all in a complex together, where BofA is the primary protein of inhibition and SpoIVFA is needed to assemble SpoIVFB and BofA in the complex. This study aims to determine a site of direct interaction between sporulation proteins, SpoIVFA and BofA, through the induction of disulfide cross-linking in the C-terminal regions of both proteins. Using a functional version of cysteine-less SpoIVFA, BofA, SpoIVFB, and Pro-$\sigma^K$ on a single plasmid, a cysteine was engineered into the C-terminal region of SpoIVFA and BofA. Copper activated 1,10-phenanthroline was used as an oxidizer to induce the formation of a disulfide bond if the cysteines in the C-terminal regions of SpoIVFA and BofA are close enough together in the complex with SpoIVFB and Pro-$\sigma^K$. When the proteins are run on a western blot, a shift in the complex size will be observed if disulfide crosslinking occurs. This result would provide direct evidence that the C-terminal regions of SpoIVFA and BofA interact in *B. subtillis*. Determining the regions of interaction lead to a better understanding of SpoIVFB regulation and other IMMPs.

21. Immunofluorescence Staining to Determine Whether K17 and p63 Function as Markers for the Periderm and Basal Cell Epithelial Layer in Canine Palates

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Palate formation is an essential process in the development of all mammals. In canines, little is known about the cell types and markers for these cells in the formation of the palate. Improper palate formation in development can lead to clefting. Cleft lip and cleft palate are common birth defects that affect thousands of newborns each year. Complications arising from these conditions later in childhood can include feeding difficulties, speech impairments, social development problems, and in some cases death.

22. Evolutionary Conservation of Epithelial Development in Canine Palate Formation

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One out of every seven hundred children born today will be impacted by orofacial clefting. With a moderately high probability those in developing countries are most affected as with lack of
medical attention to this abnormality leads to malnutrition due to inhibited breast-feeding, speech impediment and possible expiration. With incidence of medical care the malformation can be corrected this accessibility proves complication. The goal of our lab generates a fetal dog’s model in a human’s stead due to its similar size and expedited adult growth cycle of 18 months in comparison to 18 years. Subsequently imposing the necessity to research how canine oral cavity cells interact to standardized immuno-fluorescence protein markers. In humans as well as mice there are two epithelial layers found atop the palatal shelves, basal below periderm, each corresponding to p63 and K17 respectively providing a green cytoplasmic signal below a red upon a mesenchymal palate.

23. Timing of palatal closure and gestational length: a new allometric relationship?
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Allometry was defined originally as the process by which the characteristics of living creatures change with size. Now, its use has expanded to encompass all biological scaling relationships, for example the relationship between brain size and body size (Shingleton, 2010). Recently, we observed that the timing of palate fusion appeared to be logarithmically related to gestational length when we reviewed data, dating back to the early 1900s, for 16 species. We decided to take it a step further and compare a wide variety of other developmental landmarks (digit separation, hand plate formation, eyelid fusion, and timing of carnegie stages to name a few) to gestational length, to test the generalizability of our hypothesis. Since allometric relationships are often logarithmic, we also plotted the log-values of each variable and determined R2 values for each curve. We observed positive correlations in both linear and logarithmic plots, the latter being the stronger correlation, as seen with classic allometric relationships. Since this correlation is consistent across species, we hypothesize that evolutionarily conserved developmental events, such as those listed above, are related to gestational length. We call this relationship “heterochronic allometry.” The simplest interpretation for the observed relationship is that all species that fall on the line share a common pathway for embryonic development that originated in the lowest common ancestor.

24. Evaluating multiple paper filters in bacterial DNA recovery with varied water source turbidity conditions
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Collecting environmental DNA (eDNA) is a valid technique that allows for the detection and quantification of species populations. Often with eDNA, small amounts of degraded DNA fragments are present for analysis. This makes maximizing recovery of eDNA a major priority, especially in the case of recovering bacterial DNA. In this experiment, efficiency of various filter papers were tested to determine the most effective method of eDNA recovery and bacterial DNA recovery. Samples that were tested were collected from different water sources with high and
low turbidity to evaluate the efficiency of the filter papers under different conditions. The effect of the filter paper type and turbidity of the sample on eDNA and bacterial DNA was shown through filtration of the samples, followed by preservation and extraction. Extraction yields were evaluated using fluorometric quantitation. Extraction samples that were shown to contain DNA in this step were used in PCR with 16s ribosomal RNA gene primers. Amplified products from this reaction were subjected to gel electrophoresis which effectively identified samples that recovered bacterial DNA from the water sources. Glass fiber filters were identified as the type that quantified the most DNA during the fluorometric quantitation step.

