Microbiology and Molecular Genetics

Undergraduate Research Showcase 2021

Abstract Booklet

INDEX

17.	**SeGraves, Emily**	The role of mismatch repair in the formation of double stranded breaks in immunoglobulin class switch regions
18.	**Teis, Robert**	Mast cell-serotonin interactions as a potential mechanism underlying anti-anxiety effects of mast cells
19.	**Tilley, Avery**	Inferring infection risk of West Nile virus from wildlife: connecting habitat selection and seroprevalence in free-ranging white-tailed deer (*Odocoileus virginianus*)
20.	**Van Allen, Mia**	Plasmid-Mediated Transfer of Antibiotic Resistance Genes (ARGs) to Commensal and Multi-Drug Resistant Bacteria
21.	**Watson, Kimberly**	Directed Spatial Structure and Patterning in Synthetic Communities
22.	**White, Carter**	The Role of SETD2 in Canine Lymphoma

1. Crystallization of AgEC5 Protein

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Cadherins are important adhesion proteins in animal cells and have many roles in the development and embryogenesis of many organisms. E-cadherin proteins are key proteins of the cadherin family and are involved in calcium-dependent cell-cell adhesion. They form cell to cell adhesions through the formation of homodimers with other cells exhibiting E-cadherins. There are eight binding domains in this mosquito E-cadherin. However, the protein being studied in this research does not produce all eight. There are only four binding domains, (5,6,7, and 8), in this AgEC5 cadherin that is being crystallized. To accomplish this crystallization, AgEC5 plasmids with kanamycin resistance are used in Rosetta *E. coli* to produce the fifth through eighth binding domains. The *E. coli* make a perfect test subject as they can express the eukaryotic protein given to them in the plasmid when given IPTG to force this protein expression. The proteins are produced in these *E. coli* specimens and harvested for crystallization which uses multiple screening solution kits and the mosquito LCP machine. During crystallization, it is important to avoid the formation of salt crystals from compounds used in the screening kits. The goal is to crystallize this AgEC5 protein and observe the structure of this protein. The three-dimensional structure of this crystallized E-cadherin is determined using X-ray crystallography. Using the structure, the function of E-cadherins can be better analyzed and understood which would provide a deeper knowledge and understanding of cell signaling during processes like apoptosis, necrosis, and many cancers.

2. A Review of Current COVID-19 Vaccine Phase III and Emergency Use Authorization Candidates

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As the pandemic caused by SARS-CoV-2 continues into 2021, it is important to analyze available data for promising vaccine candidates. The purpose of this review is to summarize vaccine types and gather and evaluate public data regarding the safety, efficacy, and immunogenicity of twenty different COVID-19 vaccines in phase III trials as well as those authorized for emergency use. Searches were conducted using a combination of online databases including PubMed,

medRxiv, bioRxiv, NIH, and vaccine company websites. Among the vaccines reviewed, the mRNA-based vaccines (Pfizer-BioNTech and Moderna) had promising safety profiles and demonstrated effectiveness against COVID-19. However, it is still unknown if those who are vaccinated can spread the virus. Multiple viral vector-based vaccines, such as Johnson & Johnson's vaccine, are promising candidates with efficacy after the first dose. Although Moderna, Pfizer-BioNTech, and Johnson & Johnson's products are the only vaccines that have gained FDA emergency approval to date, other vaccines are also expected to be extremely effective against COVID-19. Further studies are needed to establish long term results of vaccine efficacy against SARS-CoV-2 infection and efficacy against variants of SARS-CoV-2. The COVID-19 pandemic called on scientists everywhere to expeditiously develop safe and effective vaccines, a call answered by unprecedented scientific achievement.