25. The PilZ proteins and their effect on motility of *Vibrio cholerae*

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*Vibrio cholerae* can transition between a motile and biofilm lifestyle as a behavioral response to changing environmental conditions. This process is coordinated in part by the secondary messenger c-di-GMP. *V. cholerae* has five proteins containing a PilZ domain (PlzABCDE), a receptor for c-di-GMP. However, their functions in regulating cell behavior are currently unknown. We used a reverse genetic approach to characterize the phenotypes of different *plz* mutants using soft agar motility assays and single-cell tracking. *V. cholerae* normally becomes more motile when the culture reaches the saturation phase during which the level of c-di-GMP is down-regulated. To investigate PilZ effector functions in mediating a behavioral response to high or low c-di-GMP level, we manipulated intracellular c-di-GMP levels in different *plz* mutants using inducible diguanylate cyclase (DGC) or phosphodiesterase (PDE), respectively. Motility behaviors in response to either high or low c-di-GMP level were measured by quantifying changes in the zone of spreading on soft agar or extracting swimming behaviors from single-cell tracking. Our analyses show that PlzB promotes motility, PlzD inhibits motility, and PlzE is involved in a more complex response. In particular, the absence of PlzB dampened motility response to low c-di-GMP concentrations, while the absence of PlzD enhanced motility response to low c-di-GMP concentrations. PlzA and PlzC had no effect on motility even after manipulation of intracellular c-di-GMP concentrations. Overall, PilZ proteins regulate motility in a c-di-GMP-dependent manner, but the interplay between positive and negative regulators of motility remains to be characterized.

26. Switching to Sugars: Improving Lignocellulose Pretreatments for Biofuels

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Switchgrass is a dominant prairie tallgrass native to North America and is currently a leading candidate as a source of biomass feedstock for the biofuel industry. Grasses such as switchgrass contain large quantities of glucose and xylose monomers found as polymers of cellulose and hemicellulose within their cell walls. To convert these polymers into their respective sugar monomers powerful enzyme cocktails are used to cleave the polymerizing bonds between
monomers. However, these enzymes are not effective on untreated biomass due to the presence of lignin and structure of cell wall, so the switchgrass must be treated using a chemical pretreatment to alter its cell wall structure to improve digestibility. We previously demonstrated that alkaline hydrogen peroxide (AHP) treatment with a copper bipyridine (bpy) catalyst is effective at degrading the cell walls of other lignocellulosic biomasses. However most need a bipyridine catalyst to achieve yields of 80%, but results in pretreatment switchgrass with Cu-AHP even in the absence of the bpy ligand have resulted in high glucose yields as well. The elimination of bpy from the pretreatment process has opened the possibility of optimizing an economically feasible pretreatment process for switchgrass, and has raised questions about how the pretreatment process works on molecular level. In this poster we will explore our findings about the pretreatment process of switchgrass and future research that may yield a greater understanding AHP pretreatments.

27. Characterizing Inhibitory Mechanisms of Lactobacillus murinus against Campylobacter jejuni Growth
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C57BL/6 IL-10 knock out (KO) mice are a laboratory model for investigating inflammatory bowel disease (IBD). When exposed to the foodborne pathogen Campylobacter jejuni the knockout of IL-10 blocks inflammation-suppressive mechanisms in the gut and IBD results. However, in a recent experiment where mice were inoculated with C. jejuni strain 11168, mortality rates in C57BL/6 IL-10 KO mice were low and histopathologic lesions were mild, suggesting that the mice had been protected from colonic inflammation. Coincident with this finding an unidentified bacterium appeared on TSA-CVA isolation plates cultured from the feces of both infected and sham-inoculated mice containing tryptic soy agar, defibrinated sheep’s blood, cefoperazone, vancomycin, and amphotericin. It was hypothesized that the bacterium had probiotic effects, protecting the mice from C. jejuni-induced colitis by secreting an antimicrobial agent. Matrix-assisted laser desorption/ionization time of flight mass spectrometry identified the organism as Lactobacillus murinus. Primary Bolton agar plates were streaked and incubated for 48 hours at 37°C to produce isolated colonies of C. jejuni strain 11168. 40 colonies were suspended in 240 mL Bolton broth with 0.75% agar at 48°C and 20 mL were decanted into 10 petri plates. Supernatant from L. murinus broth cultures grown in De Man, Rogosa, and Sharpe (MRS) broth at 37°C for 72 hours was added to wells cut into the Bolton agar with C. jejuni. After incubation for 48 hours at 37°C, slight clearance was observed around the wells, suggesting L. murinus secreted an inhibitory compound. Funded by NIH Grant U19AI090872

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The plant microbiome is a vast and complex community that consists of specialized microbes that are essential to the preservation of plant life on Earth. These communities have been a key causative of plant health function, and productivity over the course of millions of years. The basis on which a plant microbiome partnership is developed is largely exclusive. Previous studies have suggested that these partners are selected on a genomic level basis. The goal of this study is to investigate whether the resident microbial community of a home soil of a certain species of *Trifolium* can form a symbiotic partnership with a different species of *Trifolium*, and whether this will influence plant productivity. It is hypothesized that the rhizospheric and endophytic communities will differ between each species, while within each species, the presence of a home soil will yield higher plant productivity. To test this hypothesis, eight species of *Trifolium* from within a single ecosystem were selected from Bodega Bay, California. Soil selection was determined such that each species had two corresponding “home” soils, and two corresponding “away” soils. A soil was labeled a home soil if it was collected from directly underneath the species in question, and was labeled an away soil if it was collected from a different part of the field. Plant productivity was determined using expanded leaf count and dry shoot weight. The selection of microbial symbionts was analyzed via DNA extraction, and 16S rRNA analysis. A variance in preliminary plant productivity data between home and away groups indicates that the variable soil plays a role, however the results are not uniform across all species. Future data from DNA extraction needs to be analyzed to gain more specific insight into the construction of microbial communities involved.

**29. Baiting for bacteria with living fungal hyphae**

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Fungi are important components of functioning ecosystems, and have important applications in medicine and agriculture. In natural systems, fungal hyphae interact with various bacteria. However, exactly which bacteria are recruited to the hyphae of different fungi, and the functional impact is largely unexplored. The more we know about fungal-bacteria interactions, the more efficiently we can apply and use these consortia to benefit society. The purpose of this study is to assess whether different trophic groups of fungi recruit different bacteria to their hyphae, and to determine if soil bacteria are specialized to specific hosts or generalists. The specific strains under study are *Mortierella*, a soil fungus in the phylum Mucoromycota; *Morchella*, an edible mushroom in the phylum Ascomycota; and *Ganoderma*, a wood-rot fungus in the phylum Basidiomycota. For our experimental set up, fungal hyphae were grown across a microscope slides to provide a support for their growth. These colonized slides were then placed in a mesh bag and incubated in moist chambers of soil. After two week, the slides were removed, carefully rinsed, and then bacterial communities associated with the different fungal hyphae were examined both visually and through 16S rDNA amplicon sequencing on the Illumina MiSeq platform. Bacterial isolates from the hyphae of each fungus were also made and identified through Sanger sequencing. We hypothesize that each phylogenetic group of fungi will preferentially recruit a specific set of bacteria from a common soil.
30. The Isolation of Tellurite Sensitive Mutants of *Staphylococcus aureus* from Cystic Fibrosis Patients