3. Identifying Mechanisms of Phage Defense in *Vibrio cholerae* Using High-Throughput Barcode Sequencing

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Phage, viral parasites of bacteria, are primary drivers of bacterial evolution and ecology. My research centers on understanding how the bacterial pathogen *Vibrio cholerae*, the causative agent of the diarrheal disease cholera, defends itself against phage infection. Prior research revealed that the ability of lytic phage to infect *V. cholerae* was density-dependent such that phage infection did not occur at high cell densities. To characterize which genes are responsible for this shift in phage defense, I plan to employ a systems biology approach that utilizes transposon mutagenesis with random genetic barcodes (Bar-Seq). For this approach, hundreds of thousands of *V. cholerae* transposon mutants, each identified by 20 base pair unique barcode sequences, will be constructed, creating a system that tracks relative mutant abundance under selective conditions. The mutant library will then be challenged at high cell densities with ICP-1, ICP-2, and ICP-3, the three common lytic phage of *V. cholerae*. The fitness of each gene will be analyzed by comparing the number of hits for each barcode, representing unique transposon mutations, before and after phage infection. Mutants that exhibit decreased abundance post-infection demonstrate decreased fitness and possibly contribute to the ability of *V. cholerae* to resist phage infection at high cell densities. Once established, this barcode sequencing approach can be utilized to study phage infection of *V. cholerae* in dozens of different environments. Future research will more closely examine the function of genes identified by Bar-Seq to further elucidate their role in high cell density resistance to ICP phage infection.

4. Effects of Oxybenzone on Epithelial Ductal Development in Murine Mammary Glands

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Oxybenzone (benzophenone-3; BP-3) is a putative endocrine disrupting chemical, and common ingredient in sunscreens and many personal care products. Endocrine disrupting chemicals can interfere with the normal action of reproductive hormones. BP-3 is found in the urine of as much as 98% of the U.S. population. Given the prevalence of BP-3 exposure, published evidence that BP-3 is an endocrine disrupting chemical, and the lab's previous studies showing that a high-fat diet can promote breast cancer, we investigated the effects of BP-3 and diet on the ductal development of mammary glands in mice. To that end, BALB/c mice were fed diets with or without BP-3. The diets also varied in dietary fat. Mice were fed a continuous low-fat diet (LFD; 10% kcal fat), continuous high-fat diet (HFD; 60% kcal fat), high-fat diet switched to low-fat diet (HFD-LFD) after puberty, or low-fat diet switched to high-fat diet (LFD-HFD) after puberty (10 weeks of age). To determine if BP-3 had a continued impact after exposure ended, two additional diet groups included withdrawal of BP-3 for 2 weeks and 4 weeks before termination of the experiment. At 26 weeks of age, mammary glands were collected from the mice. Images of whole mounted mammary glands were captured using a Nikon stereo microscope at 4x magnification. The frequency of branch points in each mammary gland was calculated as a representation of glandular development, and the differences in branch point frequency between diet groups were statistically compared. Preliminary findings suggest the withdrawal of BP-3 after lengthy exposure can induce regression of mammary gland development. The results also indicate that diet has an impact on the development of mammary glands.

5. Curating a Collection of Seed Bacterial Community Members Enriched Under Maternal Plant Stress

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Many plants rely on interactions with microbes to gain essential nutrients for growth, and to promote stress tolerance. One way that plants can acquire their microbiota is via vertical transmission from the parent plant via the seed. However, there is currently a knowledge gap regarding the seed microbiome and its consequences for the next generation of plants. Pilot experiments in our lab have shown that the seeds from common bean plants (*Phaseolus vulgaris* L.) that were stressed by either drought or excess fertilizer had an altered seed microbiome as compared to control plants. Now, we seek to understand the costs and benefits of an altered seed microbiome for the stress tolerance and health of the plant. Specifically, we are creating a representative collection of bacterial community members that are enriched under these stress conditions as compared to the community found in control seeds. To understand these

members' consequences for plant outcomes under stress, we will perform controlled experiments in which we add the members to naïve plants and determine plant outcomes under stress. This research will inform which members of a seed bacterial community confer a protective, neutral or detrimental effect on plant health and aims to ultimately improve plant resilience via beneficial microbial community members. The ability to manipulate the seed microbiome to improve plant growth or resilience will benefit crop agriculture.