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*Staphylococcus aureus* is a gram-positive bacteria that is found in the nose and on the skin of approximately 30% of healthy individuals. Cystic fibrosis is a genetic disorder that causes the production of abnormally thick and sticky mucus in the lungs. This abnormal mucus layer increases susceptibility to chronic and progressive bacterial infections. As a common microbial pathogen of cystic fibrosis patients, *S. aureus* often initiates this chronic microbial infection. Immune cells, such as neutrophils, produce large quantities of reactive oxygen species (ROS) as a mechanism to destroy invading pathogens. This influx of ROS produces an imbalance in the surrounding environment causing oxidative stress to the invading bacteria. Pathogens, such *S. aureus*, that are resistant to this imbalance avoid eradication and infection can proceed. *S. aureus* was isolated from sputum samples collected from a cohort of cystic fibrosis patients from multiple time points and tested for their ability to resist oxidative stress in the form of tellurite. Tellurite is known to induce oxidative stress in several species of bacteria. Wild type *S. aureus* is resistant to the toxic effects of tellurite through a poorly defined mechanism. We screened 68 *S. aureus* clinical isolates for increased sensitivity to tellurite and identified three isolates with abnormal sensitivity when compared to laboratory strains of *S. aureus*. By identifying and characterizing naturally occurring tellurite mutants we seek to identify novel mechanisms of oxidative stress resistance utilized by *S. aureus*.

31. The Effects of Variable Dietary Fat Content on Bone Health in Developing Mice

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Obesity in both adults and children has seen a surge within the past few decades, prompting enormous amounts of studies on the associated medical risk factors. The link between high fat diets, obesity, and bone quality have been studied for many years. Research has shown that mice fed a high-fat diet present with a lower trabecular bone volume fraction (BVF) compared to controls. Despite these studies, there is little information the consequences of changing the fat content in the diets of developing mice. This study was conducted to determine the effects of variable diet conditions on bone health and body weight in mice. 3-week-old mice were initially fed either a low (LFD)- or high (HFD)- fat diet. Half of the mice underwent a diet change after 7 weeks, switching from LFD to HFD and vice versa, while the other half of these mice continued their initial diet. Consistent with literature, a HFD caused a significant decrease in BVF (mean of 1.07 BVF%/g body weight) compared to mice fed a LFD (mean of 1.92 BVF%/g, p<0.0001). Those fed a HFD also experienced an increase in pro-inflammatory cytokine expression relative to the mice fed a LFD. The administration of an initial LFD then switched to a HFD resulting in a 21% higher BVF%/g body weight compared to those mice fed only a HFD. These results suggest that an initial LFD is necessary during development in order to build and maintain a high bone volume fraction.
32. Evaluating a New Method for Detection of *Legionella pneumophila*

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*Legionella pneumophila* is the primary causative agent of Legionnaires disease, a pneumonia that can be fatal. *L. pneumophila* is found in freshwater environments, including rivers, lakes, and water cooling systems. Agar-based culturing methods are the gold-standard for identifying *L. pneumophila* in samples from these environments. However, such methods are difficult due to *L. pneumophila*’s growth requirements and long incubation period. Legiolert is a new most-probable number assay designed to specifically identify and quantify *L. pneumophila* in water samples without the difficulties of agar-based methods. However, the applicability of using Legiolert for various water types, including ambient surface water samples, is unknown. The objective of this work was to assess the ability of Legiolert, using both the potable and non-potable methods, to accurately detect and quantify *L. pneumophila* in ambient surface waters by testing nine samples from the Flint River during the 2017 summer season. The experiments showed that the potable method of Legiolert yielded false positive results for *L. pneumophila* for samples when compared to other identification techniques, including PCR. While the potable method of Legiolert results indicated that *L. pneumophila* was present in nine river water samples collected from April to August, droplet digital PCR results for these same samples were negative for *L. pneumophila*. This experiment suggests that the Legiolert potable method may be inappropriate for use with ambient surface water samples due to the possibility of false positive results.