6. Effects of modulating muscle contractions on embryo movement in early mouse pregnancy

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Early-stage mammalian embryos rely on physical and biological interactions with the maternal environment (the uterine niche) to arrive at their site of attachment. In the mouse during early pregnancy, embryos first move unidirectionally, as a cluster, away from the oviduct towards the center of the uterine horn. Next, the embryos spread out bidirectionally towards the oviduct and the cervix until they space equally and attach. We are currently investigating the role of uterine muscle contractions in the movement of embryos by asking: a) do muscle relaxing drugs prevent contractions of the uterus?, b) does modulating the contractions prevent embryo movement?, and c) which phase of embryo movement (unidirectional or bidirectional) is affected? To answer the questions, I will be using image analysis techniques to quantify uterine horn contractions. First, we record 2D videos of the muscle and graph the longitudinal movement on the x-axis and transverse movement on the y- axis. Using image analysis, I then determine the lines' slopes to calculate the intensity and magnitude of the contractions. “Contraction intensity” thus quantified, is then compared between a) uterine horns at different times during pregnancy, and b) with and without drug treatment. These quantitative data will allow me to start addressing questions regarding embryo movement and the effect of drugs on this movement. Understanding how muscle relaxation affects pregnancy in the mouse model will inform the potential for these drugs to regulate contractions that lead to spontaneous abortions and miscarriage in pregnant women.

7. Development of tetraSTR 12-plex to locate PRA-causing mutations within various purebred dog breeds

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A multitude of canine disorders can be attributed to genetic mutations, and the ability to pinpoint these mutations is crucial to the ethical and financial success of breeding. Many of the

mutations for one group of disorders in dogs known as progressive retinal atrophy (PRA), which causes declining vision leading to eventual complete vision loss, have already been located. However, with the vast nature of the canine genome and the genetic variation between different dog breeds, many of these PRA mutations have yet to be unearthed. In order to combat this problem, our research has focused on designing a simple and cost-effective genetic test that could be used to localize these mutations across a variety of different dog breeds. Using software including USCS Genome Browser and Primer3, we have successfully designed effective primer sets targeting these mutations to be used in a future one-step multiplex. Our creation of a 12-plex of tetraSTRs should simplify the search for PRA genes in different breeds, leading to the understanding and discovery of other PRA-causing mutations. By using this multiplex, researchers will likely be able to develop tests to identify carriers of these mutations, and breeders can therefore prevent the production of PRA-affected dogs leading to the eventual elimination of these specific mutations and disorders from these breeds. Future lab work will entail testing this multiplex on samples of purebred dog DNA to determine the efficacy of our primer sets.

8. FDA Approved Drug Bicalutamide Rescues Male Mice from Kennedy's Disease

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Kennedy's Disease (KD) is a neurodegenerative disorder caused by a polyglutamine expansion, or CAG repeat, in the Androgen Receptor (AR) gene. This disease affects only males, usually emerges mid-life, and is characterized by marked deficits in muscle strength and coordination. Male mice with the KD allele develop muscle weakness around puberty as testosterone levels surge, suggesting that levels of testosterone may contribute to disease development in mice with mutated AR. Bicalutamide (Casodex Brand) is a drug currently being prescribed to slow progression of prostate cancer. By binding to androgen receptors to block androgens like testosterone, bicalutamide prevents the detection and effects of testosterone. We hypothesize that giving bicalutamide to male mice carrying the mutated AR allele will slow or prevent disease progression. We monitored the integrity of their motor function over time based on measures of hang time, grip strength, and ambulatory function in an open field. When one mouse in the trial reached a certain disease threshold indicated by weakness, all animals in the trial were sacrificed and muscle size and body weight were analyzed. Disease symptoms were significantly less for mice who received bicalutamide compared to the vehicle-treated mice, demonstrating a clear benefit of this drug in a mouse model of KD. Bicalutamide is currently approved for use as a treatment for prostate cancer, and our findings suggest that this drug may be an effective therapeutic for patients afflicted by KD.