33. Combatting Drug Resistant *Enterobacter cloacae*

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Gram-negative multi-drug resistant infections are a growing problem in healthcare settings. Carbapenem Resistant Enterobacteriaceae (CRE) is a specific group of pathogens which cause these infections. CRE are especially important as they have developed resistance to carbapenems, which are typically used as last line antibiotics. With currently available antibiotics, future infections with CRE pathogens may become untreatable. Development of new drugs to fight CRE infections is hindered as the outer membrane of these Gram negative pathogens acts as a barrier making it difficult for the drugs to enter the bacterial cells and reach their target. My research is aimed at understanding and targeting the membrane of the CRE pathogen *Enterobacter cloacae* (*E. cloacae*). *E. cloacae* is an opportunistic pathogen and common commensal bacterium from the human gastrointestinal (GI) tract. It is only pathogenic however, when able to invade the bloodstream and colonize sterile organs. The mechanisms by which *E. cloacae* does this are still largely unknown. I am analyzing transposon-induced mutants of *E. cloacae* isolated in a screen for membrane-related defects. To investigate these further, I am classifying their sensitivity to a range of antibiotics (Abx), as well as to the membrane-active
compound sodium dodecyl sulfate (SDS). Mutant classes Abx-resistant (Abx\(^r\)) and SDS-sensitive (SDS\(^s\)) are being established among these generally membrane-defective isolates. Data from these assays, and knowledge of the transposon insertion site in each mutant will enable me to design and test hypotheses about underlying mechanisms of membrane integrity and antibiotic resistance.

34. Characterization of a bacterium isolated from a mosquito tree hole
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The focus of this study is to better understand the mosquito habitat for container breeders to aid in the vector control effort limiting the spread of disease. The mosquito breeding habitats were previously thought of as being nutrient limiting. The tree holes in which mosquitos lay their eggs are in fact not nutrient limiting but are reducing environments where electron acceptors are deposited into the environment via stem flow from the trees. An organism was isolated from sediment samples using anaerobic culturing with Ferric Pyrophosphate as the electron acceptor and Hydrogen gas (H\(_2\)) as the electron donor from tree holes in various wood lots on Michigan State University’s campus. This unknown organism is being treated and characterized as novel until the ribosomal RNA (rRNA) results are received. For the characterization of this bacteria, the anaerobic culturing technique was used to identify which electron acceptor and donors yield the most effective growth. The most effective combination of electron donor and electron acceptor will be used to perform other growth assays on varying conditions such as, pH fluctuations, temperature, and salt concentration. Ferric Pyrophosphate and Hydrogen gas were used to obtain a growth curve of the bacteria. The goal of this study is to determine what role the unknown organism plays in the mosquito habitat for an increased understanding in the fight against disease.

35. Identifying Interacting Proteins of Shugoshin (Sgo1) During Mitosis of Saccharomyces cerevisiae
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During cell division, the duplicated chromosomes are held together by the cohesin complex so all chromosomes of a genome can aligned at the metaphase plate for concerted separation. A key criterion for cells to coordinate the simultaneous separation of these chromosomes is to ensure each pair of sister chromosomes are under tension, which results from the tug-of-war pulling toward the two daughter cells when the sister chromosomes are still held by cohesin. After tension is detected in all chromosome pairs, cells initiate the anaphase by proteolytically cleaving the cohesin complex. The two sets of identical genomes can thus be evenly segregated. If cells initiate segregation before tension is built up, uneven distribution of genomes, i.e., aneuploidy, ensues. Aneuploidy is an underlying cause of many cancers and the majority of first-trimester spontaneous abortion in humans. The detection of tension between sister chromosomes is
mediated by the Shugoshin protein (Sgo1p) and its partner the tension sensing motif of histone H3 (TSM). Without either, cells suffer from higher rates of aneuploidy. However, how Sgo1p and TSM relay the tension status to the mitosis machinery is elusive. We discovered that cells with a defective TSM can be rescued by two very specific mutant alleles of Sgo1p: one that is truncated at the residue Y317 (Y317X) and another one bearing a single alanine mutation at residue proline 353 (P353A). The mechanism underlying the suppression by these two Sgo1p alleles gives us a unique opportunity to understand how Sgo1p and TSM convey the tension status to the cellular mitosis machinery. To this end, I set out to identify proteins that may be targeted directly by the Y317X allele of Sgo1p. I take a yeast two-hybrid (Y2H) approach to genetically screen for Y317X-specific binding proteins. The initial screen identified 17 candidates and the identities and potential functions of these candidates will be presented.