9. FDA Approved Drug Bicalutamide Rescues Male Mice from Kennedy's Disease

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Dr. Xi's lab focuses on developing *Wolbachia*-based control strategies to block dengue virus transmission in mosquitoes. Mosquito-borne diseases, such as dengue fever and ZIKA, deprive millions of lives worldwide each year. Due to the lack of effective vaccine and treatment, vector control has become a very promising aspect of achieving adequate control and prevention of mosquito-borne diseases by replacing the wild-type mosquito populations with the modified populations which couldn't carry the viruses. *Wolbachia*, an endosymbiotic bacterium, may serve as a vehicle to deliver disease-resistance genes into mosquitoes or to reduce disease transmission ability directly. Therefore, the project I took part in aims to introduce *Wolbachia* into mosquito strains to generate sterile mosquitoes, replace the natural mosquito populations with our sterile mosquitoes so that we can reduce the risk of Dengue/ZIKA, etc. spreading by reducing the population of specific mosquito strains (like Dengue/ZIKA carriers). My main tasks here were to assist this process, and the results showed that only releasing a specific ratio of male and female mosquitos into the wild environment can significantly reduce the number of target mosquito populations. Now I am helping investigate the underlying mechanisms including the effects of molting hormones and various environmental conditions factors on the duration of mosquito pupation and molting.

10. A Literature Review of Periderm in Development and Disease

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The periderm is a flat, squamous layer of epithelial cells found only on the surface of a fetus during development. To study its role in development and disease, I performed a literature search. I queried the PubMed database using the following key search terms “periderm” and one of the following terms, “proliferation” and “differentiation”. I found 160 publications. From reading these papers, I learned that the periderm originates from the fetal limbs and migrates to cover the entire surface of the fetus, including the oral cavity. Interestingly, the periderm is a transient tissue and is shed by 20-24 weeks gestation in humans. The shedding coincides with stratification and keratinization of underlying epithelial cells. A key function of periderm is to prevent adhesions between the immature epithelial layers prior to stratification. Then, programmed cell death (apoptosis) of periderm cells facilitates the adhesion of underlying basal epithelial cells to promote tissue fusion, such as between the palatal shelves in the oral cavity. The significance of periderm function is highlighted by the fact that mutations in genes required for periderm development and dissolution cause birth defects like orofacial clefting as seen in Van der Woude syndrome, popliteal pterygium syndrome, cocoon syndrome, and Bartsocas-Papas syndrome. Because of its critical functions in development and its transient location on the fetus, we hypothesize that the periderm is a good target for in utero gene therapy.

11. Sunscreen... Helpful or Harmful? Potential Modulation of Immune Funtion by BP-3 Sunscreens

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Oxybenzone (benzophenone-3; BP-3) is a common active ingredient in sunscreens and other personal care products. It is found in urine of 98% of the U.S. population. A recent study found after a single heavy application of sunscreen that levels of BP-3 in blood exceeded Food and Drug Administration guidelines for chemicals requiring more study for potential toxicity. The Schwartz lab published studies on BP-3 effects on mammary cancer risk in a *p53*-knockout mammary gland mouse model. While BP-3 effects varied depending on dietary fat, BP-3 exposure had adverse consequences for mammary cancer risk. In unpublished studies, the Schwartz lab found that BP-3 induced expression of RNAs encoding IL-4 and IL-13 in *p53*-knockout mammary glands of mice fed an adult high fat diet, conditions with the strongest BP-3 promotional effect on mammary cancer. IL-4 and IL-13 are potent immunoregulatory cytokines that can polarize macrophages to a tumor promoting phenotype, decrease the tumoricidal activity of T cells, and, apart from potentially promoting tumorigenesis, can promote allergy and asthma. We found in mice fed high fat diet that IL-4 and particularly IL-13 were strongly induced by BP-3 in parametrial fat distal to the mammary gland and likely uninfluenced by the *p53*-knockout mutation. IL-13 protein was also found in the plasma of these mice. To directly address whether IL-13 induction is independent of the *p53*-knockout, we are examining BP-3 effects on IL-4 and IL-13 expression in parametrial fat of wild type mice. A positive result will have important health implications for BP-3 exposure.

12. Improved Genetic Engineering of Gram-Negative Bacteria for Bioluminescent Assays and Antibiotic Testing

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The resistance of bacteria to antibiotics is a growing worldwide public health threat. Half of the bacteria considered by the CDC as urgent or serious threats are gastrointestinal pathogens. Sensitivity to antibiotics is conventionally assessed by the Kirby-Bauer method where bacteria are grown on agar plates with antibiotic-soaked wavers and the size of the bacteria-free zone surrounding each waver indicates each antibiotic's effectiveness. However, we want to analyze the antibacterial efficacy of candidate antibiotics in real-time in vitro and in vivo using bioluminescence imaging (BLI). However, few bacterial species, e.g. some *Vibrio cholerae* strains, have an endogenous light-emitting luciferase enzyme, whereas most other species need to be genetically engineered. For this, we are using a mobile genetic element, the transposon Tn7, to insert a set of genes (lux operon) into the bacterial genome that encode a bacterial luciferase

and the substrate synthesizing enzymes. The Tn7 plasmid, unfortunately, can only be selected for, making the removal of the plasmid backbone after transposition time-consuming. We are currently replacing the antibiotic selection marker, beta lactamase, with the tetracycline resistance marker, which can be both selected for and against. This modified plasmid will permit us to label clinical isolates of enteropathogenic *E. coli* (EPEC) and assess bacterial survival in response to various plant-derived antibiotics. Finally, in preparation for clinical trials we will assess their treatment efficacy in a mouse model of gastrointestinal infection with bioluminescent EPEC that we will also monitor by BLI.

13. Exploring Metabolic Outcomes of Putrescine in *Atropa belladonna*

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Tropane alkaloids are compounds with an eight-membered, nitrogen containing ring. Several tropane alkaloids have medicinal properties, such as scopolamine for treating motion sickness, or cocaine, which is used as a stimulant. The medicinally important tropane alkaloids scopolamine and hyoscyamine are synthesized in plants of the Solanaceae family, including *Atropa belladonna*, Deadly Nightshade. Putrescine represents a key entry point into the tropane alkaloid pathway because it is methylated by putrescine methyltransferase (PMT) to form N-methylputrescine, which will eventually form the N-methypyrrolinium cation, the first ring of the tropane alkaloid core. Putrescine is involved in cell cycle regulation and is used in several different metabolic pathways like polyamine and hydroxycinnamic acid amide (HCAA) synthesis. To gain a better understanding of the biosynthesis of tropane alkaloids, the PMT gene was silenced using virus-induced gene silencing, or VIGS, and non-targeted metabolomics analysis of PMT-silenced plants was utilized to uncover the fates of putrescine and downstream products. The purpose of this study is to determine how levels of various metabolites change when putrescine cannot be methylated, and to gain a better understanding of how putrescine's role in various metabolic pathways in *A. belladonna*. Following PMT silencing, phenyllactic acid accumulated along with non-tropane phenylacetyl-derived metabolites; there were no changes in known hydroxycinnamic acid amides (HCAAs). Future experiments will focus on identifying and characterizing the altered metabolites.

14. Differences in PFAS Serum Concentrations Across Job Occupation and Industry in the NHANES

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Per- and polyfluoroalkyl substances (PFAS), including perfluorooctanoate (PFOA) and perfluorooctanesuflonic acid (PFOS), have numerous industrial applications and are commonly

detected in blood, suggesting widespread exposure. However, occupational exposures have only been characterized for a select few jobs. Here, we use data from the National Health and Nutrition Examination Survey (NHANES) to examine trends in serum concentrations of PFAS across occupation and industry. Measurements of PFOS and PFOA in serum from the 2003-2012 NHANES surveys were assessed across industry and occupation groupings for participant's current and longest jobs using multivariate linear regression while controlling for covariates. Mean PFOA serum concentrations were predicted to be 24-32% lower than the overall population mean for those whose current or longest jobs were in the agriculture industry or whose current job was an agricultural occupation. PFOA was conversely 18% higher in those whose current job was in the transportation, warehousing, and utilities industry group, and 11% higher in those whose current job was in the professional occupational group. PFOS serum levels were also higher than the overall mean for those with a current job either in the transportation industry group (20% higher) or the professional occupation group (17%). The higher estimated serum levels for these occupations and industries may be driven by and indicative of specific jobs with particularly high exposures within these broad groupings. These results are therefore a starting point for future targeted assessments within certain jobs and working environments to better identify and more fully characterize occupational PFAS exposures.