36. The Performance and Accuracy of the CMEIAS Object Editing Plugin Tool
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Most microbial interactions exist in diverse multicellular aggregates. Digital micrograph images of microorganisms commonly contain cell aggregates with overlapping cell contacts, and this massive conformation is difficult to analyze by either artificial or mechanical means. Also, the classification and diversity of cell shapes need to be assessed for individual cells at single-cell resolution. The Object Edit plugin of CMEIAS v. 4.0 is a digital image processing tool designed to identify and split object overlaps of microbes in digital images. In this study, the accuracy of this plugin’s auto splitting function is evaluated on 25 binary and 10 gray-scale digital microbiological images. In addition, it accuracy is compared to the Watershed segmentation algorithm, the gold standard tool of digital object separation, using the same group of binary images. The occurrences of two types of common segmentation errors are measured for both programs. The type 1 error occurs when adjacent touching cells are not split where they should be (erroneously decreasing the object count), and the type 2 error occurs when a cell is split internally where it should not be (erroneously increasing the object count). The results of this study indicate that CMEIAS is more stable and performs with greater accuracy (98.36% overall accuracy), compared to the 77.03% general accuracy of the Watershed algorithm. These results will be incorporated into the user manual of the final CMEIAS release ver. 4.0.

37. An in vivo murine model of MKL3 deficient metastatic breast cancer
   Hayden Stoub and Kathleen Gallo
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Breast cancer leads the nation in newly diagnosed cases annually and is second only to lung/bronchus cancer in rates of cancer deaths. The cause of death in these cases is not due to the primary tumor, but rather metastatic growths in vital areas such as the lungs, liver, and brain. Mixed-lineage kinase-3 (MLK3) is a serine/threonine protein kinase kinase kinase with diverse
involvement in the MAPK pathways and has been shown to influence malignancy and migration behavior in mammary carcinomas. In this study we aim to elucidate phenotype differences that may arise from MLK3 knockdown in respect to carcinomas that have already gained metastatic status.

38. **Plant-microbe interactions in the rhizosphere of common bean (Phaseolus vulgaris)**

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Common bean is the most important legume crop for human and livestock food consumption worldwide. Extreme environmental conditions such as heat and drought can reduce bean yields and quality. Microbes play an important role in plant health, contributing to nutrient availability, pathogen resistance, growth promotion and stress reduction. To better understand interactions between the common bean and soil microbiota, we are investigating the root associated (rhizosphere) microbial community structure and activity over bean development. Two common bean cultivars, CELRK (California early light red kidney) and Eclipse, will be grown in soils collected from two different bean production fields in Michigan, until flowering time (~5 weeks). Soil and plant samples will be collected at 3 time points. Microbial respiration rates and soil exoenzyme activities will be quantified to determine the changes in microbial activity due to plant development. Population sizes of dormant and active cells will be determined by combining live-dead cell staining and flow cytometry. To determine which plant metabolites are released and have potential impact on changes in microbial community structure and activities, root exudates will be collected from plants growing hydroponically at comparable time points. During the experiment, microbes of the common bean rhizosphere also will be cultivated, identified and characterized for future studies. The results of this experiment will provide insights into the relationship between microbes and beans which then can be used to improve legume crop yield.

39. **Retinoblastoma Cancer Protein Regulation of Isocitrate Dehydrogenase (Idh)**

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Retinoblastoma (Rb) is a tumor suppressor and has well studied regulatory control over cell cycle genes. Rb also exhibits its function in genes outside of the cell cycle family in mechanisms that are not fully understood. Previous findings from this laboratory suggest that Rbf2 binds to the E2F site of Idh in Drosophila melanogaster embryos. Since mutation or misregulation of Idh are found in the vast majority of certain human gliomas, Rb is a known tumor suppressor protein which is dysfunctional in many cancers, and there is evidence that Rb binds to Idh from ChIP-seq data, this study was designed to determine if Rb has regulatory control over Idh through the
Rb/E2F pathway. Gamete directed CRISPR/Cas9 gene editing of the E2F binding site on Idh was coupled with co-editing of ebony (e) to produce a linked visible phenotype. A variety of genotypes were observed across the unique lineages of injected individuals, including a mutation that partially mutated the E2F site. It is hypothesized that a disruption of this regulatory site will result in an increase of Idh expression. Determination of the disrupted E2F site’s effects on Idh will be performed by qPCR, enzyme activity assay, western blot, and chromatin immunoprecipitation.