15. *GNAO1* Mutant Mice Display Cerebellar Defects Which May Explain Movement Disorders and Epilepsy

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Mutations in the gene *GNAO1* have been associated with ultra-rare (ca. 200 patients) neurologic abnormalities including movement disorders, epilepsy, and developmental delay. Patients with these mutations often display symptoms at birth or in early childhood. *GNAO1* codes for the alpha subunit (Gαo) of the G-protein Go, which is the most abundant membrane protein in the central nervous system. Our lab developed a Gnao1+/- mouse line to mimic symptoms seen in patients with loss of function (LOF) mutations in *GNAO1*. We observed that these mutant mice had reduced inhibitory signaling in the cerebellum, which is important for movement coordination. My project will determine if there are structural changes in cerebellar neuron architecture to account for the altered inhibitory signaling, providing insights into the mechanism of LOF *GNAO1* disorders and potential therapeutic opportunities. I will characterize the number of inhibitory neurons in the cerebellar molecular layer and the structure of their synapses on Purkinje cells. Preliminary studies suggest that although there are similar numbers of inhibitory interneurons in mutants, there are reductions in the size of inhibitory synapses from molecular layer neurons onto Purkinje cells. Future studies will confirm and extend these results to include other markers of cerebellar development. In my presentation, I will provide an introduction to the *GNAO1* gene and its expression product Gαo, describe what we have

observed in our mouse models, and present my project, including both pilot study results and ongoing research.

16. Characterization of gut microbiota through nutritional intake of infants with RSV in PICU

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Respiratory Syncytial Virus (RSV) is the leading cause of respiratory tract infections in young children, and can lead to deadly secondary infections, including bronchiolitis and pneumonia. There are few nutritional mediated therapies for this disease, despite evidence that nutritional therapies improve health outcomes for infants within Pediatric Intensive Care Units (PICU). Furthermore, there is little understanding of the relationship between gut microbiota composition and nutrition within the PICU. Our objective was to characterize changes in the gut microbiome diversity across two time points, during peak illness and recovery, by patient nutritional intake. Twenty patients who tested positive for RSV and ten sedation controls were enrolled for the study at Helen Devos' Children's Hospital. Patients were <6 month and were admitted to the PICU for severe bronchiolitis. Stool samples were collected at PICU admission and 72 hours, and the bacterial composition of these samples was analyzed by sequencing of the V4 region of the 16SRNA gene. Participants with RSV were grouped into three categories based on their percent caloric intake (<33%, 33-98.9%, 99-100%) in the past 24 hours, with the control infants grouped separately. RSV patients had gut bacterial communities that significantly differed from those of healthy control infants, with control infant bacterial communities containing more *Prevotella* and *Porphyromonas*. Dysbiosis of the microbiota of adult critical care patients has been well documented, including loss of commensals such as *Prevotella*. Future studies could assess whether modulation of infant PICU microbiota via nutritional therapies that promote retention of protective bacteria improve RSV patient outcomes

17. The role of mismatch repair in the formation of double stranded breaks in immunoglobulin class switch regions

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Immunoglobulin class switch recombination (CSR) is a DNA recombination event that allows B cells to switch their isotype from IgM to IgG, IgE, or IgA, altering the effector function of the antibodies they produce for better clearance of infections. In a physiological CSR reaction, activation-induced cytodine deaminase (AID) converts cytosine bases to uracil bases at immunoglobulin class switch regions. From there, uracil DNA glycosylase (UNG) removes the uracils, and then apurinic/apyrimidinic endonuclease (APE) generates nicks at the deamination

sites. These nicks lead to the formation of induce DNA double strand break (DSB), which is a key intermediate in CSR. However, the mechanism by which nicks are converted to DSBs is poorly understood. Using CRISPR technology that can generate nicks (single-strand breaks) at precise locations in DNA, the Martin lab has shown that nicks separated far away (up to 250 bp) can trigger CSR; however, how these distal nicks are converted to DSB is unknown. Using the same experimental system, we tested the hypothesis that the mismatch repair (MMR) pathway plays a pivotal role of converting distal nicks to DSB. We used a mouse B cell line (CH12F3) that is capable of robust cytokine-induced CSR in vitro and compared the difference of CSR efficiencies in genetically engineered CH12F3 cells that are proficient or deficient in MMR. We found that an essential MMR factor (MSH2) promotes CSR induced by CRISPR-mediated distal nicks, suggesting that strand excisions during MMR helps convert nicks on different DNA strands into a DSB.

18. Mast cell-serotonin interactions as a potential mechanism underlying anti-anxiety effects of mast cells

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Mast cells (MCs) are known for their involvement in peripheral disease such as allergy, but they are also present in the brain where they can modulate behavior. We recently found that the MC deficient W^{sh}/W^{sh} mice show a phenotype consistent with increased anxiety/anhedonia: they show decreased time spent in the open vs. the closed arms in the elevated plus maze test and reduced sucrose preference. These results are consistent with previous findings and suggest that MCs exert anti-anxiety and anti-depressive effects under basal or mild stress conditions. One of the potential mechanisms by which MCs could reduce anxiety is by modulating the brain serotonergic network: MCs can synthesize, store, and quickly release a variety of molecules that can directly affect the activity neuronal function, such as cytokines, histamine, and serotonin. Further, pharmacological activation or conditional knock-out of MCs affect brain serotonin content. However, it is not clear whether this contribution is a direct result of MC serotonin release or MC modulation of neural serotonergic circuits. Here, we will use immunohistochemistry for serotonin and the immediate early gene c-Fos in brain slices to examine acute responses of serotonin neurons to mild restraint stress in wild type and W^{sh}/W^{sh} mice. Preliminary data suggest that, compared to wild type, W^{sh}/W^{sh} mice show reduced number of serotonin neurons in the dorsal raphe, the largest brain serotonergic nucleus. This work will generate a new understanding of the cellular mechanisms by which mast cells could contribute to brain physiology and behavior.

19. Inferring infection risk of West Nile virus from wildlife: connecting habitat selection and seroprevalence in free-ranging white-tailed deer (*Odocoileus virginianus*)

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West Nile virus (WNV) is a mosquito-transmitted pathogen of humans, livestock, and wildlife that has extended rapidly across the United States since its initial 1999 introduction in New York City. WNV is maintained primarily in an enzootic cycle between ornithophilic mosquitoes and avian amplifying hosts, with mammals, including humans, serving as dead-end hosts. A mammalian host of particular interest is the white-tailed deer (WTD), given their role in the maintenance and distribution of WNV as identified through serosurveillance. The WTD serves as the primary mammalian blood meal for *Culex* species associated with WNV transmission, and WTD have a widespread distribution across varying habitats promoting a variety of interactions between WTD and mosquito vectors. For these reasons, it is critical to better understand the spatial ecology of WTD and how their use of habitat at a landscape level may be important for mitigating or monitoring WNV. This study seeks to evaluate WTD habitat use with respect to *Culex* habitat and whether WTD could serve as sentinel species for WNV monitoring due to advantages such as opportunistic serum collection from hunter-harvested deer. This study utilized WTD serum obtained from live, radio-collared deer and tested for antibodies to WNV using a virus neutralization test. Serum results were compared to habitat use of deer and that of known *Culex* habitat. Our findings suggest the distribution of deer may inform when and where WNV may present a risk on the landscape.