40. Electrochemical manipulation of complex III in mitoplasts

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Cancer, Parkinson’s, and diabetes are some of the metabolic diseases linked to mitochondrial dysfunction. Current techniques for studying mitochondria involve monitoring oxygen consumption rates (OCR) as a measure of their metabolism and overall mitochondrial health. Since oxygen consumption occurs at the final component of the electron transport chain (ETC), complex IV, it is difficult to use OCR to detect small changes in activities of individual upstream complexes, such as complex I, II, and III. Previous work utilizing a custom tandem electrochemical/respirometric microfluidic cell has shown that mitochondrial OCR can be stimulated by applying reducing electric potential in the presence of TMPD, which shuttles electrons between the electrode and the ETC. Here, we investigate other pathways of electrochemical manipulation of ETC, particularly that involving complex III and cytochrome c. To allow for cytochrome c to shuttle electrons similar to TMPD, we prepare mitoplasts by hypotonic swelling of mitochondria; the treatment ruptures outer mitochondrial membrane (OMM), which cytochrome c cannot cross in intact mitochondria. Removal of the OMM is confirmed by restoring the activity by addition of exogenous cytochrome c in the presence of substrates. OCR in untreated mitochondria is high and shows no response to cytochrome c, while mitoplasts have low initial OCR, which is increased when cytochrome c is added. The results show that hypotonic swelling can be used to treat mitochondria to obtain mitoplasts and that it exposes ETC for electrochemical stimulations using to the exogenous cytochrome c.

41. Characterizing a vanillate-inducible FruA transcriptional fusion

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In conditions of extreme stress, such as starvation, many bacteria form fruiting bodies within their communities. It is known that this is done through coordinated signaling. Our research goal is to characterize a vanillate-inducible fruA transcriptional fusion in wild-type and mutant Myxococcus xanthus. It is known that fruA is involved in C signaling between the cells, but the exact system by which this happens is unknown. In hopes of understanding this, we have isolated a mutant strain of Myxococcus xanthus that does not normally develop fruiting bodies. We have inserted a construct into this mutant’s DNA so that when vanillate is introduced into the system, the mutant reverts back to the wild type behavior. This is happening because the vanillate is
inducing the fruA gene in the construct. For this reason, we know that we have a system that differs only by the turning on and off of the fruA gene. We then ran experiments with our mutant and construct combination, adding vanillate at different times to determine when the induction of fruA was closest to wild type. With this information, we have done a series of experiments and assays to confirm that we have successfully rewired the FruA network in our mutant Myxococcus Xanthus.

42. Overexpression of Tup induces expression of Hid in scrib⁻/⁻ mutant cells

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There are many different types of cell-cell interactions in cancer that are important for cell proliferation, differentiation and apoptosis. Cell competition, the process by which surrounding wild type cells can induce apoptosis in mutant cells, is the interaction my project is focused on. Apoptosis of mutant cells was found to be induced by the Eiger-JNK signaling pathway. Learning about the function of this pathway could help scientists better understand tumor growth and create more effective treatment for cancer patients. A vital protein involved in this pathway called Taliup, was discovered by a lab in Japan which has led to more research to find other proapoptotic genes related to this pathway. This poster will describe the methods used to identify if Hid is a downstream protein involved in the Eiger-JNK signaling pathway, and how this is related to tumorigenesis.