20. Plasmid-Mediated Transfer of Antibiotic Resistance Genes (ARGs) to Commensal and Multi-Drug Resistant Bacteria

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Antibiotic resistant (AR) pathogens have become a major health problem: the CDC announced we are now existing in a post antibiotic era. Plasmids are the carriers of antibiotic resistance genes, and they spread between bacteria in the microbiome via a horizontal gene transfer mechanism called conjugation. During each conjugation event, plasmids enter a host cell and can express their antibiotic resistance genes, resulting in newly acquired antibiotic resistance for that cell. This process of unrestrained AR plasmid spread cultivates an evolving reservoir of antibiotic resistant pathogens and commensal bacteria in the human gut microbiome. This study aimed to replicate and observe the rate and patterns of transconjugant frequency of fluoro-tagged plasmids in combinations of commensal *E. coli* donors, pathogenic recipients

Klebsiella pneumoniae, *Citrobacter rodentium*, *Salmonella typhi* and *Staphylococcus aureus*, and lab strain *E. coli* recipients in vitro. Conjugation protocols that allowed for quantitating transconjugation events using both introduction and absence of antibiotic pressure for selection were created and employed. The transconjugant colonies were confirmed using colony PCR with primers selecting for presence of green fluorescent protein that exists within the plasmid. Fluorescent microscopy was used to observe the transconjugants plasmid directly. Using the transconjugant frequencies obtained, predictions of plasmid spread could be made that model those in the human gut microbiome. Additionally, the individual combinations of donor and recipient bacteria could give insight into strain-specific features that affect transconjugant frequency and plasmid spread effect. Understanding plasmid spread between gut microbiota is crucial to gain insight on how potential treatments can be developed to combat the spread of antibiotic resistance genes.

21. Directed Spatial Structure and Patterning in Synthetic Communities

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Microbial communities play a foundational role in system regulation. The spatial structure of these natural communities in the environment is something that cannot be controlled in synthetic cultures yet. In order to achieve this, the adhesin pair of SpyTag and SpyCatcher was used. In *Synechococcus elongatus* (*S. elongatus*), SpyTag-FLAG was used for immunofluorescence, confirming the expression, transport, and display of the protein on the outer membrane (OM) via SomA. With that confirmation, *Escherichia coli* BL21 (DE3) was induced after transformation to produce SpyTag-mNG and SpyCatcher-mNG. Sonification and purification were performed, yielding the proteins to be used in a time series binding assay. The gel showed the covalent bonding between SpyTag and SpyCatcher as the combination band of the bound proteins increased as their individual bands decreased. With these promising preliminary results, further experimentation can be performed with the goal of introducing SpyCatcher-mNG to *S. elongatus* expressing SpyTag via SomA, and introducing SpyTag-mNG to *Escherichia coli* W expressing SpyCatcher via Intimin to test direct binding of the two proteins. Further study includes having both *S. elongatus* and *Escherichia coli* W express their proteins simultaneously and combining them in co-culture to test cell binding. Through this project, specific binding can be achieved, providing a toolbox to control the aggregation of the microbes in synthetic communities.

22. The Role of SETD2 in Canine Lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is one type of common and aggressive cancer that can affect humans as well as canines. This cancer does not currently have non-toxic and/or effective treatment therapies. The epigenetic tumor suppressor gene *SETD2*, which is recurrently mutated in human DLBCL tumors, could play some role in the proliferation of DLBCL. The study of the *SETD2* gene in canine DLBCL may reveal a path towards less toxic and more effective treatments in both canine and human lymphomas. To explore this possibility, we will generate three *SETD2* knockout canine B-cell lymphoma cell lines. Methods: Two CRISPR-Cas9 constructs targeting portions of *SETD2* spaced approximately 1kb apart will be introduced into the canine lymphoma cell lines *CLBL1*, 17-71, and GL-1 by electroporation. Selection with GFP sorting and puromycin follow, then single cell cloning. Successful truncation of *SETD2* will be confirmed by Sanger sequencing. After *SETD2*(-) cells are produced and cloned, the effect of the gene's absence can be assessed. Assessment can be carried out by testing proliferative ability, resistance to chemotherapy, microsatellite instability, and alteration of DNA methylation/transcription/etc. The end goal of comparative studies between cells with and without *SETD2* is to create and hypothesize a humane and effective treatment of DLBCL in canines, which may be later compared to human cancer.

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Congratulations to all of the MMG graduating seniors!

Best Wishes for a very bright future!

Microbiology and
Molecular Genetics