43. The characterization Staphylococcus aureus isolates from cystic fibrosis patient’s sputum

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Staphylococcus aureus is a gram positive cocci that colonizes the skin and respiratory tract of ~30% of healthy individuals, and can cause diseases ranging from skin infections to sepsis. Cystic fibrosis (CF) is a genetic disorder that primarily affects the lungs, and pulmonary infections are the leading cause of death for those diagnosed with CF. In the lungs of CF patients, the normally lubricating mucus becomes thick. The increased viscosity and abundance of the mucus in the lungs encourages microbial infections. One of the earliest microbes that colonize a CF lung is S. aureus, which leads to chronic pulmonary infections. S. aureus was isolated from sixty-six CF patient sputum samples from seven different patients across multiple time points. Multiple virulence factors were characterized in these clinical isolates of S. aureus. These clinical isolates demonstrated great variability in multiple virulence factors such as hemolytic activity, antibiotic resistance, sensitivity to oxidative stress, and lipase activity. These clinical isolates S. aureus will be utilized to further define the pathogenesis of S. aureus in future studies.
44. Blinding *Mycobacterium tuberculosis*: DosRST inhibitory compounds

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*Mycobacterium tuberculosis* (Mtb) uses regulatory systems such as DosRST to perceive changes in the environment and adapt accordingly. DosRST is a two-component system consisting of a sensor kinase (DosS/T) and response regulator (DosR). This pathway allows Mtb to respond to variations in oxygen levels, and thus inhibition of DosRST may shorten TB treatment by affecting Mtb’s ability to tolerate current drugs. Three inhibitory compounds, HC104A, HC105A, and HC106A were hypothesized to modulate the heme group carried by DosS/T and prevent DosRST function. The heme must be in reduced form for DosS to be active, therefore, inhibitors that cause a shift of the heme from the reduced to oxidized state, may be inhibiting DosS function via a heme-dependent mechanism.

To test this hypothesis, recombinant, his-tagged DosS was expressed from *E. coli* and purified via metal affinity chromatography. The protein was then reduced by treatment with dithionite under anaerobic conditions, and UV-Visible spectroscopy was performed to observe the effect of each compound on redox status. HC106A caused a partial oxidation of the heme, while HC104A and HC105A exhibited no impact on redox status.

These results suggest that HC106A interacts with the sensor kinase’s heme. However, lack of complete oxidation points to a direct binding between the compound and the heme, rather than a change in redox status. This is supported by similarities between the UV-visible spectra of DosS treated with HC106A and DosS treated with carbon monoxide, which is known to bind directly to the heme.

45. Elucidating the Lanthanide-Dependent Network and its Impact on the Pyrrolo-Quinoline Quinone Role in Methanol Dehydrogenases

Zachary Jansen, Josh Lensmire, Kurt Tobin, Nathan Good, and N. Cecilia Martinez-Gomez

*Methylobacterium* Extorquens AM1 is an aerobic methylotroph bacteria that oxidizes methanol to formaldehyde. Typically, the methanol dehydrogenases used in this process need to incorporate calcium into their structure in order to function. However, it has been found that these bacteria also produce another methanol dehydrogenase that uses Lanthanides instead of calcium to power their respiration, named XoxF1. XoxF1 allows these bacteria to grow on media that does not contain calcium as long as it does contain lanthanides. The mutant forms of *Methylobacterium* Extorquens AM1, Meta1:1746 and Meta1:1747, have been found to lack the ability to grow on media containing lanthanides in the absence of calcium. The mutation in their genome can be found adjacent to the part of the genome that encodes production of PQQ, an integral part of the XoxF protein. Our hypothesis is that the mutation in Meta1:1746 and Meta1:1747 is in an uncharacterized gene that facilitates the incorporation of PQQ into the XoxF protein. Without PQQ, the XoxF genes in these mutants is unable to function, and these bacteria are then unable to use Lanthanides in place of calcium in order to grow. In order to test our hypothesis, Meta1:1746 and Meta1:1747 were transformed with a plasmid, PLB01, that encodes the genes for XoxF along with the XoxF promoter, as well as Kanamycin resistance. This plasmid forces the bacteria to overproduce XoxF. Cultures of these bacteria containing the plasmid were then
evaluated using UV/Vis spectroscopy to evaluate the incorporation of PQQ into the XoxF protein.

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Congratulations to all of the MMG graduating seniors;
Best Wishes for a very bright future!